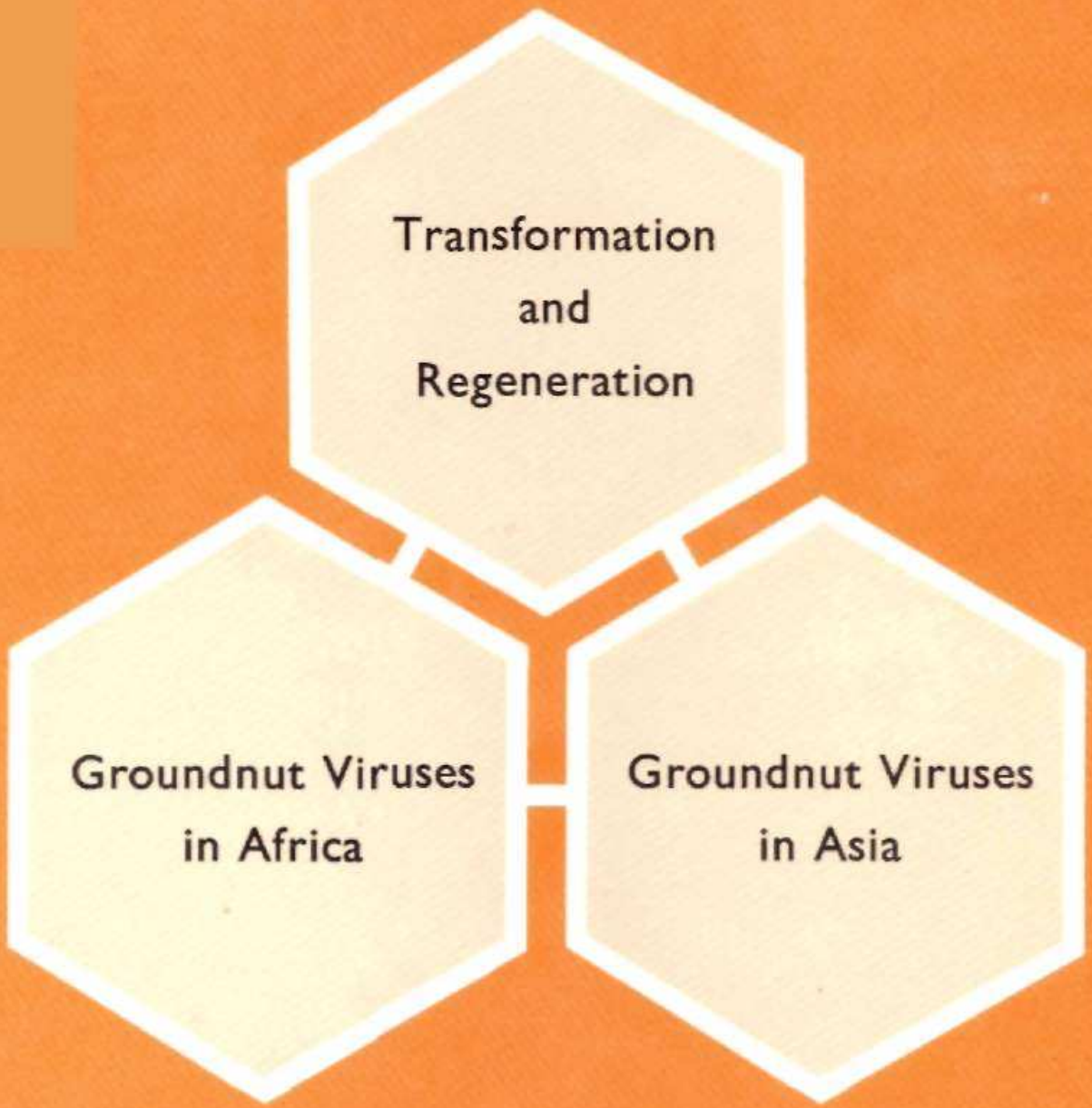
Working Together on Groundnut Virus Diseases



International Crops Research Institute for the Semi-Arid Tropics

Abstract

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At a meeting organized by ICRISAT in cooperation with the Peanut Collaborative Research Support Program (Peanut CRSP) and the Virology Department, Scottish Crop Research Institute (SCRI), UK, scientists from 11 countries, representing the three working groups—'Groundnut viruses in Asia-Pacific region', 'Groundnut viruses in Africa', and 'Transformation and regeneration of groundnut, and utilization of viral genes to induce resistance to virus diseases'—reviewed the progress made by the three working groups since their last meetings. Following general discussion, recommendations were made for global cooperative research on groundnut viruses, and specific recommendations for collaborative research were listed for each working group.

Résumé

Les groupes de travail internationaux sur les maladies virales de l'arachide: compte rendu et recommandations d'une réunion 15–19 août 1993, Institut écossais de recherche sur les cultures, Dundee, Royaume-Uni. Lors d'une réunion organisée par l'ICRISAT en collaboration avec le Programme d'appui à la recherche collaborative sur l'arachide (Peanut CRSP) et avec le Département de virologie de l'Institut écossais de recherche sur les cultures (SCRI), Royaume-Uni, des chercheurs provenant de 11 pays, représentant les trois groupes de travail ('Virus de l'arachide dans la région Asie-Pacifique', 'Virus de l'arachide en Afrique' et 'Transformation et la régénération de l'arachide et l'utilisation de gènes viraux pour induire la résistance aux maladies virales') ont fait le point des progrès effectués par les trois groupes depuis leur dernière réunion. Une discussion générale a permis la formulation de recommandations pour la recherche collaborative globale sur les virus de l'arachide. Des recommandations particulières pour la recherche collaborative ont également été dressées pour chaque groupe de travail.

Working Together on Groundnut Virus Diseases

Summary and recommendations of a meeting of international working groups on groundnut virus diseases

15-19 Aug 1993
Scottish Crop Research Institute
Dundee, UK

Edited by

D V R Reddy
D McDonald
J P Moss

ICRISAT

International Crops Research Institute for the Semi-Arid Tropics
Patancheru 502 324, Andhra Pradesh, India

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Co-sponsors

Scottish Crop Research Institute (SCRI), UK

Peanut Collaborative Research Support Program (Peanut CRSP), USA

International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), India

Contents

Opening Session

Chairperson : **A F Murant**

Welcome Address

Prof. J R Hillman
Director, Scottish Crop Research Institute, Invergowrie, Dundee DD2 5DA, UK

Professor Hillman welcomed the members of the three working groups on groundnut virus diseases and expressed his satisfaction that this important international meeting was being held in Dundee and that the Scottish Crop Research Institute (SCRI) was a co-sponsor. He was pleased to note the important contributions of scientists of the SCRI's Virology and Cellular and Molecular Genetics Departments to the international collaborative research on groundnut virus diseases and hoped that this partnership could continue to the ultimate benefit of farmers in developing countries. Professor Hillman invited the participants to visit SCRI on the following day to see current research on groundnut viruses, and to obtain a broader perspective of the research being done at Invergowrie.

Professor Hillman indicated the many places of historical and scientific interest in the area, and said that Dundee was becoming an important center for scientific research. He commended the efforts of the local organizing committee and the University of Dundee in making the arrangements for the working groups meeting, and extended his best wishes for its success.

Background to Meeting

D McDonald
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On behalf of ICRISAT I welcome you to this first joint meeting of the three working groups on groundnut virus diseases. We are most grateful to the Scottish Crop Research Institute (SCRI) and to the US Peanut Collaborative Research Support Program (Peanut CRSP) for co-sponsoring the meeting, and to all members to their participation.

The choice of Dundee for our meeting was influenced by the International Virology Congress being organized nearby in Glasgow, and by the interest of working group members in being able to interact with scientists of the SCRI who have made such notable contributions to the collaborative international research on groundnut virus diseases.

The first working group meeting on groundnut viruses in Africa was held in Georgia, USA, in 1983, and later meetings in Cambridge, UK, in 1985; in Lilongwe, Malawi, in 1987; and in Montpellier, France, in 1990. Though the group originally focussed on groundnut rosette virus disease, it now covers collaborative research on all groundnut viruses in Africa. The success of this approach led to the establishment of a second working group to coordinate research on peanut stripe virus disease in Asia, the first meeting of which was held in Malang, Indonesia, in 1987, and a second meeting at ICRISAT Center, India, in 1989.

Rapid developments in biotechnology led to our establishing a third working group to help coordinate international research on transformation and regeneration of groundnut to produce transgenic plants that express viral genes. The first meeting of this group was held in 1992 at Wageningen, in the Netherlands.

The present meeting offers the members of the three related groups the opportunity to interact to their mutual benefit. I am sure this will be a stimulating experience, and I hope that the recommendations that evolve from the meeting will provide a sound basis for continued international cooperative research to provide solutions to the serious virus diseases that constrain production of groundnut in many regions of the world.

International Working Groups on Groundnut Virus Diseases

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International working groups for research on economically important groundnut virus diseases were formed nearly ten years ago to coordinate collaborative research and technology exchange activities of concerned national agricultural research systems (NARS), mentor institutes, and regional and international research organizations. The main objectives of the working groups are listed below:

- To characterize economically important groundnut viruses occurring in Africa and in Asia and to develop tools for their identification.
- To provide diagnostic tools for the identification of groundnut viruses to researchers in developing countries.
- To organize training, especially for scientists in NARS, in the techniques for detection and identification of groundnut viruses.
- To organize meetings at regular intervals to review the progress made by the group members and to suggest future collaboration to make optimum use of the resources available.
- To make facilities in advanced virus research laboratories available to scientists in NARS.
- To strengthen research facilities in NARS, especially for virus identification.
- To coordinate publication of information and technical bulletins.
- To organize national and regional surveys for groundnut virus diseases.

The concept of international working groups to investigate virus disease problems in developing countries originated when virologists from ICRISAT and the US Peanut Collaborative Research Support Program (Peanut CRSP) met in 1982 to discuss research aimed at identifying the causal viruses of groundnut rosette disease. Though groundnut rosette disease was first reported in 1907, and subsequently was shown to be a major limiting factor to groundnut production in Africa, the causal agents of groundnut rosette disease were not characterized even by 1982. Therefore, Peanut CRSP organized the first working group meeting on groundnut rosette in 1983. This was held at the University of Georgia, Griffin, USA. The group met again in 1985 in Cambridge, UK, and in 1987 in Lilongwe, Malawi. This group activity resulted in the identification of the causal viruses of groundnut rosette, production of diagnostic aids, and analysis of various rosette-resistant groundnut genotypes for the presence of the two viruses and the satellites involved in disease production.

When the group met in 1990 in Montpellier, France, it was decided that in addition to coordinating research on groundnut rosette virus, the group should also encourage and facilitate research on other groundnut virus diseases occurring in Africa.

The success of the international working group on groundnut rosette encouraged us to form another working group, in 1987, to coordinate research into peanut stripe virus in Asia. The first meeting of this group was held in Malang, Indonesia, in 1987. This group met again in 1989 at ICRISAT Asia Center in India. It was recommended at the latter meeting that the group should coordinate research on all economically important groundnut viruses in the Asia-Pacific region.

For the benefit of those who may not be familiar with the functioning of the two international working groups, we list below the major achievements of the collaborating scientists.

