Groundnut Virus Diseases in the Asia-Pacific Region

International Crops Research Institute for the Semi-Arid Tropics

Abstract

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Virus diseases cause economically significant losses to groundnut crops. The Fourth Meeting of the International Working Group on Groundnut Viruses in the Asia-Pacific Region was organized by ICRISAT in cooperation with Khon Kaen University, Thailand, Peanut Collaborative Research Support Program (Peanut CRSP), USA, the Samuel Roberts Noble Foundation, USA, Belgian Administration for Development Cooperation, Australian Centre for International Agricultural Research, Directorate General for International Cooperation of the Ministry of Foreign Affairs, The Netherlands, and the Overseas Development Administration, UK. The Meeting was held to develop strategies for the management of groundnut virus diseases in Asia. This publication contains summaries of the papers presented at the Meeting. The first two sessions deal with genome organization of economically important groundnut viruses, and strategies for producing transgenic groundnuts with resistance to virus diseases. The third and fourth sessions cover country-specific situations for the management of groundnut viruses in Bangladesh, China, India, Indonesia, Myanmar, Nepal, Pakistan, and Vietnam. Specific recommendations for collaborative research on groundnut viruses in the Asia-Pacific Region are listed.

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Summary and recommendations of the
Fourth Meeting of the International Working Group

12-14 Mar 1995
Khon Kaen University, Thailand

Edited by

D V R Reddy
C L L Gowda

ICRISAT

International Crops Research Institute for the Semi-Arid Tropics

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Peanut CRSP

Peanut Collaborative Research Support Program

Contents

Welcome Address

C Renard[1]

On behalf of ICRISAT Asia Center, it gives me great pleasure to welcome all participants to the Fourth International Working Group Meeting on Groundnut Virus Diseases in Asia-Pacific Region. We are grateful to Khon Kaen University for agreeing to host this Meeting, and for the help they have rendered with various arrangements. I am very pleased that a number of scientists from the national agricultural research systems of the important groundnut-growing countries in Asia are represented at this Meeting, and I welcome them. I am also especially pleased that many reputed plant virologists from mentor institutions have been able to attend;

Virus diseases are considered to be economically important to groundnut production in Asia. I am confident, that by the end of this Meeting the Group should be in a position to assist in developing strategies for the control of these diseases. I would like to extend my best wishes for the success of the Meeting.

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Introduction and Objectives

D V R Reddy[1]

The first international working group on groundnut viruses was formed in 1983 to coordinate collaborative research and technology-exchange activities. The first meeting of the international working group to investigate virus disease problems in the Asia-Pacific region was held in 1987 in Malang, Indonesia. The group met again in 1989 at ICRISAT Asia Center, India, and in 1993 in Dundee, Scotland. This group activity resulted in:

- The characterization of the peanut stripe virus (PStV) and its isolates;
- Surveys for groundnut viruses in China, India, Indonesia, Philippines, and Thailand;
- Screening of over 10 000 cultivated groundnut genotypes for PStV resistance;
- The discovery that PStV can cause significant crop losses in groundnut; and
- The organization of training courses in China, India, Indonesia, and Thailand.

The major objectives of this meeting are to:

- Discuss the progress made in the diagnosis and management of economically important groundnut viruses, which include peanut bud necrosis virus (PBNV), peanut clump virus (PCV), peanut stripe virus (PStV), and peanut mottle virus (PMV).
- Discuss how the diagnostic tools currently available can be accessed by scientists in the National Agricultural Research Systems (NARS).
- Strengthen research facilities in NARS, especially for virus identification.
- Discuss protocols to be followed for eliminating seedborne viruses in germplasm and to facilitate exchange of virus-free seed material.
- Assist in obtaining research grants for scientists in NARS.
- Get acquainted with the ecoregional initiatives and the role of the group members.
- Know the current progress on the development of sensitive diagnostic tools for diseases and the development of virus resistance by nonconventional approaches.
- Formulate recommendations and work plans which will provide a sound basis for continued international cooperative research on economically important groundnut virus diseases in the Asia-Pacific region.

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Role of CLAN in the Functioning of International Working Groups

C L L Gowda[1], A Ramakrishna[1], D V R Reddy[2], and C Renard[3]

The Cereals and Legumes Asia Network (CLAN) was formed in 1992 to serve as a research and technology exchange network for Asia involving ICRISAT's mandate crops (sorghum, pearl millet, fingermillet, chickpea, pigeonpea, and groundnut). It was formed by merging the erstwhile Asian Grain Legumes Network (AGLN) and the Cooperative Cereals Research Network (CCRN). CLAN consists mainly of scientists and administrators from national research programs in Asian countries, who are willing to commit resources to undertake collaborative research, participate in network activities, and share the results of technology. CLAN's ultimate goal is to improve the well-being of farmers by improving the production and productivity of crops in a sustainable manner (Gowda 1993).

Agricultural research in developing countries is facing an acute paucity of funds, and research administrators and scientists are being asked to cut costs and maximize the cost effectiveness of research and technology exchange. Laboratories and/or institutions are unable to take up comprehensive studies due to the scarcity of funds, facilities, and expertise. Therefore, it is not surprising that scientists are joining hands to share research agendas.

The concept of a Working Group (WG) is not new; scientists around the world have long been pooling their resources and sharing the results of their studies, either formally or informally. For instance, in India, the Coordinated Research Programs brought together scientists from different research organizations to review the research done, plan future research, and share the planned research activities. Many other countries have similar collaborative ventures. In the international arena, collaborative research networks such as CLAN encourage and support a few international working groups in a more formal manner. Working Groups are a means of using funds, facilities, and staff more efficiently and effectively (Faris et al. 1992).

Working Groups are defined as a group of committed scientists with a common interest in addressing high-priority regional problems. Members of a WG are expected to commit time and resources to work together, share research responsibilities, and exchange results. Working Groups bring together expertise from developed and developing countries, international research centers, and nongovernmental organizations, to form the critical mass needed to achieve the objectives.

The membership of a WG may include scientists from national programs, international and regional institutions, and advanced research laboratories. Each WG

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nominates a Technical Coordinator (TC) to liaise, coordinate, and harmonize research. The TC is usually an active scientist in the subject and can be from any one of the institutions mentioned above. Figure 1 shows an example of the structure of a Working Group. The TC is usually supported by a network or institution that provides the necessary administrative and logistic support.