- Identification of causal viruses of groundnut rosette (groundnut rosette assistor luteovirus, groundnut rosette virus and its satellites).
- Production of diagnostic aids (polyclonal antibodies for the assistor luteovirus, and cDNA probes for the rosette virus and the satellite RNA).
- The discovery that rosette-resistant genotypes are resistant only to the rosette virus and its satellite RNA but not to the assistor virus.
- Sequencing of the major portion of RNA 2 of peanut clump virus from West Africa and India.
- Preparation of constructs of the coat protein gene of peanut clump virus for transformation and in vitro expression.
- Surveys for groundnut viruses in West Africa showed that peanut clump virus is economically important in the region; that peanut stripe virus was detected in Senegal in germplasm imported from the People's Republic of China; and that cowpea mild mottle virus is distributed widely in groundnut.
- Surveys for groundnut viruses in Asia showed that peanut stripe virus is present in all the major groundnut-producing countries in the region; that peanut bud necrosis virus is widely distributed in India and is economically important in India, Nepal, and Sri Lanka; that cowpea mild mottle virus occurs in several countries; and that many viruses infect groundnut under field conditions.
- Characterization of peanut stripe virus and its isolates.
- Over 10 000 groundnut genotypes were screened for resistance to peanut stripe virus in Indonesia.
- The discovery that peanut stripe virus can cause significant crop losses in groundnut.
- Organization of training courses in the detection of groundnut viruses.
- Supply of diagnostic tools to scientists in NARS in Asia and Africa.
- Providing access to facilities in advanced virus laboratories in UK, France, Germany, and USA for scientists from developing countries.
- Assistance in obtaining research grants for scientists in NARS.

Substantial progress achieved in biotechnology in the utilization of viral genes to induce resistance to virus diseases has stimulated several institutions in developed countries to follow this approach to obtain resistance to groundnut virus diseases—hence the formation of a third international working group, comprising scientists representing all the major research groups currently involved in the regeneration and transformation of groundnut, and utilization of viral genes to induce resistance to virus diseases. This group met for the first time at Wageningen, the Netherlands, in

1992. The major objectives of the meeting are given by McDonald (1992). As anticipated, considerable advances have been made since this group met in 1992.

This meeting here in Dundee is unique in that it brings together, for the first time, all the three international working groups involved with groundnut viruses. Factors that favored Dundee as the venue include:

- opportunity for participants to get acquainted with current research on groundnut viruses at SCRI,
- economy in travel costs as several working group members were attending the International Virology Congress in Glasgow,
- opportunity for members, especially from the developing countries, to cooperate in identifying viruses in plant material brought by them to the meeting, and
- opportunity for scientists in NARS to interact with leading plant virologists from laboratories in advanced countries.

Collaborative Research Through Networks: A 10th Anniversary Examination of the Groundnut Virus Working Group

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The 1993 Dundee meeting marks the 10th anniversary of the Groundnut Virus Working Group. In May 1993 a planning conference was held at the Georgia Experiment Station, the University of Georgia, Griffin, Georgia, USA, to establish a cooperative research effort to help solve the problem of groundnut rosette virus disease. The impetus for the meeting was the new Peanut Collaborative Research Support Program (Peanut CRSP) and the Virus Project led by Dr J W Demski at the Georgia Experiment Station in cooperation with Drs S M Misari and O A Ansa at Ahmadu Bello University, Nigeria. Dr D V R Reddy, ICRISAT and Dr R Casper, Biologische Bundensanstalt fur Land-und Forstwirtschaft, completed this initial group.

Collaborative research continued on rosette virus and expanded to include the Scottish Crop Research Institute, and another planning/working group meeting was held in Cambridge, UK, in 1985. The participating groups had interest in other viruses, one being peanut stripe virus described in 1982 and found in several countries. A Peanut Stripe Virus Coordinators' Research Meeting was held in 1987, broadening the scope of the group to include more viruses and more collaborating institutions. Khon Kaen University,Thailand; the Centre de cooperation Internationale en recherche agronomique pour le developpement, France; the Agency for Agricultural Research and Development, Indonesia; and the Australian Centre for International Agricultural Research, Australia were represented. A second meeting on peanut stripe virus followed at ICRISAT Asia Center in 1989 with India and China represented. The 1993 Dundee meeting has a wide representation and is multidisciplinary; the working groups have matured into strong entities for coordinating, planning, and conducting priority virus research that has potential for impact.

The Working Group concept provides:

- priority problem identification and strategies for addressing the problem by scientists with varied experience and expertise,
- economy in resource utilization through pooled resources,
- cooperating teams of scientists with increased probability of success as compared with scientists working in isolation, and
- stimulus for better technology exchange leading to increased incomes, greater availability of food, and a better quality of life.

Donors should continue to support networks or working groups because of the comparative advantage a working group brings to bear on identifying priority problems, developing strategies toward solving the problems, and providing solutions to

the problems. International interdisciplinary programs can have greater impacts on the problems of food, income, equity, and quality of life.

Acknowledgement

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Group Meeting on Transformation and Regeneration of Groundnut, and Utilization of Viral Genes to Induce Resistance to Virus Diseases

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Chairperson: **B D Harrison**

Rapporteur: **R A Naidu**

Transformation of Groundnut: Research at ICRISAT Asia Center

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Several biotic constraints are known to cause heavy economic losses to the groundnut crop. Transformation with a range of genes offers unique possibilities to develop groundnuts resistant to some of these constraints. However, engineering new traits in this crop has been limited due to the nonavailability of a transformation methodology. Although the susceptibility of groundnut to *Agrobacterium* infection offers some hope of using this technique for transformation, it is not easy to regenerate plants from transformed cells. A major obstacle in the development of a genetic transformation strategy has been the lack of an efficient tissue culture system to obtain a high frequency of shoot bud regeneration. Several reports have illustrated the regeneration of shoots from diverse types of explants, but the frequency of response is often low and strongly dependent on genotype.

We have developed a shoot regeneration system that provides efficient shoot formation from cotyledon explants of mature seeds of cv JL 24. The de-embryonated cotyledon explants were cultured on shoot induction medium (SIM) [Murashige and Skoogs (MS) containing an auxin and a cytokinin]. Within 2 weeks, up to 90% of the explants produced numerous multiple shoots at the cut ends. After 4 weeks on SIM, the explants with shoot buds were transferred to hormone-free medium for 2-3 passages of 4 weeks each until the shoot buds developed into shoots. At this stage, the individual shoots were clonally propagated through nodal explants on MS medium containing 5 µM α-naphthalene acetic acid (NAA) and transplanted to pots containing sand and vermiculite (1:1). All the plants survived in the greenhouse and produced normal seeds.

The protocol described above was used for *Agrobacterium tumefaciens* mediated genetic transformation. Two strains of disarmed *A. tumefaciens,* C 58 and LBA 4404, containing NPT II and GUS-Intron genes in a binary vector, were used. The cotyledon explants from presoaked seeds of cv JL 24 were co-cultured with the bacterial strains on SIM for 3 days and subsequently subcultured onto selection medium containing SIM + 125 mg L^{-1} cefotaxime + 250 mg L^{-1} carbenicillin for removal of the bacteria and 25 mg L^{-1} kanamycin as selection agent. After 4 weeks, 70% of the explants produced numerous shoot buds, which were then subcultured on hormone-free medium containing 50 mg L^{-1} kanamycin. After two passages of 4 weeks each, the individual shoots were clonally propagated through nodal explants; the rooted explants were then transplanted.

Leaves from the shoots growing in vitro were histochemically analyzed for activity of the β-glucuronidase (GUS) gene. The DNA isolated from putative transformants was analyzed for presence of the GUS gene through polymerase chain reaction (PCR) amplification. These analyses showed a positive GUS activity and amplification of 1.2

kb GUS gene fragment respectively. Preliminary results of Southern blot hybridization have also indicated a positive hybridization with the GUS probe. We are now conducting tests for the presence of GUS and NPT II genes in the seed collected from transformed plants.

Transformation of Groundnut with Tomato Spotted Wilt Virus Genes

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Agrobacterium tumefaciens containing the tomato spotted wilt virus coat protein and the GUS reporter genes was used to transform groundnut shoot apex explants of cv Florunner. Groundnut seeds were surface disinfected in soapy water for 1 h and in 20% (v/v) Chlorox® for 5 min and then cultured on MS inorganic salts, 8 g L^{-1} TC agar at pH 5.7. After 4 to 5 days, the shoot apex was excised and cultured on MS inorganic salts, thiamine, i-inositol, 30 g L^{-1} sucrose, 8 g L^{-1} agar, and 0.1 mg L^{-1} benzyl adenine (BA). After 2 days, the shoot apices were inoculated with the *Agrobacterium* suspension culture. Two days later, the shoot apices were transferred to the same medium with 250 mg L^{-1} augmentin to control the *Agrobacterium* growth. In 4 to 5 weeks, the shoot apices are generally elongated, form roots, and are ready for transfer into a potting mix. Over 50 plants were obtained, one of which gave a GUS-positive reaction in leaf tissue. An enzyme-linked immunosorbent assay (ELISA) test was also positive on the same plant. A Southern blot on leaf tissue of the same plant was also positive. None of the other plants gave any positive indication of having incorporated the foreign genes. Only two seeds were obtained from the plant that had tested positive and both were negative for GUS in ELISA tests. This experiment is being repeated on a larger scale.

The advantage of this approach to transformation of groundnut is that it uses the shoot apical meristem as the explant. Difficulties experienced in handling some of the groundnut cultivars when they have to be grown in tissue culture are thus avoided. Somaclonal variation will not occur due to tissue culture since a preformed apical meristem is the explant. Additionally, the method is simple and rapid. However, the utility of this technique remains to be tested in groundnut though preliminary results are very encouraging.

This research was funded by the Texas Peanut Producers Board.

Development of Transformation Systems for Wild and Cultivated Groundnuts for the Integration of Virus Coat Protein Genes

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Protoplasts provide an ideal experimental system for studies on genetic transformation. An efficient plant regeneration system from protoplasts of a perennial wild species, *Arachis paraguariensis,* has been developed by using a nurse culture method. Protoplasts isolated from cell suspensions were immobilized in agarose medium and co-cultured with nurse cells. Up to 20% of the protoplasts formed microcalli within 3 weeks. Multiple shoots were produced from protoplast-derived callus colonies within 2 months after their transfer to plant regeneration medium. Modifications to this method have resulted in successful regeneration of plants from immature cotyledon-derived protoplasts of cultivated groundnut. Shoot bud formation was observed in protoplast-derived callus from 7 out of 12 groundnut cultivars and breeding lines after transfer to MS medium containing 2,4-dichlorophenoxyacetic acid (2,4-D), benzyl adenine (BA), gibberellic acid (GA 3), abscisic acid (ABA), and zeatin. However, the frequency of plant regeneration remained low. Protoplast-derived groundnut plants were normal in appearance and produced seeds in the greenhouse. Efforts are currently being made to improve the plant regeneration frequency.

Conditions for efficient polyethylene glycol (PEG) and electroporation-mediated transformation are being defined by monitoring protoplast regeneration frequency and transient GUS expression. A number of DNase inhibitors were tested for their effect on transformation efficiency. DNA plasmids containing different selectable marker genes were introduced into groundnut protoplasts for the evaluation of selection efficacy and effects on plant regeneration. Virus coat protein genes were introduced into groundnut protoplasts. Putative transgenic plants are being analyzed for DNA integration and coat protein expression.

An efficient system for plant regeneration from hypocotyl tissue has been developed by using thidiazuron (TDZ) to provide an alternative approach to groundnut transformation, using tissue electroporation, *Agrobacterium,* and microprojectile bombardment. Hypocotyl tissue from mature seeds was exposed to TDZ for 1 week and then cultured in hormone-free MS medium for 5 weeks. Over 200 shoots developed from a single seed through organogenesis. Plantlets were readily obtained within 2 to 3 months by subculturing individual shoots and rooting in MS medium containing NAA. Regenerated plants were phenotypically normal and produced seeds in the greenhouse. Comparable regeneration frequencies were observed from different groundnut genotypes, including 10 major US cultivars and A *glabrata.* Hygromycin-

resistant explants were recovered after electroporation with plasmids containing the HPT gene. About 40% of the seeds inoculated with *Agrobacterium* containing a binary vector with kanamycin resistance and/or GUS genes produced normal plantlets in the presence of high concentrations of kanamycin. GUS gene expression was also observed in growing plantlets after histochemical assay for GUS activity. Efforts are being made to identify true transformants by DNA analysis.

Development of Methods for Genetic Transformation of Groundnut with Special Reference to Plant Viral Genes

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In recent years significant progress has been made towards using viral genes for transformation of plants to induce resistance to virus infection. In an Overseas Development Administration (ODA)-funded project, we are attempting to produce transgenic groundnut plants with resistance to groundnut rosette assistor virus and Indian peanut clump virus by utilizing the coat protein or satellite-mediated resistance mechanisms. We have established efficient and reliable plant regeneration systems for both mature cotyledons and immature leaves for several Indian and African genotypes. *Agrobacterium*-mediated transformation has been successfully used to demonstrate both transient and stable expression of the marker genes such as GUS and NPT II in the transformed groundnut tissues. However, our attempts to obtain transgenic plants from these transgenic tissues have not been successful to date. Biolistic-mediated transformation has also been used to show transient expression of the GUS gene in both cotyledon and leaf tissues and attempts are being made to regenerate transgenic plants from them. Two wild groundnuts, *Arachis paraguariensis* and *A. villosulicarpa,* have been efficiently regenerated from leaf tissues. *Arachis villosulicarpa* has also been transformed by *Agrobacterium-mediated* transformation. Therefore, *A. villosulicarpa* can be used as a model plant to study viral resistance mechanisms in the absence of a transformation system for the cultivated groundnut plants. *Nicotiana benthamiana* shows easily recognizable symptoms when infected with the Indian peanut clump virus (IPCV) and thus can be used to assess the coat protein (CP)-mediated resistance mechanism of the virus. Indeed, we have introduced the coat protein gene of IPCV by *Agrobacterium*-mediated transformation into *N. benthamiana* plants. Several kanamycin-resistant transgenic plants have been produced and are being analyzed for the expression of the IPCV CP gene and CP-mediated resistance.

Transformation of Groundnut: Research at the Samuel Roberts Noble Foundation

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Attempts to regenerate transgenic plants from leaf, petiole, or cotyledon explants of groundnut cultivars Okrun and Spanco using published regeneration protocols have been unsuccessful. The regeneration protocols yielded somatic embryos or shoots differentiated from a de novo formed meristem and not from the callus. The regeneration frequency was very low. Results from our transformation experiments indicate that the meristematic tissue or its precursors are not amenable for genetic transformation. However, transformed callus can be obtained from groundnut hypocotyl segments via *Agrobacterium*-mediated DNA transfer. The transformed callus was obtained using the binary vector pKYLX71GUS containing the GUS reporter gene driven by the CAMV 35S promoter or the binary vector p5'9 containing the full length peanut stripe virus coat protein gene driven by CAMV 35S promoter (Franklin et al. In press). Stable integration and expression of the foreign genes in these transformed callus cultures were confirmed by Southern and Western blot analyses as well as GUS histochemical assay. Unfortunately, the transformed callus does not have the potential for regeneration. These results indicate that, in groundnut, the transformable tissue (i.e., callus) is not regenerable, and the regenerable tissue (i.e., meristematic tissue) is not transformable.

To develop a regeneration system capable of producing groundnut plants at a higher frequency, preferably through a callus stage, we tested the influence of different explant sources and several plant growth regulators (PGR). Of all the explants tested, plumular explants from imbibed groundnut (cvs Okrun and Spanco) seeds produced the highest numbers (7-10) of plantlets per explant. With respect to PGR treatments, we observed that the pretreatment of plumular explants with two novel 'auxin-like' PGRs (Ponsamuel 1990), namely phenylboronic acid (PBOA) and tetraphenylboron (TPB), gave the best results. The plumular explants were first cultured (pretreatment) on MS basal medium containing 10 µM of PBOA or TPB for 10 days and then transferred to regeneration medium containing MS-salts and vitamins + 30 µM 6-benzylaminopurine + 5 µM α-naphthalene acetic acid. Shoot regeneration occurred from all pretreated plumular explants by 30 days after the transfer to the regeneration medium. Explants cultured directly on regeneration medium produced 60-70% fewer plantlets than did the explants pretreated with PBOA or TPB. Pretreatment of explants with other auxins such as NAA, 2,4-dichlorophenoxyacetic acid, β-napthoxyacetic acid, and picloram produced fewer shoots or nonregenerable callus. Histological studies indicated that shoot regeneration occurred from callus produced at the cut end of the plumular explants. We are now attempting to integrate this regeneration system with *Agrobacterium* or biolistic bombardment mediated DNA transfer to obtain transgenic groundnut plants.

Development of Novel Genes and a Groundnut Transformation System for Peanut Stripe Virus Resistance

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Peanut stripe virus (PStV) is a major constraint to groundnut production in Southeast Asia and its accidental introduction into Australia could pose a serious quarantine risk. We aim to generate genetically engineered groundnut cultivars that are resistant to PStV. The novel disease resistance genes to be introduced into groundnut are based on genes taken from the viral pathogen. PStV coat protein gene constructs have been cloned into a plant expression vector cassette and gene expression will be assessed in vitro prior to transformation of regenerable groundnut tissues. Efficient stable transformation of groundnut tissues has been achieved by particle bombardment, but more work is required on efficient selection and regeneration systems to recover transgenic plants.

Group Meeting on Virus Diseases in Africa

Chairpersons: **A F Murant and S M Misari**
Rapporteurs: **M A Mayo and X Zeyong**

Insect Vectors of Groundnut Viruses

J W Todd[1], A K Culbreath[1], and J W Demski[2]

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Three hundred and eighty-one species of animals are known to transmit at least one plant virus, and there are 298 arthropod-borne viruses. Insects are without question the largest group of virus vectors. The family Aphididae in the order Homoptera contains 193 vector species, and the family Thripidae in the order Thysanoptera contains eight species that are extremely important as vectors of at least three groundnut viruses in the newly named tospovirus group.

Of 23 taxonomically characterized viruses that occur on groundnut naturally, six are believed to be economically important. The potyviruses, peanut mottle virus (PMV) and peanut stripe virus (PStV), are endemic in the majority of groundnut-production areas of the world. The tospovirus group contains three viruses known to infect groundnut. Two of these, peanut bud necrosis virus (PBNV) and tomato spotted wilt virus (TSWV), are becoming more widespread and may be very severe in a given locality. Peanut clump in India and West Africa is caused by a soilborne furovirus, transmitted by the fungus *Polymyxa graminis.* Groundnut rosette assistor virus (GRAV) is a luteovirus. GRAV coat protein is necessary for the aphid transmission of groundnut rosette virus (GRV) and its satellite RNA. The satellite RNA was shown to be involved in symptom expression of groundnut rosette virus. GRAV and GRV are transmitted persistently (circulative) by *Aphis craccivora* and, to a lesser extent, by *Aphis gossypii* and *Myzus persicae.* Peanut mottle and peanut stripe viruses are transmitted by many aphid species, but in a nonpersistent manner.

Among the tospoviruses, PBNV and TSWV are thought to be the most damaging. Their vectors are primarily *Thrips palmi* (PBNV in portions of Southeast Asia), and *Frankliniella fusca,* and/or *F. occidentalis* (TSWV, cosmopolitan). A third member of the tospovirus group, peanut yellow spot virus (PYSV), the vector of which is *Scirtothrips dorsalis,* is considered to be less important as a yield-limiting factor because the virus is not systemically translocated in the plant and produces symptoms only at the site of inoculation.

Recent work on various aspects of vector ecology and TSWV epidemiology in Georgia has shown that an extremely low percentage of adult thrips initially colonizing groundnut fields in early spring sowings are viruliferous. Also, TSWV has been detected by ELISA in overwintering *F. fusca* adults, particularly brachypterous individuals. Furthermore, the presence of the virus in volunteer groundnut in both the fall and spring seasons suggests that TSWV may be able to pass the winter in the host plant and in the vector (thrips).

Asymptomatic infection of groundnut by TSWV has also been reported from Georgia, and preliminary results indicate that thrips larvae may be able to acquire the virus from asymptomatic plants (A K Culbreath, J W Todd, and J W Demski, unpublished data). These results indicate that virus incidence in groundnut fields has probably

been underestimated; thrips may possibly acquire the virus from a much larger number of plants in a given area.

Vector management studies in Georgia and elsewhere (unpublished data) show that although satisfactory reduction in thrips populations is usually achieved by spraying insecticides, reductions in TSWV incidence have been minimal and are usually statistically insignificant. However, reductions in TSWV incidence, up to 50% of that in control blocks, following intensive insecticidal sprays have been reported from several crops. Under similar conditions, viruses nonpersistently transmitted by aphids could not be controlled. These results may be more meaningful when one considers that differential control efficacy among adult and larval stages is also commonly noted. In-furrow applications of systemic insecticides at sowing are very efficacious against the larval stages of *F. fusca* primarily, but are less so against the adults of *F. fusca* and *F. occidentalis.* These results indicate a predominance of early primary infection and/or continuous or sporadic primary infection over a longer period of time than originally postulated. Also, though some degree of secondary spread can be assumed, the low level of thrips reproduction in treated fields relative to the timing and severity of symptom expression indicates that primary infection over an extended period of time is more likely.

Screening for TSWV resistance among advanced breeding lines from Georgia and Florida and germplasm from the collection of the Plant Introduction Station of USDA at Griffin, Georgia, has identified several promising accessions. The cultivar Southern Runner and the breeding line GAT 2741 have both demonstrated usable levels of resistance to TSWV. The nature of the resistance in these accessions is not currently known; however, thrips resistance has been shown not to be a factor in the manifestation of resistance to TSWV by cv Southern Runner since comparable thrips populations and damage levels have been noted on susceptible and resistant lines. Resistant varieties are the single most promising approach towards management of this problem in the foreseeable future; therefore, considerable effort is being directed towards identification and development of varieties resistant to TSWV.

Current Research on Groundnut Rosette Disease at SCRI

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Work at the Scottish Crop Research Institute (SCRI) on groundnut rosette disease has been funded for several years from grants provided by the UK Overseas Development Administration and administered through the Natural Resources Institute, Chatham and the Center for Arid Zone Studies, Bangor.

Previous work has shown that the disease, which is of major importance in Africa, is caused by a virus complex in which groundnut rosette virus (GRV) depends on groundnut rosette assistor luteovirus (GRAV) for transmission by *Aphis craccivora.* Moreover, GRV has a 0.9 kb satellite RNA (RNA 3), which is the actual cause of the disease symptoms. Different forms of the disease (e.g., chlorotic and green) are caused by variants of the satellite. Other satellite variants induce few or no symptoms in groundnut, yellow blotch, instead of mild mottle, in *Nicotiana benthamiana.* Mild forms of the satellite protect groundnut plants from the virulent forms. In addition, the satellite mediates the GRAV-dependent aphid transmission of GRV, in some way which currently is not understood. GRAV is a luteovirus and can be detected by polyclonal antisera and by some monoclonal antibodies (McAbs) to potato leafroll luteovirus (PLRV). A polyclonal antiserum and several McAbs have been produced for detecting GRAV; they all reacted with PLRV. Nonetheless, these reagents are proving useful not only in the diagnosis of GRAV but also in epidemiological studies, for example in tests to find possible native hosts of GRAV in Africa, and in tests of groundnut germplasm and breeding lines for resistance to GRAV.

The nucleotide sequence of a ca 700 bp cDNA clone containing the coat protein (CP) gene of GRAV has been determined. It has extensive homology with the CP sequences of other luteoviruses: 65% in case of PLRV, 69% in case of beet western yellows virus, and 45% in case of barley yellow dwarf virus (PAV strain). This GRAV CP gene fragment has been cloned into a pROK vector for *Agrobacterium-mediated* transformation of plants.