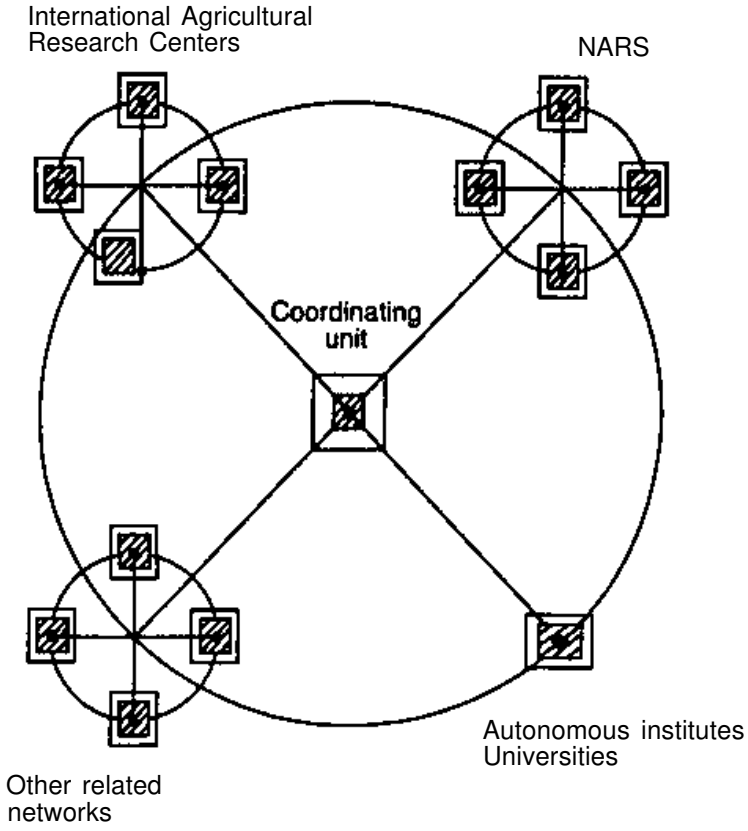

Figure 1. Structure of a Working Group

Working Group on Asia* Pacific Groundnut Viruses

The Working Group on Asia-Pacific Groundnut Viruses is supported by CLAN. Peanut stripe virus (PStV) entered the USA through seed imported from China, and in a span of 4 years, it was found in all the major groundnut-growing areas. It was apparent that the virus, earlier described as peanut mottle virus (PMV) from China, Indonesia, Malaysia, Philippines, and Thailand, was indeed PStV. The importance of PStV in these countries was ascertained following extensive surveys and crop loss estimates. Considering the need expressed by the members of the network to address the problem, AGLN played a major role in establishing a Working Group for PStV.

The first meeting of the group was held in June 1987 at Malang, Indonesia, and was cosponsored by AGLN, the Indonesian national program, Australian Centre for International Agricultural Research (ACIAR), International Development Research Center (IDRC), and Food and Agriculture Organization of the United Nations (FAO). This meeting, which brought together scientists working on PStV in Asia and USA, reviewed the available information on the disease, identified future needs, and prepared a plan to conduct joint research to tackle the virus. Naming of the virus, which was earlier referred to as PeMoV, was clarified as PStV. A subsequent publication (Demski et al. 1993) provided details on virus identification and requirements for future research. The group recommended that future research on PStV should be coordinated, and AGLN was requested to provide the necessary logistics and coordination (ICRISAT 1988).

Subsequently, the AGLN (and later CLAN) coordination unit provided the support for research and facilitated the dissemination of information and exchange of technology in the WG.

During the second meeting on PStV held in August 1989, it was agreed to extend the Working Group's activities to other economically important groundnut viruses in the Asia-Pacific region, and the group was renamed as "Working Group on Groundnut Viruses for the Asia-Pacific Region". The network provides financial support for organizing WG meetings, facilitating contacts among network members, and liaising with other organizations (such as ACIAR, IDRC, FAO, and Peanut CRSP) to carry out joint research, provide seed money or support funding to in-country or third-country research activities, training courses in detecting and diagnosing virus diseases (in 1987, 1990, 1993, and 1995), publication of proceedings (ICRISAT 1988; ICRISAT 1989; Reddy et al. 1994) and information bulletins (Demski et al. 1993).

Another WG for groundnut viruses in Africa has been operating in the African region, and is based on similar principles. There being common members in both the Asia and Africa WGs, it has helped strengthen bonds between them. Both the WGs served as a model for other scientists to emulate the methodologies and procedures developed.

The success of these two WGs has led to the formation of a third working group on "Transformation and Regeneration of Groundnut and Utilization of Viral Genes to Induce Resistance to Virus Diseases". The ACIAR-Indonesia collaborative project on transformation of groundnut with the coat protein gene of PStV to induce resistance to the virus is linked to this WG.

A meeting of the three WGs was held in August 1993, at Dundee, UK. Its participants recommended continued collaboration between the members of the three WGs in order to derive mutual benefits and scientific advancement leading to control strategies to manage the viral diseases (Reddy et al. 1994).

These examples illustrate how CLAN (earlier AGLN) was able to provide a common forum for formulating joint research plans, providing additional funds for research, and facilitating the exchange of information and research results. The Working Group on groundnut viruses (and its predecessor PStV WG) have pioneered the WG concept. This WG has been a model for several other WGs within CLAN and outside in the agricultural research world. The scientists involved in this WG could, therefore, be proud of their achievements and the outcome of their efforts.

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Groundnut Transformation Research in Griffin, Georgia

Zhijian Li[1], Ming Cheng[1], Aiqiu Xing[1], R L Jarret[2], R Pittman[2], and J W Demski[1]

Over the past 3 years, our research efforts have focussed on the development of a system for groundnut regeneration and transformation. In this report we summarize our results on the successful regeneration of groundnut plants from isolated protoplasts; transformation of groundnut protoplasts using electroporation; and production of fertile transgenic groundnut plants using an *Agrobacterium*-mediated transformation system.

Previous studies on protoplast regeneration using *Arachis paraguariensis* (Li et al. 1993) have provided important insights into the principal factors affecting the regeneration of protoplasts of *Arachis* spp, and facilitated the extension of this technology to cultivated groundnut. High levels of protoplast colony formation (up to 19%) were reproducibly obtained from protoplasts isolated from immature cotyledons of a variety of American groundnut cultivars and breeding lines using a nurse culture approach. Our initial efforts to define a shoot induction medium for protoplast-derived callus colonies resulted in a low frequency (about 2%) of recovery of plantlets from several US cultivars. Protoplast-derived plants, grown to maturity in the greenhouse, were fertile and produced viable seeds. Details of the protocols for protoplast isolation, culture, and regeneration have been compiled (Li et al. 1996).

Recent modifications to our protoplast regeneration protocol, including the use of thidiazuron to enhance regeneration, have resulted in a dramatic increase in plant regeneration frequencies. Frequencies of 74.3% and 81.2% of protoplast colonies were noted in cvs EC 5 and Florunner, respectively. These differentiated and subsequently gave rise to shoots within three months (unpublished data).

Protoplast-mediated transformation in groundnut using electroporation

Electroporation has been used to transform groundnut protoplasts because this technique causes less physical damage to the protoplasts, as compared to polyethylene glycol-mediated approaches (Li et al. in press). In order to identify more effective conditions for successful transformation of groundnut protoplasts using electroporation, a poration medium containing glycine, or its derivative glycylglycine (glygly), as an electroporation buffer reagent, was tested. Results indicated that the use of a glycine-based poration medium improved protoplast viability and resulted in an 8-430-fold increase in transient β-glucuronidase (GUS) expression, when compared to other commonly-employed poration media (Li et al. in

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press). Efficient transformation of groundnut protoplasts was achieved using a poration medium containing 50 mM glycine or 10 mM glygly, 70 mM potassium glutamate, and 0.4 M mannitol at pH 7.3.

To date, a large number of transformed groundnut protoplast-derived callus colonies have been recovered. DNA analysis by Southern hybridization of callus colonies derived from protoplasts transformed with the plasmid pBI426 containing the fusion gene for GUS and neomycin phospho-transferase II *(nptII)* confirmed the integration of the transferred genes into the groundnut genome. In our efforts to produce transgenic groundnut plants with resistance to peanut stripe virus (PStV), plasmids containing the PStV coat protein gene have been introduced into groundnut protoplasts. High levels of expression of virus coat protein genes have been confirmed by ELISA in transformed groundnut protoplast-derived callus colonies. Transgenic groundnut plants are being regenerated from transformed protoplasts. Greenhouse studies will be conducted to determine the fecundity of these transgenic groundnut plants.

Agrobacterium-mediated transformation of groundnut

Based on a previously defined protocol for *in vitro* plant regeneration from seedling explants of groundnut cultivar New Mexico Valencia A, a procedure for efficient transformation by *Agrobacterium tumefaciens* strain EHA105 incited with tobacco extracts has been developed. A large number of transgenic groundnut plants containing the GUS and *nptII* genes were produced. Recovered transgenic groundnut plants grown to maturity were fertile and produced viable seeds. DNA analysis by Southern hybridization indicated that the transferred genes were integrated into the nuclear genome of primary- and second-generation transgenic groundnut plants. Histochemical analysis of GUS expression demonstrated that the GUS gene in most of the transgenic groundnut plants was transmitted to the progeny in a single locus in a Mendelian pattern. The development of this successful groundnut transformation protocol using *Agrobacterium* reduced the duration of *in vitro* culture and enabled us to obtain transgenic groundnut plants in a relatively short time. Current efforts are being focussed on utilizing this system of transformation of groundnut using various virus coat protein genes.

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The Genome Organization of Indian Peanut Clump Virus: the Potential for Improved Diagnostics and Transgenic Resistance

M A Mayo[1], J S Miller[1], R A Naidu[2], S V Wesley[2], and D V R Reddy[2]

Indian peanut clump virus (IPCV) causes an economically significant disease in groundnut in several pans of India. The virus is transmitted by a soil fungus *Polymyxa graminis,* which can remain in the soil for many years. Diagnosis of the virus is complicated by the presence of at least three IPCV serotypes which react only weakly, if at all, with heterologous antibodies. No resistance against IPCV has been found in groundnut germplasm, which has necessitated the search for nonconventional sources of resistance.

To tackle this problem, research has been undertaken at the Scottish Crop Research Institute (SCRI), funded by the Overseas Development Administration (ODA), UK. The work, being done in collaboration with ICRISAT virologists, has been based on the determination of the nucleotide sequences of the components of the IPCV genome.

The sequence of the smaller genome RNA (RNA 2) has shown that it contains five genes, of which the coat protein is the 5'-most. Comparison of the sequence of IPCV RNA 2 with the RNA 2 of peanut clump virus (PCV) from West Africa has shown that the two are similar but not identical and that the translation products of the different genes in the RNA are 29-89% identical in sequence, depending on which genes are compared. The sequence of the larger RNA (RNA 1) revealed a different picture. This RNA contains three genes but these differ little in sequence (75-95% identical) between IPCV and PCV. It appears that the viruses have diverged greatly in RNA 2 but little in RNA 1.

The utility of cloned cDNA from different parts of the genome as diagnostic probes was tested by conducting hybridization tests between cDNA and RNA extracted from infected plants. cDNA corresponding to the coat protein gene in RNA 2 reacted only with RNA extracted from plants infected with the homologous virus. In contrast, cDNA corresponding to RNA 1, and in particular that corresponding to the 3'-terminal sequences of RNA 1, reacted readily with RNA extracted from plants infected with three serotypes of IPCV or with PCV. This probe will be used in future survey work.

In earlier work, the coat protein gene of IPCV (H serotype) was inserted into a plant transformation vector. *Nicotiana benthamiana* was transformed utilizing *Agrobacterium tumefaciens* containing this vector, and plants were regenerated from the transformed callus. Some lines of these transformed plants make readily detectable amounts of IPCV coat protein whereas others do not. These plants are now ready for testing to assess their resistance to infection by IPCV.

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Genome Organization of Tospoviruses and the Potential of Some of their Genes to Develop Transgenic Resistance in Plants

D Peters, M Prins, and R Goldbach[1]

Tospoviruses are the causal agents of a number of serious plant diseases, and are a major threat to the cultivation of tobacco in eastern Europe, tomato in Brazil, and groundnut in India and other countries in South Asia. Considerable progress has been made in understanding the molecular biology and mode of transmission of these viruses. Recently published reports describe the use of engineered host resistance to combat these viruses.

Molecular studies at Wageningen revealed that in tospoviruses, genetic information is divided among three RNA molecules. The largest (L) RNA encodes the putative RNA polymerase. The M RNA encodes the precursor to the two glycoproteins (G1 and G2) and a nonstructural (NSm) protein. Evidence accumulated so far indicates that NSm is involved in a tubule-guided cell-to-cell virus translocation. The smallest (S) RNA encodes two proteins, the nucleocapsid (N) protein and a nonstructural (NSs) protein that can form large aggregates in the cytoplasm of infected cells.

Different regions of the tospovirus genome have been studied for their potential to convert susceptible plant species into resistant ones by genetic engineering. High levels of resistance (immunity) have been obtained by expressing the tomato spotted wilt virus (TSWV) nucleoprotein (N) gene in transgenic tobacco and tomato plants. During the course of studies on the mechanisms of this resistance, similar levels of protection were also found when an untranslatable N gene was expressed. This observation indicates that the resistance is, at least for a major part, RNA-mediated. This resistance was not broken when inoculated with viruliferous thrips.

Plants carrying the TSWV N gene were resistant to TSWV isolates, but not to the closely related tomato chlorosis spot and groundnut ringspot tospoviruses. The resistance could, however, be broadened by transforming tobacco plants with a DNA construct comprising the tandemly cloned N genes of these three viruses. Each gene was provided with a copy of the CAMV 35S promotor and the terminator. A transgenic tobacco line was obtained that exhibited high levels of resistance to these three tospoviruses. However, a few lines showed resistance to one or two of these viruses, but not to all three. The results demonstrate that resistance can be achieved by introducing several genes to one locus in the plant genome; this approach could help create broad-spectrum resistance in transgenic plants.

A second potential source of resistance involves the NSm protein gene. Recent studies have shown that this protein could be associated with plasmodesmata in infected plants. This result suggests that the NSm protein is involved in the cell-to-

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cell movement of tospoviruses. Further analysis of the functional properties of this protein has strengthened our idea. Expression of the NSm gene in protoplasts transfected with an expression vector containing this NSm gene sequence, resulted in the synthesis of tubular structures extending from the surface of these cells. Transforming plants with a modified gene that produces a defective protein may result in the impairment of virus transport from cell to cell. Transgenic plants expressing this gene were found to be highly resistant to infection with the virus. Expression of untranslatable as well as anti-sense RNA of the NSm gene resulted in resistance in levels as high as those expressing translatable sequences. The results obtained also indicate that the resistance mediated by the NSm gene is accomplished by the expression of transcripts rather than protein.

The results show that transformation of plants with the N and NSm gene is a successful way to induce high levels of resistance or complete immunity to tospovirus infections. However, studies conducted with the other TSWV genes did not result in transgenic plants showing resistance to TSWV. This shows that the resistance induced by the N and NSm gene has to be explained by specific mechanisms underlying or related to their function during the infection process. Apparently, they play a vital role in infecting plants.

Update on Groundnut Transformation and Evidence for Mechanism of Induced Pathogen-derived Resistance to Peanut Stripe Virus in *Nicotiana benthamiana*

B Cassidy and J Ponsamuel[1]

Current efforts to transform groundnut *(Arachis hypogaea)* are based on an efficient regeneration protocol. This protocol utilizes immature embryos from the fifth developmental class of groundnut seeds (Williams and Drexler 1981). The embryonic axis is dissected from the seed and includes a small amount of cotyledon tissue. The shoot and radicle are cut off perpendicular to the axis. The remaining embryo explant is cocultivated with *Agrobacterium* containing a Ti plasmid carrying a selectable marker gene and a portion of a virus genome. Experiments utilizing the Bar gene as a selectable marker are being conducted. Putative transformed plants have been selected for resistance to Bialophos$^{(R)}$ and will be used for further analysis.

The mechanism of pathogen-derived resistance to peanut stripe virus (PStV) is being investigated. Numerous lines of transformed *Nicotiana benthamiana* expressing open reading frames (ORFs) of the PStV genome, cytoplasmic inclusion protein (CI), viral replicase (NIb), coat protein (CP), and a cassette containing the CI, NIa, and NIb (CNI) have been produced. These plants exhibited two types of resistance. Approximately 10% of the lines exhibited no viral symptoms and contained no detectable virus when challenged with PStV by mechanical transmission. The remainder of the transgenic lines exhibited viral symptoms initially, but subsequently produced leaves that were symptomless and did not contain PStV. We describe this as inducible resistance. The levels of PStV CP transgene protein and mRNA were found to be greatly reduced or nonexistent in the virus-free systemic leaves following the induction of resistance. The virus-free systemic tissue was not susceptible to a second inoculation of PStV but was susceptible to a second inoculation of another potyvirus, tobacco etch virus.