By using the double-stranded form of the GRV satellite RNA as a template for reverse transcription, nucleotide sequences of 10 clones were obtained, representing four satellite variants: mc3 and yb3, from Malawian GRV isolates causing respectively chlorotic rosette in groundnut and yellow blotch symptoms in *N. benthamiana;* and ng3 and nm3, from Nigerian GRV isolates causing respectively green rosette and very mild chlorotic mottle in groundnut. Variant mc3 had 95% sequence identity with yb3, but only 88% with ng3 and nm3. In turn, ng3 and nm3 had only 90% sequence identity with each other. Thus, sequence differences among the satellite variants were associated both with symptom differences and with geographical origin. Little or no homology was detected with other published virus or satellite sequences. Five

open reading frames (ORFs) were present in mc3, two in one strand and three in the other. The largest could encode a polypeptide of 124 amino acids and the others could encode polypeptides of 30-63 amino acids. Only one of these ORFs was present in all the four variants. The relevance of these differences to the observed phenotypic effects of the satellites (symptom induction, cross protection, and mediation of the GRAV-dependent aphid transmission of GRV) is of considerable interest. This can now be investigated with the aid of biologically active transcripts that have been developed from some of the satellite cDNA clones.

Current Research on Groundnut Viruses at LPRC-CIRAD/ORSTOM

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Surveys for groundnut viruses were carried out in four countries in West Africa: Senegal [Centre de cooperation internationale en recherche agronomique pour le developpement (CIRAD)-Institut francais de recherche scientifique pour le developpement en cooperation (ORSTOM), 1986-90), Niger (ICRISAT-ORSTOM, 1989), and Burkina Faso and Mali (CIRAD-ICRISAT, 1991). Typical peanut clump virus (PCV) symptoms were observed in all the countries surveyed. However, PCV was also detected in groundnuts exhibiting a large range of symptoms, with or without stunting, that included yellow line patterns, yellow specking, chlorotic rings or line patterns, mottle, mild mosaic, etc. It is worth noting that a high percentage of plants showing variation in symptoms were found at research stations and seed multiplication farms in Senegal, Mali, and Burkina Faso. Forty-one isolates of PCV from these countries were studied for diversity in serological reactions. From ELISA, by using eight monoclonal antibodies, five or six serogroups (the sixth class containing a single member) were identified. PCV isolates in each serogroup did not produce similar symptoms. Additionally, no definite pattern was observed with regard to their distribution.

We tried to understand this wide variability in PCV by studying its genome organization. The virus contains two major species of RNA, and a number of minor bands, shorter than RNA 2, that could be encapsidated as subgenomic RNAs.

The complete nucleotide sequence of RNA 2 has been determined. It is 4503 nucleotides long and contains six open reading frames (ORFs). The ORF 1 at the 5' end corresponds to the cistron for the coat protein. ORFs 3, 4, and 5 resemble the 'Triple Gene Block' found in other furo and hordeiviruses. The mechanism of expression of P 39, the putative translation product of the second ORF, is not known. But it is remarkable that in some isolates of PCV, RNA 2 has undergone large deletions within ORF 2: a 402 nucleotides deletion in one isolate from Thysse Kaymor (Senegal) with symptoms of stunting but light green leaves and a 968 nucleotide deletion in one isolate from Pout (Senegal) without any clumping and presenting only chlorotic rings or a line pattern.

These studies indicate wide variation among PCV isolates and justify further efforts to understand the molecular basis of symptom variation.

However, it should be added that not all 'clumped' groundnut plants are infected by PCV. At least three other viruses (one with spherical particles and two with flexuous particles) have been found to be associated with stunting symptoms resembling clump disease.

The Peanut CRSP Virus Project

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The Peanut CRSP was initiated in 1980, through funds provided by the Agency for International Development (AID). Dr C R Jackson, former Director of the Georgia Experiment Station in Griffin, and Dr D G Cummins wrote the proposal which was approved in 1981. Eleven projects on various aspects of groundnut production and utilization were awarded funding. One of these projects was on viruses. It was started in 1982 with a mandate to support research in the developing countries and to provide training for cooperators in the host countries. The funds for the project were received by the University of Georgia (UGA). After deducting the administrative costs, the remaining funds were split between the host countries and the UGA.

Technology transfer was an important component of this project. To achieve the transfer, cooperators from the host countries were provided with funds to attend scientific meetings and make visits up to 3 weeks to the research labs at UGA. Graduate Assistantships were provided to facilitate advanced training in virology.

Currently, the Virus Project is functioning mainly in Thailand and Nigeria. The main objectives of the Project in Nigeria are:

- to breed for improved lines that are resistant to groundnut rosette, with special emphasis on short-duration genotypes;
- to cooperate with other breeders in West Africa, including personnel at ICRISAT and the Peanut CRSP Project in Burkina Faso, in the production of rosette-resistant cultivars;
- to demonstrate the effectiveness of rosette-resistant cultivars; and
- to multiply the seed of rosette resistant cultivars.

The project leader in Nigeria is Dr P E Olorunju.

In Thailand the objectives are:

- to identify the viruses infecting groundnut in Southeast Asia;
- to compile epidemiological data on economically important viruses; and
- to experiment with methods that could reduce or prevent the virus infection and its spread in groundnut crops.

The project leader in Thailand is Dr S Wongkaew.

In addition to cooperation with the host countries, collaborative links have been established with other US and international institutes. Networking increases awareness of other projects and leads to reduced costs and more efficient research.

Peanut CRSP plays a significant role in the working group activity established for groundnut viruses. This approach has lead to reduced costs and efficient utilization of manpower and resources.

Biological and Molecular Diversity Among the Tospoviruses: Groundnut, a Possible Generator of Diversity

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Advances in the understanding of the tomato spotted wilt virus (TSWV) group or tospoviruses over the last five years have begun to reveal a heretofore unrecognized level of diversity among them. Although TSWV was thought to be a monotypic taxon of viruses, the discovery of impatiens necrotic spot virus (INSV) exposed the first of several distinct viruses to be described that were similar to TSWV. TSWV and INSV were sufficiently well characterized for them to be appropriately classified as a new genus of tospovirus in the Bunyaviridae. Since then, three members of the tospovirus genus have been associated with groundnut: TSWV in North America; groundnut ringspot virus in South Africa; and peanut bud necrosis virus in India. A fourth virus, peanut yellow spot, also found in Southeast Asia, has not been fully characterized.

We have found considerable diversity among TSWV isolates occurring in groundnut in the United States. They vary in many biological characters such as subtle differences in host range and symptom severity. Although each isolate infects groundnut, the severity of symptoms is variable among isolates but relatively stable from inoculation to inoculation. We have also been interested in the molecular basis of variability in the nucleocapsid *n gene.* Our interest is derived from our previous investigations, which determined that the *n gene* was the least conserved among the major structural proteins. Therefore, the *n gene* is the primary marker being used to establish taxonomic relationships. In addition, the *n gene* has been used to confer resistance by transformation of plants with this gene. In these studies, we have compared nucleotide sequences of the *n gene* of various isolates with their serological reactions. We developed a panel of over 60 monoclonal antibodies from which four were selected that reacted to different epitopes on the TSWV *n gene.* From these studies we have been able to establish affinity groups (serotypes) within TSWV (= serogroup or species).

Current efforts include investigations to understand the diversity of tospoviruses and involve improved diagnostic procedures, identification of atypical viruses, and development and identification of resistant germplasm.

New Aspects in Tospovirology: Virus-Vector Relationships and Transgenic Resistance

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The present studies on tospoviruses at the Wageningen Agricultural University are aimed at understanding their molecular biology. These studies have revealed that the genome of the tospoviruses encodes for at least six proteins. One protein, with a molecular weight of 332 kd, is encoded by the L RNA and represents the putative transcriptase. Three glycoproteins, namely G1, G2, and a nonstructural protein, designated NSm, with a size of 33.6 kd, are encoded by the M RNA. The NSm protein may function as the movement or transport protein. Transfection of protoplasts with an NSm clone resulted in the production of several hairlike structures. The S RNA encodes for the nucleocapsid protein (N) and a nonstructural protein (NSs) that produces fibrillar inclusions in plant cells as revealed in EM studies.

To enhance the efficiency of studies on the transmission of tospoviruses, a method was developed to rear large numbers of thrips in protected conditions. Adults were allowed to oviposit on bean pods, or on other fruits with a long shelf-life, in glass jars covered with tissue paper. Cohorts of larvae of known age could be obtained by retrieving the fruits daily from the jars and supplying the adults with fresh fruits. Addition of pollen to these cultures enhanced egg production. In addition, a local lesion assay to follow virus transmission was developed using leaf disks of petunia (*Petunia hybrida*), which produces local lesions within 3 days after infection. Leaf disks are placed in an Eppendorf tube for 1 day with the thrips to be tested. The thrips are then transferred to another leaf disk, while the inoculated disks are placed on water for symptom development. Using this approach it was established that the majority of the thrips became viruliferous in the second larvae stage. The LP50 values at this stage for impatiens necrotic spot virus were 157 h at 20 °C, 103 h at 24 °C, and 82 h at 27 °C. For tomato spotted wilt virus (TSWV), the values were 171 h at 20 °C, 109 h at 24 °C, and 84 h at 27 °C.

This procedure facilitates the recovery of thrips specimens used in the tests. The use of leaf disks could be extended to those plants that do not produce local lesions on the inoculated leaf, but cause systemic infections. Infections have then to be assayed a few days after inoculation, using ELISA.

One day after acquisition, an increase in the amount of the NSs and N protein was demonstrated in *Frankliniella occidentalis,* indicating TSWV multiplication. This conclusion is also supported by the observation that the N and NSs proteins accumulate in midgut cells and in the salivary glands. A massive number of virus particles could be located in the salivary ducts, demonstrating TSWV replication in salivary glands.

Tobacco (*Nicotiana tabacum*) and tomato (*Lycopersicon esculentum*) plants were transformed with viral nucleoprotein gene. Further analysis of the resistance thus

conferred has shown that it is not protein-mediated but is, at least for a major part, RNA-mediated. The resistance was also observed when the plants were inoculated with TSWV by thrips, but was ineffective when the plants were inoculated with two other tospoviruses, tomato chlorotic spot and groundnut ringspot. To analyze the underlying mechanism by which the engineered resistance works, other parts of the TSWV genome (coding and noncoding, positive and complementary sense) are being transcriptionally expressed in tobacco plants and will be tested for their potential to induce resistance.

Screening for Resistance to Groundnut Rosette Virus Disease

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Rosette is the most important virus disease of groundnut in Africa south of the Sahara and on its offshore islands. Although rosette epidemics are sporadic, yield losses approach 100% whenever the disease occurs in epidemic proportions. Two types of rosette are recognized on the basis of foliar symptoms, chlorotic rosette and green rosette (Gibbons 1977). Chlorotic rosette is the more prevalent type in eastern and southern Africa, while green rosette is the more common in West Africa. However, recent surveys in southern Africa have indicated the presence of green rosette in Angola, Swaziland, and northern Malawi (Subrahmanyam, unpublished). Groundnut rosette is caused by a complex of three agents, namely groundnut rosette virus (GRV) and its satellite RNA, and groundnut rosette assistor virus (GRAV) (Reddy et al. 1985, Murant et al. 1988). The disease is transmitted by *Aphis craccivora.*

In this paper we report current research in the SADC/ICRISAT Groundnut Project on identification of genetic resistance in groundnut germplasm originating from South America and Africa.