We propose a mechanism for this induced resistance in which the combination of PStV CP transgene expression and infection by PStV combine to produce a response in the newly-developing cells that results in the tagging of both the transgene mRNA and the infecting virus, which leads to their degradation. Further experiments to understand the requirement for high sequence identity between the transgene and the infecting virus are being conducted.

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Current Research on Improved Molecular Diagnosis and Control of Groundnut Viruses

R G Dietzgen[1], P Y Teycheney[1], M Livingstone[2], and R G Birch[2]

With the aim of obtaining coat protein gene-mediated resistance to peanut stripe virus (PStV), four modified PStV and one peanut mottle virus (PMV) coat protein (CP) constructs were expressed using combined *in vitro* transcription/translation in a rabbit reticulocyte lysate system. Analysis of the transcription and translation products showed that all the constructs directed the synthesis of transcripts and proteins of the expected size, and that the translation products could be detected by virus-specific antisera. *Nicotiana benthamiana* plants are being regenerated following leaf disc transformation using *Agrobacterium tumefaciens* harboring all PStV and PMV constructs cloned into the pBIN19 binary vector.

To produce transgenic groundnut plants, the efficiency of four potential transformation systems is being investigated for commercial groundnut cultivars: (i) particle bombardment of embryonic leaflets, (ii) meristem bombardment, (iii) bombardment of embryogenic callus or immature embryos, and (iv) *Agrobacterium-mediated* transformation of leaf discs or embryonic leaflets.

The highlights of our studies are:

- Expression of the luceferose (luc) reporter gene was observed for 7 weeks in nonselected callus formed on bombarded embryogenic leaflets. From the 5000 embryonic leaflets bombarded with pGN1 and cultured on a sublethal level of kanamycin (50 mg L^{-1}), 300 shoots were obtained, 10 of which formed roots. Of these, 3 lines are available for assay by Southern analysis.
- Preliminary results based on transient expression in bombarded meristems appear to be promising, provided care is taken to avoid meristem damage during dissection and bombardment.
- Somatic embryogenesis was observed in immature embryos of groundnut cvs Gajah and NC 7 cultured on MS medium supplemented with 1 mg L^{-1} picloram. Prolonged expression of the luc and uid A reporter genes was observed (2-3 weeks after bombardment) in somatic embryos and embryogenic callus from immature embryos. Somatic embryos (cvs Gajah and NC 7) were shown to proliferate in liquid culture, and the resulting embryogenic callus was shown to have the potential to regenerate *in vitro.*
- Antibiotic-resistant callus was selected following *Agrobacterium-mediatcd* transformation of leaf discs, but for the cultivars we tested, this callus was not capable of regeneration into plants.

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Preliminary results, utilizing the multiple reverse transcriptase-polymerase chain reaction (RT-PCR) method showed that PStV could be detected in groundnut seeds. A novel virus release method which does not require grinding of plant tissue was employed for RT-PCR template preparation. A dot blot nucleic acid hybridization assay was developed for the detection of PStV, PMV, and cucumber mosaic virus (differentiation of both serogroups) using PCR DIG-labelled DNA probes and colorimetric or chemiluminescent substrate.

Use of Serology to Identify Viruliferous Thrips that Transmit Tomato Spotted Wilt Virus and the Use of RT-PCR to Detect Peanut Stripe Virus in Groundnut Seed

J L Sherwood[1]

Detection of viruses in their primary source is imperative for the development of effective tactics and strategies for disease management. Tomato spotted wilt tospovirus (TSWV) replicates in and is transmitted by the western flower thrips *(Franklinella occidentalis).* Monoclonal antibodies (McAbs) were made to the nonstructural protein (NSs) encoded by the small RNA of TSWV. NSs is produced in the thrips vector in which TSWV has replicated. McAbs were used in antigen-coated plate enzyme-linked immunosorbent assay (ACP-ELISA) with the Zwitterionic detergent Empigen-BB (E-BB) at 0.1% (a.i.) in the antibody dilution buffer in order to reduce nonspecific binding which results in high absorbance readings of control samples, especially when thrips were used in ACP-ELISA. With E-BB, a 10-fold difference in absorbance values was observed between adult thrips fed on healthy plants and adult thrips fed on virus-infected plants as larvae, compared with ACP-ELISA with Tween-20, in which there was only a three-fold difference.

The utility of ACP-ELISA in identifying viruliferous thrips was compared with transmission of TSWV by thrips to *Petunia grandiflora.* The two assays were in agreement 92% of the time. The errors were divided: 6% occurred when ACP-ELISA detected thrips with NSs but the thrips were not identified as transmitters in the plant transmission assay, and 2% occurred when ACP-ELISA did not detect thrips that were positive in the plant transmission assay.

Double antibody sandwich (DAS)-ELISA and ACP-ELISA with antibodies to NSs of TSWV were also conducted with leaf tissue infected with Impatiens necrotic spot virus (INSV) and peanut bud necrosis virus (PBNV). Both INSV and PBNV gave positive reactions in DAS- and ACP-ELISA (3 to 4 times the healthy control) with antibodies to TSWV-NSs, but the absorbance was less than the reaction obtained with TSWV (10 times the healthy control). These findings indicate that ACP-ELISA with E-BB is a useful technique for identifying viruliferous thrips and has potential for use in forecasting to manage TSWV epidemics. However, production of antibodies to NSs of the tospovirus of interest may be needed to effectively use this assay.

Serological techniques have been useful in detecting peanut stripe virus (PStV) in a single seed, but are not sensitive enough for screening of seed-lots. The reverse transcriptase-polymerase chain reaction (RT-PCR) is a very sensitive assay, but detection of PStV by RT-PCR and other assays have not been compared. Using a

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modification of the method for extraction of viral RNA from plant tissue and a set of primers that yielded an approximately 400 bp PCR product, detection of PStV was examined by RT-PCR, ELISA, and grow-out tests. The primers used were 5-AAGCCGTTCATC ACAATT-3' (for RT-PCR) and 5'-TCAGGGAGCAGCA-CA-3' (for PCR). Seedlings of the cultivars Florunner and Pronto were mechanically inoculated with PStV at the 3-leaf stage and grown in baskets (approximately 45 cm x 45 cm x 30 cm) to maturity. Of 200 seeds of each cultivar tested, some that were positive by ELISA (OD_{405} < 0.100) were assayed by RT-PCR and grow-out tests. All seeds positive by ELISA were also positive by RT-PCR. For the cv Florunner, seven seeds negative by ELISA were also negative in grow-out tests, but two were positive by RT-PCR. For the cv Pronto, of 26 seeds negative by ELISA, one was positive in the grow-out test, and 16 were positive by RT-PCR. Apparently, RT-PCR may detect viral RNA in groundnut seed that does not lead to infection. Further evaluation of RT-PCR is under progress.

Components of Resistance to Peanut Bud Necrosis Virus

A A M Buiel[1,2], D V R Reddy[1], D Peters[3], and J E Parlevliet[2]

Peanut bud necrosis disease caused by peanut bud necrosis virus (PBNV), is a major constraint to groundnut production in South Asia. It has been reported from China, India, Nepal, Sri Lanka, and Thailand. The virus is persistently transmitted by *Thrips palmi* Karny, but is not transmitted by seed. Breeding for resistance to both the virus and the vector is the most promising approach to control the disease. Field resistance has been observed in some groundnut genotypes, and is expressed as a lower infection rate and fewer infected plants.

Studies using sap inoculation tests have revealed a general adult-plant resistance independent of genotype. For instance, it was observed that with increasing leaf age or plant age, the number of infected plants became fewer in all genotypes. To exclude factor of adult-plant resistance, only the unfolded third leaf (in order of appearance) was used in the inoculation tests. Using this method, virus-resistant genotypes were identified e.g., ICGVs 86029, 86031, 86363, 86388, and 86430. Genotypes with field resistance but without virus resistance were assumed to have vector resistance. This was confirmed in field experiments in which low thrips populations were observed on these genotypes.

In our study, we examined the virus resistance mechanisms causing reduced incidence. Specifically, we studied the development of virus concentration in the mechanically sap-inoculated leaf and the spread of the virus to systemically infected leaves, in order to investigate whether reduced incidence is a result of resistance to multiplication or restriction of systemic translocation.

Virus multiplication was inhibited in the inoculated leaves of resistant genotypes. Yet, when systemic infection occurred in the resistant genotypes, the virus concentration in the systemically infected leaves was comparable with that in the susceptible genotypes. Virus concentration was positively correlated with the severity of the symptoms on the systemically infected leaf in both the resistant and susceptible genotypes. These results indicate that resistance inhibits virus multiplication at the site of infection. Thus, resistance decreases the chances of systemic infection, resulting in reduced disease incidence in a population of resistant genotypes.

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Current Research on Breeding for Resistance to Groundnut Viruses at ICRISAT Asia Center

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Diseases of groundnut *(Arachis hypogaea* L.) caused by peanut bud necrosis virus (PBNV), peanut mottle virus (PMV), peanut stripe virus (PStV), and Indian peanut clump virus (IPCV) are economically important in the Asia-Pacific region. At ICRISAT Asia Center (IAC), groundnut breeding activity has focussed on germplasm screening and development of improved germplasm with resistance to peanut bud necrosis disease (PBND). Over 8000 accessions of cultivated groundnut *(A. hypogaea)* and 19 accessions of wild *Arachis* species were screened for resistance to PBND under field conditions. Of these, ICGs 848, 851, 852, 862, 869, 885, 2271, 2306, 2307, 2323, 2741, 3042, 3806, 3873, 5024, 5030, 5043, 5044, 6135, 6317, 6323, 7676, and 7892 showed consistently low PBND incidence compared with the susceptible control JL 24. They all belong to the subspecies *hypogaea.* Five accessions of the wild species *A. duranensis* (30064, 30065, 36002, 36002-2, and 36005), and one accession each of *A. volida* (30011), *A. correntina* (9530), and *A. monticola* (30063) showed resistance to PBND.

PBNV is transmitted by *Thrips palmi.* Resistance to PBND could be due to resistance to the vector and/or the virus. Therefore, the strategy adopted for breeding for resistance involves the incorporation of resistance to both vector and virus into improved genetic backgrounds. Several high-yielding lines with resistance to PBND have been developed at IAC. In the majority of these lines, resistance to PBND is due to nonpreference by the vector. All the improved lines tested so far, including interspecific derivatives, are susceptible to PBNV when mechanically inoculated at a relatively high virus concentration (10^{-1} dilution of extract). However, ICGV 86031 and ICGV 86388 showed resistance when inoculated at a low virus concentration (10^{2} or lower dilution of extract). High-yielding cultivars (ICGVs 87123, 87128, 87189, and 876141) with field resistance to PBND are now being grown in India.

PMV is seedborne and is transmitted by many aphids. None of the 3000 cultivated groundnut germplasm lines screened so far have shown resistance to it under field conditions. However, NC Ac 2240 and NC Ac 2243 consistently showed significantly lower yield losses than the susceptible controls. Therefore they are regarded as tolerant to PMV. NC Ac 17090 and NC Ac 17133 (RF), the two rust- and late leaf spot-resistant sources, did not transmit the virus through seeds when more than 20 000 were tested. A limited breeding program was initiated to combine the tolerance and non-seed-transmission traits with improved genetic backgrounds. Several advanced breeding lines are now available for further screening.

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Our efforts in the past to locate resistance to IPCV and PStV in cultivated groundnut germplasm have failed. Resistance to PStV in some accessions of wild *Arachis* species has been reported by other workers. However, attempts have not been made to transfer this resistance to cultivated groundnut. The development of transgenic plants containing virus genes offers exciting prospects for inducing resistance to IPCV and PStV.

Strategies for Management of Indian Peanut Clump Virus

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Peanut clump virus is a furovirus affecting groundnut in West African countries (WAPCV) and India (IPCV). Since the virus is seedborne in *Arachis hypogaea, Eleusine coracana, Pennisetum glaucum,* and *Setaria italica,* use of virus-free seeds is essential to prevent the spread of the disease. *Polymyxa* spp, a soil-inhabiting fungus, transmits the virus to groundnut and also to various other crops and weeds.

Cynodon dactylon, Dactyloctenium aegyptium, Digitaria ciliaris, Eleusine coracana, Eragrostis ciliaris, E. tremula, E. uniloides, Pennisetum glaucum, Setaria italica, Sorghum bicolor, S. sudanense, Triticum aestivum, and *Zea mays* have been found to be naturally infected by both the virus and the fungus.

The virus is often detected in groundnut and in some weeds e.g., *Celosia argentea* and *Oldenlandia corymbosa.* Interestingly, these hosts have not been found to be colonized by the fungus.

Polymyxa cystosori have seldom been observed in the roots of *A. hypogaea.* An experiment conducted at ICRISAT Asia Center (IAC) has indicated that it is possible to reduce disease incidence by growing a crop nonpreferred by *Polymyxa* before raising groundnut in the rainy season. Such a crop would induce the germination of the resting spores, leading to a reduction in the inoculum of the fungus in the soil. Nevertheless, it is advisable not to grow groundnut continuously because this may drastically increase pressure from other pathogens.

In Rajasthan, in fields where peanut clump disease is very severe, a collaborative trial is in progress with the Rajasthan Agricultural University, in which groundnut is being rotated with chickpea, mustard, and sunflower instead of wheat, which is the most commonly grown crop in the postrainy season. It was found that the weeds *Cyperus rotundus* and *C. diffusus* usually have a high degree of infection by the fungus. However PCV was not detected in them. This is an important observation. If *Polymyxa* spp infecting *Cyperus* spp is the same fungus that transmits the virus to groundnut, then this raises important possibilities. If the fungus can multiply in a host that is immune to virus infection, it may be rendered nonviruliferous. It is absolutely essential to identify a crop plant with the same features. Growing such a crop before groundnut will lead to a gradual reduction of the virus inoculum and a concomitant reduction in disease incidence.

Cynodon dactylon is a pernicious weed commonly found in farmers' fields which can reproduce vegetatively through rhizomes. All the rhizomes arising from an infected plant contain the virus. Such rhizomes can be reservoirs of the virus. Since they tend to spread in the soil, the new roots arising from them can provide viral

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inoculum to the nonviruliferous *Polymyxa* and thus create a new nucleus of the disease. Therefore, clean cultivation appears to be necessary for the management of the disease.

Groundnut is usually not affected by the disease during the postrainy season as IPCV transmission is correlated with temperature. When temperatures are below 25°C, only negligible disease incidence has been observed (Reddy et al. 1988). When groundnut was raised in the rainy season, if sowing was delayed beyond the onset of the monsoon rains, most of the groundnut plants escaped the disease. In summer, the soil temperature can easily reach 45°C. This dry heat is likely to break the dormancy of the spores of *Polymyxa* spp and the first rains may induce the production of primary zoospores which infect even nonpreferred hosts. However, preferred hosts of *Polymyxa* spp such as sorghum, finger millet, pearl millet, and wheat can be infected throughout the year. Wheat was found to be infected even when the soil temperature varied from 17 to 24°C whereas the optimum temperature required for *Polymyxa* spp was found to be between 25 and 30°C for the Indian isolates (A. Legreve, personal communication).