Sources of resistance to rosette were first discovered in 1952, when an epidemic of the disease destroyed a large collection of groundnut germplasm in Senegal, with the exception of few germplasm lines originating from the frontier region between Burkina Faso and Cote d'Ivoire (Sauger and Catharinet 1954). These lines have been extensively used in rosette resistance breeding programs throughout Africa. Resistance was effective against both chlorotic rosette and green rosette and is governed by two independent recessive genes (Nigam and Bock 1990). *Arachis chacoense* has been reported to be highly resistant to both GRV and GRAV in greenhouse tests (Murant et al. 1991). Recently, an interspecific hybrid derivative, 83/372-2-22-BI, originating from a cross between *Arachis hypogaea* and *A. chacoense,* has been reported to be resistant to groundnut rosette in Malawi (Moss et al. 1993). Most of the rosette-resistant breeding lines are long-duration Virginia types.

The SADC/ICRISAT Groundnut Project located at Chitedze, Malawi, is currently investigating the possibilities of broadening the genetic base of rosette resistance through identification and utilization of new sources of resistance. Effective field screening methods have been developed for large-scale evaluation of groundnut genotypes against rosette (Bock 1985). In collaboration with the Genetic Resources Division at ICRISAT Asia Center in India, 1406 South American, 801 West African, and 230 southern African germplasm lines were evaluated over three crop seasons (1990-93) for rosette resistance using the infector-row technique (Bock 1985). Seeds of each entry were sown in single row plots. Infector rows of a rosette-susceptible

cultivar, Malimba, were sown after every two test rows. To minimize the chances of any escape, each infector row was examined approximately 2 weeks later, and the plants that were free from rosette symptoms were infested with viruliferous aphids. This resulted in 100% chlorotic rosette incidence in the rosette-susceptible lines. The majority of the infected plants had died by the end of the season.

Of the 1406 South American lines, only one entry, ICG 11044 (PI 162525), a long-duration, Virginia bunch type originating from Argentina, showed a high degree of resistance to rosette. This appears to be the first report of the occurrence of resistance to groundnut rosette in germplasm obtained from South America, and it is important to determine the origin of this line as all the currently known *A. hypogaea* lines resistant to rosette have originated from Africa.

We have confirmed rosette resistance in the varieties RG 1 (ICGM 493) and RRI/6 (ICGM 497) that were bred in Malawi. Seventy-nine of the 801 West African lines evaluated have been identified as having a high degree of resistance to rosette. Of these, 12 (9 from Burkina Faso, 2 from Nigeria, and 1 from Senegal) are short-duration Spanish types and the remainder are long-duration Virginia types.

Identification of rosette resistance in Spanish type groundnuts is expected to accelerate the development of high-yielding short-duration rosette-resistant groundnut genotypes, which are in demand for many countries in Africa. Progress in this area has so far been slow because of the limited range of reliable sources of resistance in Spanish types.

Evaluation of germplasm for resistance to rosette will continue. Resistant genotypes identified in field screening will be further tested in the laboratory for their reaction against GRV and/or GRAV components. We intend to document and maintain all the available sources of rosette resistance at the SADC/ICRISAT Groundnut Project in Malawi for exchange and distribution to researchers in Africa and elsewhere. Utilization of additional sources of rosette resistance in the breeding program, especially short-duration Spanish types, will receive high priority.

Groundnut Rosette Virus: Progress in Transferring Rosette Disease Resistance to Short-duration Cultivars

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We in the SADC/ICRISAT Groundnut Project believe that utilization of genetic resistance to groundnut rosette remains the most effective method of minimizing yield losses due to this important disease. Therefore, the development of agronomically acceptable, rosette-resistant groundnut cultivars that are adapted to the region is the most important objective of the Project on rosette disease.

Until recently, sources of resistance to rosette disease were confined exclusively to long-duration Virginia genotypes.

Because both rosette resistance and season length are governed by double recessive genes, recovery of short-duration resistant segregants is small when long-duration resistance sources are used in hybridization.

Harkness (1977) reported a low recovery of resistant plants from Virginia x Spanish crosses and suggested that double-recessive plants may succumb to heavy inoculation pressure in the early stages of growth. He also suggested that double-recessive genotypes may not confer resistance in all nuclear backgrounds. We have also recorded infrequent recovery of short-duration resistant plants in crosses between Virginia and Spanish parents. In many cases recovery has been less frequent than expected theoretically. Therefore, although not proved, we cannot exclude the linkage of resistance to long duration as a possible reason for infrequent recombination.

Until 1987/88, the only sources of resistance available for use as parents in our hybridization program were of the long-duration type. We have made extensive use of 48-36, RMP 40, RMP 91, and RMP 93, introduced from Senegal, and RG 1, which was bred in Malawi and released in the early 1970s. Some of these have variegated (red/white) seed, which are not satisfactory for confectionery use.

Rosette-resistant virginia-type entries developed by the Project were evaluated in yield trials at Chitedze for the first time in the 1989/90 season. Since then, we have evaluated over 50 advanced breeding lines and a number of these have significantly outyielded RG 1, the rosette-resistant control. Some have larger seed than RG 1, and have acceptable seed color.

All these trials were conducted under conditions of low or negligible incidence of rosette disease. However, in 1992/93, we began to evaluate the performance of several of these high-yielding rosette-resistant lines, along with local rosette-susceptible and rosette-resistant genotypes, in on-farm trials in three agroecological zones in Malawi.

The few rosette-resistant short-duration selections recovered from earlier crosses have low yield potential and often do not have true Spanish characteristics, although duration is similar to that of local Spanish control cultivars. Two such selections, ICGX-SM 82040/7/4 and ICGX-SM 82051/7/1, were used as parents in crosses in 1989/90. The F_3 population from the crosses from the latter appeared to be segregating for duration and resistance. An additional selection, ICGX-SM 82040/8/1, was confirmed to be resistant in 1992/93. Twenty-five F_7 bulk selections from 1986 crosses are currently being multiplied in a winter nursery for yield evaluation in 1993/94.

In 1984 we introduced two rosette-resistant Spanish genotypes, KH 149A and KH 241D, from Senegal. These were screened for resistance in 1986/87, and only very few symptomless plants were recovered from KH 241D. This was sufficient, however, to start a purified nucleus source of resistance in short-duration background. We have used KH 241D extensively in our hybridization program since 1987/88.

Two other sources of resistance, both Spanish type (Bockelee-Morvan 1988), were screened in 1992/93 but were found to be susceptible.

We screened 801 West African germplasm accessions in 1992/93 and identified a further 12 resistant Spanish genotypes.

The first F_3 populations from crosses involving KH 241D were screened in the rosette nursery in 1990/91. Incidence of rosette was high in the rosette nursery in 1990/91, reaching 80% in resistant genotypes such as RG 1, and this resulted in the elimination of some plants that may have been resistant. We screened a duplicate set of these populations in 1991/92 and selected a number of resistant plants, most of which were confirmed as resistant in 1992/93.

Twenty-five F_5 selections from the 1987 crosses and 7 F_5 selections from 1988 crosses will undergo further selection for yield and agronomic suitability in 1993/94.

The low frequency of recovery of short-duration segregants from crosses between rosette-resistant Virginia parents and susceptible Spanish lines is certainly due to the mode of inheritance of both characteristics and their possible linkage. However, probability of recovery is now much greater since we began using KH 241D, and should improve with the use of the new rosette-resistant Spanish genotypes identified in 1992/93.

Breeding for Resistance to Groundnut Rosette Virus in West Africa—Current Research in Nigeria

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Breeding for resistance to groundnut rosette virus (GRV) disease continues to be one of the major objectives of the groundnut breeding program at the Institute for Agricultural Research (IAR), Samaru. The program to develop rosette-resistant varieties was initiated at IAR in 1963 using rosette-resistant genotypes 52-11 and 52-14. Results on various crosses made and their performance were reported by Harkness (1977), Boye-Goni et al. (1989), and Olorunju and Misari (1992). This paper reports our current research on the development of agronomically acceptable high-yielding, and adapted rosette-resistant cultivars.

Genetic resistance to rosette disease has been demonstrated only in the medium- and long-duration cultivars. The sources of resistance used in our breeding program were long-season varieties (52-11, 52-14, RMP 91, and 69-101) from West Africa. Many rosette-resistant varieties released were of medium- or long-duration types. The current releases are SAMNUT 10, SAMNUT 11, and SAMNUT 16 (Boye-Goni et al. 1989).

In 1987, we used RMP 12 and RG 1 as sources of resistance in crosses with short-, medium-, and long-duration genotypes. Disease reactions, inheritance of resistance, and screening procedures were reported by Olorunju et al. (1991). Results from the cross RMP 12 x M 1204.781 suggested that the resistance was different from the double recessive type observed with other resistant x susceptible crosses. An experiment involving five of these genotypes (RMP 12, RG 1, M 1204.781, ICGS(E) 56, and JL 24) was conducted. Field screening procedure was as described by Bock and Nigam (1988). Results of the first year showed a trend similar to that reported previously by Olorunju et al. (1991). Although the pedigree of M 1204.781 was traced, it did not give any clue to the peculiarity of the cross RMP 12 x M 1204.781.

Breeding lines from the 1987 crosses involving resistant and susceptible genotypes were included in a preliminary trial in 1992 for yield evaluation of rosette-resistant genotypes. Overall mean for all the 13 lines was 2.6 t ha^{-1}; mean yield for the lines involving RMP 12 was 2.8 t ha^{-1}; whereas those involving RG 1 had a mean yield of 2.3 t ha^{-1}. Breeding lines UGA 2, UGA 6, and UGA 13 performed well with yields exceeding 3 t ha^{-1}. They outyielded the local controls RMP 12 (2.9 t ha^{-1}) and MS 54.76 (2.4 t ha^{-1}). All the 13 lines are highly resistant to groundnut rosette. These lines are being prepared for the state trials to check their suitability to different ecological zones.

Attempts to develop short-duration rosette-resistant lines were initiated in 1964. Harkness (1977) reported crosses made between rosette-resistant genotypes and short-duration Spanish genotypes. Recovery of resistant plants from these crosses was

low. They were mostly long- and medium-duration genotypes of poor agronomic characteristics.

In 1980, crosses were made in Kano between KH 149A (short-duration rosette-resistant line) and selections from Virginia x Spanish crosses. About 50 lines were selected and screened for resistance for 2 years using the procedure of Bock and Nigam (1988). One line (K 51.85) had no rosette, 8 had less than 5% rosetted plants, while 14 had 6-10% rosetted plants. It was however observed that the highly resistant lines among these were poor yielders (23-38 pods per stand) whereas those which showed low rosette incidence (less than 10%) had high yields. All the 23 selected lines are now being tested for yield in the Sudan Savannah ecological zone. Selections have also been made from the crosses made in 1987 involving short-duration genotypes. New crosses have been made involving resistant selections from the UGA selection and it is hoped that rosette-resistant short-duration cultivars with acceptable yield and seed size will be produced soon.

Although we are making some progress, we continue to face problems in producing short-duration rosette-resistant lines with good agronomic characteristics that are acceptable to the farmers. Until nonconventional approaches such as utilization of viral genes for inducing resistance become available, we will continue to put our efforts into producing short-duration rosette-resistant cultivars by conventional breeding.

Preliminary Studies on Viruses of Groundnuts in South Africa

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South Africa is self sufficient in groundnuts and also exports this commodity. The average production during 1980-90 was 150 000 t year^{-1}, with peaks reaching 244 000 t year^{-1}. The gross value of the crop represents approximately 1% of the gross value of all cultivated crops in South Africa.

In South Africa, two groups are collaborating on different aspects of viruses of groundnuts. At the Plant Protection Research Institute (PPRI), interest in viruses of groundnuts is part of a wider program to determine the virus status of legumes in South Africa in order to assist the quarantine authorities. At the Grain Crops Institute (GCI), the role of thrips as vectors of TSWV in groundnut is being investigated. Both programs were initiated only in 1990.