These results indicate that if a preferred host is grown under irrigated conditions, *Polymyxa* spp carrying IPCV can infect crops during the postrainy season. In this case, the germination of resting spores is likely to be induced by the root exudates of the preferred hosts.

Various chemicals (dibromochloropropane, carbofuran, and aldicarb) have proved efficient in reducing IPCV incidence. Whether their use is economical or not is yet to be ascertained. Some of them are considered hazardous to humans. Solarization can effectively reduce disease incidence but the effect is not permanent.

More than 8000 germplasm lines were tested under field conditions in Ludhiana and Bapatla but none of them showed resistance or tolerance. In future, transformation of groundnut by the introduction of the gene coding for the coat protein of the virus may lead to resistance. At present, a well chosen crop rotation system that avoids cereal crops before groundnut, delayed sowing, using nonpreferred hosts of *Polymyxa* spp or crops that are immune to the virus before raising groundnut, clean cultivation, and use of virus-free seeds are among the practices directly available to the farmer to reduce disease incidence.

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Current Research on Peanut Stripe Virus and Measures to Contain its Spread in India

M S Basu[1]

Peanut stripe virus (PStV) is currently considered to be one of the most economically important groundnut viruses in South Asia and Southeast Asia. In India, it is regarded as a quarantine disease. It was first observed in 1987 in eight entries originating from Junagadh, Gujarat, which had been grown for multilocational evaluation under the All India Coordinated Research Project on Oilseeds (AICORPO). To prevent the virus from spreading further and to eliminate chances of its establishment, all the genotypes in the varietal trials at all the 34 test locations in the country were destroyed. This was followed by regular monitoring and destruction of all suspected samples. Thus, within two years after its first appearance, the virus was eradicated from all the groundnut research centers in the country except Junagadh and its vicinity.

In India, peanut stripe virus isolate produces green blotches on young leaves which persist in the older leaves. Seed transmission frequency has been found to range from 12% (JL 24) to 29% (Kadiri 3). The virus may be transmitted by *Aphis craccivora* and *A. glycines* in a nonpersistent manner. Higher incidence of the virus was observed in the postrainy season than in the rainy season. This may be due to the presence of larger numbers of aphids during the postrainy season.

No reliable data are available on crop losses, under either greenhouse or field conditions.

Besides suspension of seed production activities at Junagadh, a ban has been imposed on the movement of seed/plant materials from there to elsewhere in the country. Farmers' fields around Junagadh are being closely monitored by staff trained in the identification of PStV. The staff of ICRISAT Asia Center and the Directorate of Plant Protection, Quarantine, and Storage have been helping in the monitoring of PStV in Junagadh as well as in other parts of India. Measures are being taken to eradicate the virus in Gujarat, and therefore from all of India.

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Working Group Approach to Finding Solutions for Groundnut Virus Diseases in the Asia-Pacific Region

Sopone Wongkaew[1]

Tackling virus diseases calls for a multidisciplinary approach as in the case of other plant diseases. At the national level, a virologist needs to collaborate with entomologists and plant breeders in order to secure data that could be used to formulate a practical disease control strategy. Precise identification of the causal virus/es is necessary before formulating control measures. To accomplish this, trained personnel and elaborate laboratory equipment are essential. However, such facilities may not be available in most of the developing countries in the Asia-Pacific region. This problem can be overcome by scientists sharing data or working together as a group. International institutes could play the role of mediator or coordinator to facilitate the formation of such groups.

At present, there are two working groups tackling problems relating to groundnut viruses in Africa and the Asia-Pacific region. Although remarkable progress has been made, there are still many obstacles to be overcome before economical and effective control measures can be recommended for important viruses such as peanut stripe virus (PStV). More active involvement of the national agricultural research systems (NARS) in the working group will accelerate progress towards such a goal. Linkages should be established between NARS in the Asia-Pacific region and mentor institutes and international agricultural research centers. This would facilitate collaborative research and technology exchange activities. The following steps should be taken:

- Select one of the countries in the region possessing the necessary facilities and expertise to act as a nodal center for technology transfer.
- Identify the most serious virus disease problem in the region.
- Exchange available information on groundnut virus diseases.
- Share the available resources.

Since peanut bud necrosis virus (PBNV) is one of the most important viruses in many Asian countries, it may be justified to propose a working group for this virus. As there is sufficient evidence to support the assumption that the virus is present only in Asia, the Asia-Pacific Working Group should take up the task of finding a solution to it.

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Current Research on Groundnut Virus Diseases in China

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Current research on groundnut virus diseases in China is focussed on three major economically important viruses: peanut stripe virus (PStV), cucumber mosaic virus (CMV), and peanut stunt virus (PSV). Disease surveys conducted from 1992 to 1994 revealed that PStV was prevalent in all groundnut fields in seven counties in Hubei and Shandong provinces. Severe PSV epidemics were recorded in Qianan and Luanxian counties in Hubei province in 1992. CMV occurred in all seven counties, and reached epidemic proportions in Qianan and Luanxian counties of Hubei province in 1994, and in Qixia, Penglai, Muping, Fousan, and Laiyan counties in Shandong province in 1993 and 1994.

We worked in cooperation with local extension agencies to develop integrated management practices for groundnut virus diseases, mainly focussing on CMV, in various counties in Shandong province. Assessment of crop losses showed that early infection (<60 days after sowing) by CMV reduced pod yields by 33.3% in polythene mulched fields and by 45% in nonmulched groundnut fields. The average seed transmission rates for CMV and PStV from seeds collected in the Yantai area, tested by ELISA and grow-out tests, were 0.14% and 0.36% in 1993 and 0.95% and 0.38% in 1994, respectively. This indicated that seed transmission may be the major primary source of inoculum for CMV and PStV.

Application of polythene mulch reduced CMV incidence in farmers' fields in Yantai. Removal of CMV-infected seedlings (from seed transmission) at an early stage reduced CMV incidence to 12.7% compared with 33% in control plots in a field trial in Qixia in 1993. Integrated management practices recommended for CMV in Yantai include obtaining seeds from fields with low or no CMV infection, preferably from polythene sheet mulched fields; application of polythene mulch; and removal of CMV-infected seedlings at an early stage. These practices were tested in farmers' fields in four villages in 1993 and 1994. It was found that in farmers' control plots CMV incidence was in the range of 75 to 100% whereas it was only 2.3 to 29.0% in farmers' fields where integrated management practices were adopted.

Seven PSV isolates including six Chinese isolates, namely PSV-Mi, PSV 1, PSV 13, PSV-P, PSV-R, and PSV-F and one American isolate, PSV-E, were classified into three groups depending on host reaction. All the six Chinese isolates in groups I and II differed from PSV-E (group III), in that they induced systemic infection in *Chenopodium amaranticolor* and *C. quinoa.* The Chinese isolates could be serologically distinguished from PSV-E by the spur formation in agar gel diffusion tests. To study the epidemiology of PSV, disease incidence was correlated with the numbers

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of aphids collected in yellow pans in Kaifen county, Henan province, from 1988 to 1993. PSV incidence was positively correlated with the number of flight aphids trapped, and negatively correlated with rainfall during the growing season. Low rainfall and high aphid populations caused PSV epidemics in 1988, 1991, and 1992.

Cultivated groundnut genotypes (3142 accessions) were evaluated for resistance to PStV by natural infection in fields from 1983 to 1994 in Wuchang. Most of them were infected by PStV up to 100% by mid growing season. None was found to be resistant. Application of polythene sheet mulch and use of virus-free seeds showed effective control of PStV in field trials. PStV incidence could be reduced to 27.1% as compared with 96.7% in control fields. Recommended management practices for PStV control include use of seeds with low or no seed transmission, isolation from commercial groundnut fields, and mulching with polythene sheet. However, application of these measures for the control of PStV disease in areas with large-scale commercial groundnut production still remains a problem.