Current research at PPRI includes:

- characterization of viruses that occur in South Africa,
- establishment of reference cultures,
- preparation of diagnostic aids for local and exotic viruses, and
- determination of economic importance.

In South Africa, the virus status of legumes, and of groundnuts in particular, is largely unknown.

Although research on groundnut viruses was initiated in South Africa a long time ago, some of the economically important viruses are yet to be characterized. As early as the 1920s, Storey and Bottomley (1928), investigated groundnut rosette virus disease in South Africa. Klesser (1968), from PPRI, reported a number of viruses of groundnuts in the 1960s. Despite the lack of facilities for electron microscopy and serology, she did pioneering work on viruses of groundnuts. She tested the symptomatology, host range, graft and sap transmission, insect transmission, and physical properties (DEP, LIV, and TIP) of various viruses she isolated. She reported groundnut ringspot, groundnut green rosette, chlorotic rosette, veinbanding, and tomato spotted wilt viruses. Unfortunately, none of the virus cultures were preserved and as a result the viruses reported have remained largely uncharacterized till today.

We initiated research on groundnut viruses in 1990 with surveys, followed by the development of diagnostic aids. We produced antisera to a local alfalfa mosaic virus isolate (82/8) from lucerne (*Medicago sativa*), to a bean yellow mosaic isolate (92/751) from french beans (*Phaseolus vulgaris),* to the nucleocapsid protein of a local TSWV-L isolate (91/348) from peas (*Pisum sativum*) Goszczynski, to a local

peanut mottle virus isolate (88/63) from soybeans (*Glycine max*), and to cucumber mosaic virus (86/212) isolated from tomato (*Lycopersicon esculentum*).

Antisera donated by a number of colleagues abroad since the project began [cowpea mild mottle virus (Brunt), groundnut rosette assistor virus (Murant), Indian peanut clump virus (Reddy), peanut bud necrosis virus (Reddy), peanut clump virus (Thouvenel), peanut mottle virus-N (Kuhn), peanut stripe virus (Demski), peanut stunt virus (PSV)-W (Tolin), PSV-E (Barnett), and PSV (Ghabrial)], have generally not been accompanied by positive controls due to quarantine regulations. As a result, these antisera were used only in immune electron microscopy (IEM) to confirm the results of other tests.

Field surveys were launched in 1991 to determine the occurrence and importance of viruses. About 350 groundnut plants with various abnormalities have been collected and tested since then. A number of these plants reacted with TSWV-L antisera and, on inoculation onto a set of diagnostic hosts, resulted in symptoms similar to those caused by the TSWV-L isolate. In a few cases, inoculated plants tested positive for TSWV-L. Interestingly, a number of plants which showed symptoms of TSWV did not react strongly with TSWV-L antisera, but reacted weakly with TSWV-BR 01 and TSWV-I antisera. It was subsequently demonstrated that two of these isolates belonged to the recently proposed groundnut ringspot tospovirus group (see Peters et al., pages 30-31 in this proceedings). Nearly 10% of the plants contained potyvirus particles, and the majority of them reacted with PeMotV antiserum. Based on dsRNA profiles, transmission by *Aphis craccivora,* and reaction with groundnut rosette assistor antisera, it was also shown that a number of plants contained groundnut chlorotic rosette virus.

We have thus far confirmed previous reports that TSWV and GRV occur in South Africa, and we detected peanut mottle virus for the first time on groundnuts in South Africa. Recently, groundnut plants with symptoms resembling those produced by the tospoviruses were observed in the Western Transvaal area. The large number of samples in which viruses could not be detected is undoubtedly due to the collection of plants with nutrient and genetic disorders or abnormalities not necessarily associated with a virus infection, or due to viruses for which we currently do not have diagnostic aids (e.g., peanut yellow spot tospovirus and groundnut bud necrosis tospovirus).

As we did not have the means of detecting and identifying the groundnut ringspot virus specifically, we tried to develop an ELISA for this virus serotype. Attempts to produce a polyclonal serum to isolate 90/13, one of the groundnut ringspot virus isolates, have thus far resulted in sera with low titers and fairly high nonspecific reactions.

However, the virus we would like to talk about mainly is an as yet unidentified isometric virus isolated in 1992 from groundnuts in the Western Transvaal (isolate 92/475). The original field-collected groundnut plant had a number of reduced young leaves with some malformation and some chlorotic patches. On the older leaves very mild chlorotic ringspots were detected. Initially, under an electron microscope, only potyvirus-like particles, which did not react with PeMotV antiserum in ELISA, were detected. Following sap inoculation, only isometric virus particles were isolated by

two local lesion transfers on *Chenopodium quinoa* and reinoculated onto groundnut, where the original symptoms, barring the ringspots on older leaves, were reproduced. The particles were spherical and 25 nm in diameter. The isolated virus was tested in IEM against a number of antisera (southern bean mosaic virus, cucumber mosaic virus, tomato aspermy virus, peanut stunt virus, tomato bushy stunt virus, and tobacco necrosis virus-A and -D) and by ELISA (brome mosaic virus, cowpea chlorotic mottle virus, cucumber mosaic virus -86/212, and a number of peanut stunt antisera) with no positive reaction. The virus could be purified with fairly high yields from *C. quinoa* using a purification procedure that involved extracts into 0.5M citrate, a 6.5 pH buffer, precipitation by polyethylene glycol, and fractionation on sucrose gradients. This procedure had previously worked very well for CMV-86/212. An antiserum was produced and a $F(ab')_2$ ELISA developed. A single coat protein of 28 kda, and three RNA bands (ca 2.18 kb, 3.01 kb, and 3.31 kb) could be obtained from the pure virus. The data suggest that the virus belongs to the cucumovirus group.

Very little information is currently available on the transmission of TSWV on groundnuts by thrips. At GCI, trap heights and color, counting procedures, preparation of samples for identification, preservation of reference collections, thrip culture, etc., are currently being used to study the thrips vectors of TSWV. Preliminary data indicate that, during the 1992/93 season, the relatively low incidence of the tospoviruses was due to the late appearance of thrips (9 weeks after the sowing of groundnuts).

Thrips species thus far identified (Dr Zur Strassen, Germany) in groundnut fields in South Africa include *Frankliniella schultzei, Scirtothrips* sp, *Aeleothrips brevicornis,* and *Thrips tabaci* (to be confirmed).

Interestingly, the major vector of peanut bud necrosis virus, *Thrips palmi,* was not observed.

Future objectives for research on groundnut viruses are as follows:

- To develop a detection system for groundnut ringspot tospovirus.
- To identify 92/475, and to perform serological tests with antisera to other members of this group.
- To produce a nonradioactively labeled probe from dsRNA to detect GRV, and to study its epidemiology.
- To obtain antisera (with positive controls) to some of the other viruses of groundnuts, e.g., groundnut eyespot potyvirus and groundnut crinkle carlavirus, as we have some indications of other viruses present, e.g., a potyvirus-like particle that did not react with PeMotV was also found initially in sample 90/475.

We believe that the viruses of groundnuts currently known to occur in other countries are also likely to be found in South Africa. However, due to agricultural isolation, the presence of large areas with natural legumes, and the omnipresence of insect vectors, we expect to find a number of viruses unique to South Africa.

With the privatization of agricultural research it is currently not known if enough resources will be provided to tackle problems due to viruses. We are eager to collaborate with other laboratories where research is being done on groundnut viruses.

Group Meeting on Virus Diseases in Asia

Chairpersons: **J W Demski and M Dollet**

Rapporteurs: **D V R Reddy and S Wongkaew**

New Developments in Techniques for Virus Detection

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Biochemical tests for the detection of plant viruses can be divided into two broad groups, namely those based on the detection of virus nucleic acid and those based on the tests for antibodies (or serological tests), which usually detect virus capsid protein (Torrance 1992a, 1992b). Serological tests, especially enzyme immunoassays (EIA), are the most commonly used for large-scale detection and diagnosis of plant viruses. However, advances are being made rapidly in sensitive tests based on application of the polymerase chain reaction (PCR) to plant virus detection. This brief report focuses on a few recent modifications and refinements to EIA and nucleic acid detection techniques.

Of the recent modifications to EIA, the incorporation of penicillinase instead of alkaline phosphatase as the reporter enzyme (Sudarshana and Reddy 1989, Singh and Barker 1991) has proved useful in India and other developing countries where supplies of alkaline phosphatase are sometimes prohibitively expensive. In the tests reported, the detection limits of the two enzymes were similar (Singh and Barker 1991). In this assay, the penicillin G substrate is converted to nontoxic penicilloic acid, and the reaction can be monitored by the change in color of bromothymol blue pH indicator to yellow. The color changes occur over a narrow pH range and hence the results can be assessed easily by eye; expensive plate readers are not required.

Another form of EIA, using enzyme cycling, has been used to increase the sensitivity of alkaline phosphatase EIA (Torrance 1987). In this method only the substrate reaction differs. The method involves two stages; in the first, NADP is converted to NAD by alkaline phosphatase; in the second incubation step, a pair of enzymes is used to amplify the product of the alkaline phosphatase reaction (Johannsson and Bates 1988). Amplified EIA has increased the sensitivity of detection of barley yellow dwarf virus (BYDV) and potato leaf roll virus (PLRV), making it possible to detect the virus even in individual vector aphids (Torrance 1987, van den Heuvel and Peters 1989).

As with all amplification methods, any increase in sensitivity requires the use of specific reagents, or the amplification step will also enhance the background nonspecific signal. Precautions must be taken to minimize antibody binding to components of host-plant origin, e.g. utilization of monoclonal antibodies (McAbs). Also, improved methods of antibody-enzyme coupling should be investigated (Torrance 1987). The commonly used one-step glutaraldehyde method produces large aggregates that may bind nonspecifically to the sample wells, causing increased background reactions. Several heterobifunctional cross-linking reagents are available commercially (Pierce) which allow well-defined and high-quality antibody-enzyme conjugates.

Although not suited to testing a large number of samples, a simple, specific, and sensitive method to locate the virus in plant cells is available, which is called tissue printing. In this method, transverse sections of plant stems (or whole leaves) are pressed to the surface of nitrocellulose membranes. The membranes are washed and incubated with virus-specific antibodies linked to alkaline phosphatase. The bound enzyme is revealed by incubating the membrane with an insoluble enzyme substrate. This method was used to locate PLRV in phloem cells (P Derrick, personal communication).

The most useful methods for the routine detection of plant viruses are those that are (1) sensitive (i.e., can detect about 1 ng virus); (2) not affected by components of plant sap; (3) easily adapted for rapid throughput of large numbers of samples; (4) limited to a few working steps; and (5) not dependent on highly trained personnel.

The different forms of EIA generally fulfil all of the above criteria. However, in certain situations such as detection of viroids, satellite RNAs, or defective forms of virus infection such as the NM-form of tobacco rattle virus (where no capsid protein is produced), other methods based on the detection of nucleic acid must be used.

The original hybridization tests used radioisotope (e.g., P^{32}) labeled cDNA probes for detection of complementary sequences (Salazar and Querci 1992). This was a disadvantage for routine diagnostic work because compliance with regulations governing the use of radioisotopes usually required separate restricted laboratory facilities. Also, the probes had a short shelf life. Promising recent developments using non-radioactive molecules such as biotin or digoxigenin, a steroid hapten (DIG system from Boehringer Mannheim GmbH), to label the nucleic acid have helped to address these problems. Digoxigenin-labeled molecules are versatile and there are many test formats based on labeled probes (Anonymous 1993). Digoxigenin probes have been used to detect the RNA of potato mop-top, potato leafroll, and tobacco rattle viruses in infected plants, and transcript RNA in transgenic plants (H Barker, personal communication). Probes of groundnut rosette virus satellite cDNA labeled with digoxigenin were as sensitive as radioactive P^{32}-labeled probes and worked well with crude sap extracts from *Nicotiana benthamiana.* Moreover, with some additional sap extraction procedures to prevent nonspecific reactions, the digoxigenin probes could be used to detect these sequences in the leaves of groundnuts (Blok, Robinson, and Murant, unpublished results). Detection systems using probes labeled with digoxigenin are safer, have fewer restrictions to their use, and have a longer shelf life compared to radio-labeled probes.