Requirements for the Management of Groundnut Viruses in India

D V R Reddy[1]

The main options available for the integrated management of groundnut virus diseases are cultural practices, host-plant resistance, and direct vector control. In order to formulate suitable cultural practices, it is essential to understand the epidemiology of the particular disease, which includes the ecology of the principal vector/s. It is well known that adoption of several methods of control is preferable to reliance on only one. It is important to emphasize that precise diagnosis of the causal virus/es is essential in order to devise control strategies.

Although many viruses infect groundnut under natural conditions, currently peanut bud necrosis virus (PBNV) and Indian peanut clump virus (IPCV) are considered to be economically important. Peanut stripe virus (PStV) and cowpea mild mottle virus (CMMV) occur in India and have the potential to cause economic losses. Substantial progress has been made in understanding the genome organization of IPCV (Mayo et al. page 11, these proceedings), PStV (Cassidy and Ponsamuel, page 14, these proceedings), and PBNV. The genome of CMMV is currently being sequenced. Excellent sources of resistance have been located for PBNV (Buiel et al. page 19, these proceedings and Dwivedi et al. page 20, these proceedings). Future research on PBNV will focus on epidemiology and incorporation of resistance into short-duration groundnuts through nonconventional approaches.

More than 8000 genotypes, including 100 *Arachis* species, were screened at Ludhiana and Bapatla for resistance to IPCV. None was found to be resistant. The two main options available for control of IPCV are adoption of suitable cultural practices and development of host-plant resistance through nonconventional approaches. An understanding of the epidemiology of IPCV is essential for formulating cultural practices. Details of the research currently being done on this aspect are discussed by Delfosse et al. (page 22, these proceedings). Results of this investigation will lead to formulation of cultural practices for effective control of IPCV.

PStV is currently known to be restricted to Gujarat state in India. Attempts are being made to eradicate the virus. Since the virus is economically important in several countries in Southeast Asia, incorporation of resistance through nonconventional approaches should be given high priority. Efforts by Dietzgen et al. (page 15, these proceedings) and Cassidy and Ponsamual (page 14, these proceedings) in this direction will lead to availability of groundnut genotypes resistant to PStV.

Sequencing of the genome of CMMV is currently being done at the University of Florida at Lake Alfred. The virus has a very wide host range, and is transmitted with a high level of efficiency by the whitefly *Bemisia tabaci*. From the limited surveys done, it appears to be widely distributed in India and in many groundnut-growing countries in Asia and Africa (Reddy 1991). However, extensive surveys are needed to determine its economic importance.

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growing countries in Asia and Africa (Reddy 1991). However, extensive surveys are needed to determine its economic importance.

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Current Status of Research on Legume Viruses with Special Reference to Groundnut Viruses in Nepal

B P Sharma[1]

Phytopathological research in Nepal has primarily focussed on diseases of fungal origin. Diseases caused by viruses have not received much attention although they have been reported on legume crops. Their identification has been based mainly on visual symptoms under natural conditions. Mung bean yellow mosaic gemini virus has been reported on green gram, black gram, pigeonpea, soybean, and cowpea; pea seedborne mosaic virus on lentil and peas; cucumber mosaic virus and pea leaf roll virus on chickpea; soybean mosaic virus and tobacco ring spot virus on soybean; southern bean mosaic virus and cowpea aphidborne mosaic virus on cowpea; sterility mosaic on pigeonpea; and bud necrosis virus on groundnut.

Peanut bud necrosis virus (PBNV), which is widely prevalent, has been shown to cause substantial yield losses. A yield loss assessment study conducted between 1992 and 1995 at Nawalpur revealed that PBNV infection during the early stage of crop growth (prior to 30 days after sowing) could cause complete loss of pod yield. The pod yield loss was found to be negatively correlated ($r = 0.98$) with the age at which the plants were infected. The overall cumulative disease incidence was 29%, and pod yield loss reached 27.7% in the majority of the fields surveyed.

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Preliminary Studies on Viruses of Groundnut in Myanmar

Day May Khin[1]

Groundnut *(Arachis hypogaea* L.) is the major source of edible oil in Myanmar. It is grown as a rainfed crop during the monsoon over an area of 0.26 m ha, and as a winter crop after rice over 0.22 m ha. Roasted groundnut seeds are used in confectionery. The most commonly grown variety is the Spanish type.

Insect pests, diseases, rodents, and sometimes drought are the major yield-limiting factors in groundnut. Leaf spot, collar rot, and rust are the major diseases affecting the crop. Symptoms of virus diseases have also been observed for a long time in the groundnut-growing areas of Myanmar. However, identification of these diseases is yet to be undertaken.

Despite the lack of facilities for electron microscopy and to conduct serological tests, work on symptomatology, host range, graft, sap, and insect transmission of groundnut viruses is possible.

Future research should include characterization of economically important groundnut viruses occurring in Myanmar and preparation of diagnostic aids. Research on groundnut viruses was initiated in 1995/96 with field surveys and detection by serological methods.

It is quite likely that the groundnut viruses currently known to occur in neighboring countries are also found in Myanmar. Since legumes are cultivated over large areas and there is an omnipresence of insect vectors, a number of viruses unique to Southeast Asia are expected to be found in Myanmar.

Our own meagre resources render it difficult to tackle the virus problems owing to the high cost of equipment, chemicals, and consumables. Hence, any collaboration with other laboratories involved in groundnut virus research will be appreciated.

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Research on Legume Virus Diseases in Vietnam

Tran Thi Thuan and Ha Minh Trung[1]

Groundnut is the main legume crop in Vietnam occupying more than 200 000 ha, followed by soybean (150 000 ha) and mung bean (60 000 ha). Although the climatic conditions and soils in the country are suitable for growing legumes, the average pod yield is rather low (about 1 t ha^{-1}). This is ascribed to the lack of improved varieties, inadequate irrigation facilities, and biotic constraints. Until recendy, little attention had been paid to research on groundnut diseases.

Systematic surveys on the incidence of various diseases of groundnut have been conducted since 1990 in collaboration with ICRISAT. However, these surveys have been restricted to fungal and bacterial diseases. Identification of viruses occurring on groundnut has not yet been undertaken. Reports have until now been based mainly on the observation of field symptoms. Surveys conducted in the northern provinces in 1967/68 indicated that peanut stunt (attributed to *Arachis* virus Smith) was the only one present under field conditions.

In surveys conducted by the National Institute of Plant Protection (NIPP) in 1992, plants showing symptoms of peanut bud necrosis virus and peanut stripe virus were observed. Witches' broom symptoms also were observed in some locations. However, no attempt has been made to identify the causal virus in each case.

It is necessary to conduct surveys on the distribution of legume virus diseases, including those of groundnut, in order to determine their economic importance. Groundnut viruses have been known to be economically important in neighboring China and Thailand. Since seed is expected to be imported into Vietnam from these countries, it is imperative to establish a laboratory to test exotic germplasm. Research should be initiated on viruses that are widely distributed. Vietnam needs trained virologists and funds to establish facilities for virus research.

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Virus Diseases of Groundnut in Indonesia with Special Reference to Peanut Stripe Virus

Yuliantoro Baliadi and N Saleh[1]

Several virus diseases of groundnut have been reported from Indonesia. These are peanut mosaic transmitted by leaf hoppers, peanut leaf curl and rugose, peanut mottle virus (PMV), cowpea mild mottle virus (CMMV), peanut bud necrosis virus (PBNV), and peanut stripe virus (PStV). Except PStV, all of them are considered to be of minor importance. This conclusion is based on the disease incidence of these viruses, their spread and distribution pattern, and potential to cause severe yield losses.