Since most plant viruses contain RNA, particularly the RT-PCR (reverse transcription reaction followed by PCR), is proving to be very sensitive and useful for the detection of virus and viroid nucleic acid sequences (Henson and French 1993). In the PCR reaction it is possible to amplify one copy of a particular DNA sequence about one million times (Saiki et al. 1988). PCR methods rely on the prior knowledge of at least some of the nucleotide sequence of the virus or viroid. Primers, or short oligonucleotides that are complementary to nucleotide sequences on each side of the sequence (5' and 3') to be amplified, are chosen so that a specific part of the genome is amplified. In many reports on viruses, this region has been part or all of the coat protein gene. The reaction products are analyzed by agarose gel electrophoresis.

Many research papers have been published concerning use of RT-PCR for virus or viroid detection (Henson and French 1993). The advantages of RT-PCR for virus detection are its extreme sensitivity and the fact that it does not incorporate radioactive labels. Also, the potential exists to develop tests specific to a virus group or strain, by using primers based on different parts of the genome (Hadidi and Yang 1990, Langeveld et al. 1991, Robertson et al. 1991). PCR tests have the potential to allow further analysis of the genetic variation among isolates or strains identified because the PCR products can be examined for restriction fragment length polymorphisms (Robertson et al. 1991), or their nucleotide sequences can be determined. Nevertheless, there are some disadvantages too: sample preparation can be laborious, and certain plant constituents can inhibit the enzyme reaction. Also, it is a laboratory-based test that uses expensive enzymes, skilled personnel, and special equipment. At the present time it is not suited to throughput of large sample numbers.

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Current Research on Indian Peanut Clump Virus at SCRI

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Earlier research has shown that Indian peanut clump virus (IPCV) is typical of furoviruses in its particle morphology, its transmission by a fungus vector, and its bipartite ssRNA genome. It has also been shown that geographically distinct isolates are also serologically distinct, although nucleic acid hybridization tests have shown some cross-reactivity between them. In vitro translation showed that the ca 6 kb RNA 1 encoded an M_r 143 000 protein but no small proteins and that the ca 4 kb RNA 2 encoded the coat protein and little else. Small amounts of lower molecular weight RNA were detected in RNA from purified virus particles.

The disease caused by IPCV has a significant economic impact and no resistance has been found among 10 000 lines of groundnut germplasm tested. Further molecular work with IPCV was therefore deemed necessary to attain two main objectives: (1) to obtain one or more virus genes that could be used to transform groundnut plants to induce transgenic resistance, and (2) to obtain cDNA probes for diagnostic work in order to circumvent the serological diversity found in field isolates. Two projects have been funded by the UK Overseas Development Administration to attain these objectives.

In the first project, cDNA that represented the majority of RNA 2 was obtained. This region included two open-reading frames (i.e., genes) and the 5'-noncoding region. The 5'-most gene was found to encode the coat protein. cDNA corresponding to the coat protein gene, together with the 5'-noncoding sequence, was inserted into the plant transformation vector pROK II downstream of the 35S cauliflower mosaic virus promoter. *Nicotiana benthamiana* tissue has been transformed by *Agrobacterium tumefaciens* containing this vector and will be tested for expression once the plants have been regenerated.

The amino acid sequence of IPCV coat protein was compared with those of other viruses with rod-shaped particles and was found to be 61% identical to the coat protein of peanut clump virus from West Africa, 37% identical to the coat protein of barley stripe mosaic virus, but not detectably related to coat proteins of other viruses, including furoviruses. This supports earlier suggestions that IPCV and PCV are distinct viruses, and raises the possibility of a taxonomic link between the two viruses causing peanut clump disease and the barley stripe mosaic virus, which belongs to a different virus group.

In the second project, cDNA clones have been obtained, which represent most of RNA 1, and about 70% of this cDNA has been sequenced. Results of preliminary experiments done at ICRISAT Asia Center suggest that cDNA from RNA 1 reacts with RNA from all serotypes of IPCV, whereas cDNA from RNA 2 corresponding to the coat protein gene does not cross-react among the serotypes.

Nonradioactive Detection of Peanut Mottle and Peanut Stripe Viruses with cRNA Probes Transcribed from Cloned 3' Regions of the Viral Genomes

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The 3' terminal nucleotides of the genomic RNA of an Australian strain of peanut mottle virus (PMV) and an Indonesian isolate of peanut stripe virus (PStV) were cloned and sequenced. The low degree of similarity in their coat proteins and 3' untranslated regions confirmed the classification of PStV and PMV as distinct potyviruses. Digoxigenin-labeled cRNA probes transcribed from these clones specifically detected picogram amounts of PStV or PMV in infected groundnut and bean leaf extracts.

We have purified PMV and PStV and prepared complementary DNA (cDNA) libraries from the viral genomic RNAs of both the viruses. Recombinant clones containing potyviral 3' terminal sequences were identified by hybridization with digoxigenin (DIG)-labeled oligo (dT). The 3' terminal 1247 nt of PMV (EMBL accession X73422) and 1388 nt of PStV (Z21700) were determined. These regions included part of the nuclear inclusion body *b gene,* the 861 and 864 nt which encode the respective coat proteins, and the 3' untranslated regions.

The coat proteins of PMV and PStV shared 66.7% of the amino acid sequence identity whereas the 3' untranslated regions of both the viruses were similar only to the extent of 33.3%. These results confirmed the classification of PStV and PMV as distinct members of the family Potyviridae. Pairwise comparisons of the amino acid sequence of the coat proteins of PStV and PMV strains showed a close similarity among all the PStV isolates and a distant relationship to PMV. However, the coat protein of the M strain of PMV (Gunasinghe et al. 1992) showed a 97.9-98.9% sequence identity with the PStV isolates. This virus appears to have been misidentified, and should be classified as a strain of PStV.

A dot blot nucleic acid hybridization system was developed for the diagnosis of PStV and PMV in plant tissues. The use of crude leaf extracts, DIG-labeled cRNA probes, and chemiluminescent detection make this system rapid, sensitive, and easy to use. RNA probes 1000 nt were transcribed from cDNA clones that contained sequences corresponding to the 3' termini (including the coat protein gene) of PStV and PMV. Both viruses were detected in the picogram range and in 5000-fold dilutions of infected leaf extracts. No cross hybridization was detectable between PStV and PMV with either probe. PStV cRNA probes, but not the PMV probe, hybridized with members of the bean common mosaic virus subgroup and with passionfruit woodiness virus.

On Efforts to Improve Detection of Selected Groundnut Viruses and Expression of Tomato Spotted Wilt Virus Antibodies in Plants

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Our initial interest in the detection of selected viruses of groundnut was to examine the utility of monoclonal antibodies (McAbs) for the detection of peanut mottle virus (PMV, Sherwood et al. 1987), tomato spotted wilt virus (TSWV, Sherwood et al. 1989), and peanut stripe virus (PStV, Culver et al. 1987, Culver and Sherwood 1988, Culver et al. 1989). Limited quantities of culture supernatant from these McAbs are available upon request. Serological techniques have worked well for detection of PStV in single seeds, but are not sensitive enough for routine screening of seed lots. An assay that could amplify and specifically detect the viral nucleic acid could circumvent this problem. Hence, the possibility of using the polymerase chain reaction (PCR) for the specific amplification of PStV in seed was examined. A modification of the method of Langeveld et al. (1991) gave the best results out of five methods to obtain viral RNA suitable for cDNA synthesis. The cDNA was amplified in the PCR using primers based on the sequence of the viral RNA around the coat protein region (Cassidy et al. 1993). Of the sets of primers utilized, a set that resulted in an approximately 400 base pair product gave the most consistent results and detected as little as 16 pg of the virus. Similar results were obtained with an extract of seed to which purified virus was added. Seed obtained from PStV infected groundnut was assayed by ELISA (Culver and Sherwood 1988). Seeds positive in ELISA also tested positive by PCR. The sensitivity of PCR for detection of PStV-infected seed, mixed with healthy seed, is being evaluated at the Oklahoma State University and at the University of Georgia (by J.W Demski).

Another area of interest is the expression of antibodies *in planta* as a novel means to study virus-host interaction. Polyclonal antibodies and McAbs are being made for the structural and nonstructural proteins of TSWV in cooperation with T German (University of Wisconsin) and D Ullman (University of Hawaii). TSWV, the type member of the genus tospovirus in the family Bunyaviridae, consists of three linear ssRNA molecules. Nucleocapsid (N) protein, produced from the S RNA, encapsidates the viral nucleic acid and may be involved in the regulation of viral nucleic acid replication. cDNAs coding for the H and L chains of a McAb to the N protein were produced by first-strand cDNA synthesis followed by PCR cloning. DNAs coding for the H and L chain were inserted into the binary vectors for *Agrobacterium-medteted* plant transformation. Regenerated plants (R_0) contained either H or L chain constructs as tested by PCR or Southern blots. Northern blots of R_0 plants indicated H and L transcripts of the predicted size. Plants transformed with the L construct are producing L chain protein at 1 to 42 µg mg^{-1} plant protein. Additional transformations are being made to facilitate H chain production. In addition, with a single chain

antibody construct, the effects of leader sequences that differ in their hydrophilic nature are being examined. Although our primary focus at this time is on the antibody for the N protein, McAbs are being made for the other proteins of TSWV that have either been isolated from virions or expressed in bacterial expression vectors. As the transformation of plants for expression of antibodies becomes more routine in the laboratory, the expression of antibodies to these other proteins will be examined.

Peanut Stripe Virus: Genome Organization and Resistance Conferred by the Expression of the Coat Protein Gene in *Nicotiana benthamiana*

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Peanut stripe virus (PStV) is an important potyvirus infecting groundnuts and other legumes in India, China, and Southeast Asia. It was first reported in the United States in 1984. In order to develop resistant cultivars of groundnuts, a molecular approach was followed.

PStV was purified from *Nicotiana benthamiana* and RNA extracted by standard procedures. Complementary DNA (cDNA) to the viral RNA was synthesized using reverse transcriptase and DNA polymerase. Several strategies were used to determine the complete genome sequence.

Analysis of the nucleotide sequence revealed that the genome was 10 048 nucleotides long and codes for a polyprotein of 3224 amino acids (~370 kda). Two possible initiation codons at positions 134-136 and 146-148 were identified. The proposed cleavage sites for the eight viral encoded proteins are in good agreement with other potyviral sequences. The extent of similarity among PStV gene products was compared with that among several other fully sequenced potyviral genomes. Soybean mosaic virus (SMV) shared the highest degree of similarity with PStV (38-83%).

As yet, groundnut remains recalcitrant to transformation and regeneration; therefore, *N. benthamiana,* a host for PStV, has been used to study coat protein-mediated resistance (CPMR). Three PStV coat protein (CP) constructs (full length - FL; putative 16, putative 109) had previously been demonstrated to synthesize the expected peptides in *Escherichia coli. In planta,* some lines of each of the constructs did not synthesize detectable levels of CP. No protein product from PStV CP-putative 109 has been detected in plant lines. Regardless of expression levels, our results showed a distinct delay in initial symptoms and a clear recovery of the upper leaves from plants expressing each of the PStV constructs. There does not appear to be any correlation between the level of CP protein expressed and the degree of resistance seen. One plant line that did not produce detectable levels of CP (CP-putative 16) was immune to PStV inoculation.