PStV is considered to be economically important in all the major groundnut-growing areas of Indonesia. Assessment of crop losses showed that early infection by PStV reduced pod yields by up to 50% under field conditions.

PStV posed a serious threat to groundnut production in Indonesia in 1987. Since then, several projects have been undertaken to identify the virus, its epidemiology, and devise strategies for its management.

The cultivar Kidang was found to be a better source of purified PStV than cv Gajah. Indirect ELISA proved to be more sensitive than direct ELISA.

Seed transmission of PStV was found to vary from 1.4 to 3.3% depending on the variety. It was influenced by the age at which the plants were infected. Plants infected early had a higher rate of seed transmission. It was 3.62% if the plants were infected one week after sowing, and 0.45% if they were infected when they were five week old.

More than 12 aphid species were found to transmit PStV. The population density and feeding habits of *Aphis craccivora* influenced epidemics. Both *A. craccivora* and *A. glycine* could transmit PStV from soybean to groundnut and from groundnut to groundnut but their transmission efficiency differed. While *A. glycine* was more efficient in transmitting PStV from soybean to groundnut, *A. craccivora* was more efficient in transmitting it from groundnut to groundnut.

Epidemiological studies on PStV in Indonesia have shown that the incidence of the virus was high only in the postrainy season. The primary source of PStV inoculum appeared to be infected seed, with subsequent dissemination being done by aphids. PStV belongs to the compound interest disease (CID) category. The infection rate (r) of PStV in all locations varied between 0.26 and 0.56 per plant per week. The value (r) indicated the incidence of PStV. The first symptom appeared close to the source of the inoculum and it spread up to a distance of 8.35 m 5-6 weeks after sowing.

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Though the cultivation of PStV-resistant groundnut cultivars is seen as the most effective way of PStV management, attempts to obtain such genotypes have not been successful.

Sowing of virus-free groundnut seeds in areas where PStV is not known to be endemic resulted in very low PStV incidence. EC 36892, an aphid-resistant groundnut genotype, showed lower aphid population and yield loss compared to var Gajah. However, it failed to show resistance to the virus in sap inoculations and aphid transmission tests. In areas where PStV is endemic, sowing of virus-free seed did not result in low PStV incidence.

PStV management through vector control (insecticides), cultural practices such as mixed cropping, intercropping with trap crops (cowpea and mung bean), and barrier crops (corn), roguing of infected plants, weeding, sanitation, and rice straw or polythene sheet mulching require further investigation. Since PStV-resistant sources are not currently available and the PStV genome is fully sequenced, attempts are being made in a collaborative project supported by funds from the Australian Centre for International Agricultural Research (ACIAR), to develop transgenic groundnut plants expressing PStV coat protein genes.

Integrated Management of Groundnut Viruses in Bangladesh

Firoza Khatun, F U Mian, and H U Ahmed[1]

Legumes are an important group of food crops in Bangladesh next to rice and wheat. Pulses are grown over an area of 0.7 million ha and groundnut over 0.4 million ha. At present, groundnut is the second most important oilseed crop after rape seed, and there is ample scope for expanding its cultivation.

Virus diseases are economically important throughout the pulse- and groundnut-growing areas of the country. So far, 19 viral diseases have been recorded on eight legume crops, including groundnut. The groundnut virus diseases that are suspected to occur in Bangladesh are bud necrosis (caused by peanut bud necrosis virus - PBNV), mosaic (causal virus not known), peanut stripe (peanut stripe virus), and peanut mottle (peanut mottle virus).

Research on viruses in Bangladesh has been limited. Disease incidence, crop losses, vector and seed transmission, epidemiology, and control aspects of the viruses occurring in the country have not been investigated. Limited trials have been initiated to screen germplasm for resistance. Of the 25 entries of groundnut screened against PBNV and mosaic virus diseases under field conditions, two were found to be tolerant to PBNV and three to mosaic virus.

In order to develop integrated management practices for virus diseases of groundnut and other legume crops in Bangladesh, emphasis must be laid on:

- Surveys for virus diseases to determine the incidence and distribution;
- Identification of the principal vectors of economically important virus diseases;
- Development of disease-resistant cultivars and cultural practices that can reduce disease incidence;
- Establishment of laboratory and greenhouse facilities for the characterization and detection of viruses; and
- Training of staff in the identification and detection of viruses.

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Recommendations for global cooperation in research on groundnut viruses in the Asia-Pacific Region

- The supply of resistance material produced either by conventional or nonconventional methods (transgenic lines) should be given a high priority. The Working Group endorses transgenic approach.
- Support for virus diagnosis is needed, preferably in the form of kits containing antisera and other reagents. Nucleic acid probes should be provided or developed only for those viruses that might not be distinguished by serology, e.g., peanut clump virus.
- Diagnostic surveys should be conducted in the major groundnut producing regions of each country. ICRISAT should provide a survey protocol that facilitates harmonization of the results obtained in different countries. ICRISAT should solicit information from members on surveys that have been conducted, and disseminate information to other member countries of the Working Group.
- It is essential to generate data on epidemiology in order to develop integrated management practices.
- ICRISAT should conduct training courses, similar to the highly successful one held in Khon Kaen, for individuals or groups from developing countries. Funds for conducting such courses are to be identified from donor agencies by ICRISAT. Such countries as China, Thailand, and India should take the initiative in the provision of diagnostic aids, and technology transfer to other Working Group members in the Region.
- Regulations for utilizing transgenic lines in different countries within the Asia-Pacific Region are to be obtained and distributed to the member countries.
- ICRISAT should solicit information from group members on their research on groundnut viruses and publish it at least once a year preferably as an informal newsletter.
- ICRISAT should take the initiative in preparing such material as case histories, risk assessments etc. to help members of the Working Group to approach their own governments with proposals for virus research support.
- If any seed-transmitted viruses are intercepted (identified) ICRISAT should be informed. ICRISAT in turn will inform FAO to disseminate the information through their channels.
- ICRISAT should take the initiative to develop consortium-based projects to target funds from such sources as the Asian Development Bank (ADB), World Bank, etc.
- The next Working Group will be held in China in 1998. Professor Xu Zeyong will help choose the venue and make arrangements.
- The value of obtaining training in the diagnosis and identification of viruses at ICRISAT Asia Center and in advanced laboratories in developed countries was recognized by the Group. It may be desirable that a central register of training possibilities is held by ICRISAT and provided to members, so that when they travel they can visit appropriate laboratories.

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About ICRISAT

The semi-arid tropics (SAT) encompasses parts of 48 developing countries including most of India, parts of southeast Asia, a swathe across sub-Saharan Africa, much of southern and eastern Africa, and parts of Latin America. Many of these countries are among the poorest in the world. Approximately one-sixth of the world's population lives in the SAT, which is typified by unpredictable weather, limited and erratic rainfall, and nutrient-poor soils.

ICRISAT's mandate crops are sorghum, pearl millet, finger millet, chickpea, pigeonpea, and groundnut; these six crops are vital to life for the ever-increasing populations of the semi-arid tropics. ICRISAT's mission is to conduct research which can lead to enhanced sustainable production of these crops and to improved management of the limited natural resources of the SAT. ICRISAT communicates information on technologies as they are developed through workshops, networks, training, library services, and publishing.

ICRISAT was established in 1972. It is one of 16 nonprofit, research and training centers funded through the Consultative Group on International Agricultural Research (CGIAR). The CGIAR is an informal association of approximately 50 public and private sector donors; it is co-sponsored by the Food and Agriculture Organization of the United Nations (FAO), the United Nations Development Programme (UNDP), the United Nations Environment Programme (UNEP), and the World Bank.

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