Symptomless systemic leaves, which we refer to as recovered leaves, were further analyzed. We found that this tissue had very low or undetectable levels of virus and, surprisingly, reduced or no expression of the CP transgene. Upon reinoculating the recovered leaves, low levels of virus replication were detected in them but not in leaves produced subsequently.

Efforts to introduce the PStV CP gene into *A. hypogaea* are in progress. Mechanisms underlying CPMR are also being investigated.

Ecology of *Polymyxa graminis*

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The plasmodiophoromycete fungus *Polymyxa graminis* Ledingham was reported to be the natural vector of the peanut clump virus (PCV) in West Africa (Thouvenel et al. 1988), and of the Indian peanut clump virus (IPCV) (Ratna et al. 1991). Evidence that the fungus transmits the virus was based on the observation that air-dried soil remained infective for at least 1 year, and on the presence of cystosori of *P. graminis* in the roots of various hosts of PCV and IPCV collected from virus-infested soils. Additionally, the presence of P. *graminis* in roots of virus-infected plants could be correlated with virus transmission (Reddy et al. 1988). *Polymyxa graminis* has also been found to be associated with the transmission of 11 other rod-shaped or filamentous viruses (Maraite 1991). To obtain convincing evidence of virus transmission by P. *graminis* it is essential to culture the fungus and utilize zoospores produced in vitro in transmission tests.

Polymyxa graminis populations obtained from IPCV-infested soils in India were found to infect both monocotyledonous and dicotyledonous plants including sugar beet (*Beta vulgaris),* a host of *P. betae* in temperate countries (Ratna et al. 1991). In contrast, *P. graminis* isolates from Belgian soils have much narrower host ranges, often being restricted to barley *(Hordeum vulgare)* (Bastin et al. 1989). Cystosori were observed in roots of IPCV-infected groundnuts in India, but not in West Africa, where it is believed that the fungus acquires PCV from a graminaceous host such as *Sorghum arundinaceum* and transmits it to groundnut in a manner that has not been resolved so far. Groundnut roots naturally infected with PCV were not effective as sources of infection in PCV transmission tests, whereas roots of *Sorghum arundinaceum* were. Pearl millet (*Pennisetum glaucum)* is a host of both *P. graminis* and IPCV in India, but is considered to be a non-host in West Africa. Therefore it may be a suitable crop in the farming system to reduce PCV incidence. These differences suggest that the isolates of *P. graminis* in India are likely to be different from those in West Africa. However, these differences could also be due to variations in the technique. When high concentrations of inocula of *P. graminis* isolates from Belgium were used under laboratory conditions it was possible to infect hosts that did not support multiplication of *P. graminis* under field conditions. Therefore the presence of cystosori in the roots is, by itself, not an adequate criterion for determining the natural host range of the fungus.

Much diversity is currently known to exist among *P. graminis* isolates occurring in Belgium. Therefore, it is essential to characterize precisely the isolates of *P. graminis* that occur in India, to understand the epidemiology of clump disease. The life cycle of *P. graminis* in India and the diversity among its isolates have not been investigated. Cystosori are highly resistant to various adverse soil conditions. They are disseminated in soil containing root debris. In temperate countries, viral diseases transmitted

by *Polymyxa* spp also occur at the same location in the field in succeeding crops. The inoculum potential of *P. graminis,* assessed by the most probable number method, could reach 100 infective units per gram of dry soil. Though the inoculum potential of *P. graminis* from Indian soils has not been determined, it appears to be low from preliminary experiments conducted in Belgium. Despite the wide host range of both the IPCV and the vector, the rapid turnover of organic matter in tropical soils may be contributing to the restricted spread of the clump disease. On the other hand, the narrow host range of *P. graminis* in temperate countries may contribute to the slow spread of the fungus.

The period of dormancy, and factors contributing to its breakdown, have yet to be determined for Indian isolates of *P. graminis.* For Belgian isolates, soil moisture with matrix potential of -100 mb is required for infection but, for the isolates in tropical countries, the soil moisture requirements need to be analyzed. The optimum temperature required for zoospore release in temperate countries is below 20°C, whereas temperatures above 25°C are required for this process in isolates from tropical countries (Maraite et al. 1988).

Preferred hosts of *P. graminis* in temperate countries, such as wheat *(Triticum* spp), are seldom rotated with groundnut in the major groundnut-growing areas of India. Therefore, the ecology of the *P. graminis* isolates in India is likely to be different from that of isolates from temperate countries.

A collaborative project between the University Catholique de Louvain (UCL) and ICRISAT to study the diversity among *P. graminis* isolates in India and West Africa, and optimum conditions required for transmission of IPCV, has been initiated with funds provided by the Administration generale de la cooperation au developpement (AGCD) of Belgian government.

Current Research on Groundnut Viruses at ICRISAT Asia Center

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In this paper, we focus on research carried out on peanut bud necrosis, peanut clump, and peanut stripe viruses at the ICRISAT Asia Center. Though several widely distributed minor virus diseases of groundnut such as cowpea mild mottle virus and peanut chlorotic streak virus have been investigated, current research on these viruses has been restricted to their characterization, and to development of precise diagnostic aids.

PBNV was found to be serologically distinct from tomato spotted wilt virus (TSWV). The virus-vector relationships have been determined, and the virus is shown to be transmitted efficiently by *Thrips palmi.* Three RNA species have been clearly resolved in agarose gels under denaturing conditions. RNA 3 (the smallest) was cloned into pUC119 vector and a ca 700 bp specific clone was identified. Research is in progress to sequence all the three species of the nucleic acid. Sources of resistance to both the virus and the vector have been identified in *Arachis hypogaea* and are currently being used to develop varieties with durable resistance.

Extensive surveys for peanut stripe virus (PStV) have been conducted in various research stations in India since 1987. The virus was detected only in plants grown from seeds that originated in Junagadh, Gujarat. It was detected in several farmers' fields located near Junagadh (R D V J Prasada Rao and M S Basu, personal communication). Surveys will be continued to monitor the distribution of the virus in India.

The coat protein gene of PStV obtained from the S R Noble Foundation, Oklahoma (Cassidy et al. 1993) was transferred to pETI5b expression vector and expressed in *Escherichia coli.* The viral coat protein produced was separated by gel electrophoresis and utilized in producing polyclonal antisera of extremely high quality.

In collaboration with scientists of the Scottish Crop Research Institute, the RNA 2 of Indian peanut clump virus was partially sequenced (Wesley et al. 1994). A cDNA probe derived from the RNA 1 could be used to detect all the currently known IPCV serotypes. Digoxigenin-labeled probes could detect as low as 10 picogram of IPCV RNA. A procedure for the polymerase chain reaction was developed to enable us to detect very low quantities of the virus in groundnut tissue. Specific primers that flank the capsid protein gene have been used to amplify the coat protein gene. A plant

expression vector has been constructed, using the coat protein gene, that is suitable for transforming groundnut through *Agrobacterium.*

Our future research on IPCV will be concentrated on elucidation of the genome organization of IPCV, utilization of viral gene constructs for transforming groundnut, and testing of transgenic plants for resistance to IPCV and to the PCV that occurs in West Africa.

A collaborative project on the diversity of *Polymyxa graminis* isolates in India and their ecology has been initiated with Dr H Maraite, Head, Unite de phytopathologie, Faculte des sciences agronomiques, Universite Catholique de Louvain, Belgium, with funds provided by the Belgian government.

The Epidemiology and Control of Viruses of Groundnut

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Groundnut is grown in many countries of the old and new worlds and in very diverse agroecological conditions, including humid and less humid environments in the tropics and in higher latitudes during the summer months. In some areas, monoculture on large mechanized farms is common whereas in others, the crop is grown mainly on small holdings, often mixed with other crops.

In these circumstances, it is hardly surprising that the many viruses that have been encountered in groundnut belong to different groups and exploit various means of spread and perennation. This diversity is apparent from a list of 14 of the most important viruses reported and their means of spread:

1. Aphidborne: persistent
   - Groundnut rosette umbravirus
   - Groundnut rosette assistor luteovirus
   - Groundnut streak necrosis virus

2. Aphidborne: nonpersistent
   - Peanut stunt cucumovirus (seedborne)
   - Peanut stripe potyvirus (seedborne)
   - Peanut mottle potyvirus (seedborne)
   - Cucumber mosaic cucumovirus (seedborne)

3. Thripsborne
   - Tomato spotted wilt tospovirus
   - Peanut bud necrosis tospovirus
   - Groundnut ringspot tospovirus
   - Groundnut chlorotic spot tospovirus

4. Whiteflyborne
   - Cowpea mild mottle carlavirus

5. Fungusborne
   - Indian peanut clump furovirus (seedborne)
   - African peanut clump furovirus (seedborne)

Detailed epidemiological information is required on these and other viruses of groundnut in order to develop effective control measures. However, such studies have been restricted to only some of the viruses encountered and to few of the environments in which they occur. This is largely inevitable given the limited virological expertise available in many tropical countries and the current preoccupation of many virologists in developed countries with the structure and biochemical properties

of viruses. Moreover, epidemiological studies, especially covering several production systems, require a great deal of manpower, land, and other resources.

Despite these difficulties, some progress has been made and it is known that the thripsborne viruses cause diseases of the monocyclic type because spread is mainly from outside sources of infection and there is little or no secondary spread within crops. By contrast, the aphidborne viruses that are transmitted nonpersistently spread readily within crops and cause typical polycyclic diseases. Viruses with a soil-inhabiting fungus vector do not seem to fit readily into either category, but there is little information on their ecological behavior and on the role of the large number of hosts these viruses infect, which include sorghum *{Sorghum bicoior)* and other graminaceous hosts.

There is great diversity in the sources of infection from which the initial spread of virus occurs. These can be crop, weed or wild plants within or alongside crops. Such nearby sources pose the greatest threat. However, there is some evidence that groundnut rosette and bud necrosis viruses can be spread far by insect vectors to initiate outbreaks in entirely new areas. This possibility merits further attention. There are many other unresolved epidemiological problems. For example, there is little information on the effects of intercrops on insect vector populations and virus spread. This emphasizes the need for additional epidemiological information on the whole range of groundnut viruses to complement the detailed studies now in progress in various laboratories on their physico-chemical properties.

Groundnut Virus Research in Thailand

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Among the six viruses reported from Thailand, peanut stripe (PStV), peanut bud necrosis (PBNV), and peanut mottle viruses (PMV) are considered to be economically important. Various aspects currently under investigation are identification of strains, epidemiology, and measures for disease management.

Evidence has been obtained to show that PStV has been occurring in Thailand since 1972. Seven PStV isolates that produced different symptoms on groundnut have been identified. They also differed in the frequencies of seed and aphid transmission. An isolate that produced mild symptoms could cross-protect groundnut against infection by other isolates. In institutional productions, isolates that produced blotch or mild mottle symptoms occurred frequently. Interestingly, both these isolates produced severe symptoms on a range of soybean (*Glycine max*) cultivars. The blotch and mild mottle isolates are transmitted to the extent of 6% and 16% respectively through groundnut seed. The necrotic and stripe isolates could reduce yield in groundnut by up to 79%, and showed seed transmission rates of 8% and 13% respectively. In disease surveys, PStV was found to be more widely distributed than PMV. Nevertheless, their combined incidence on farmers' fields was less than 5%.

Since symptoms produced by PStV and PMV are similar to some extent, methods for their differentiation have been standardized. They can be easily distinguished by symptoms on a range of diagnostic hosts and by examining the inclusion bodies, produced in leaves, under a light microscope. Since none of the insecticides tried reduced the incidence of PStV, efforts are currently being made to identify groundnut cultivars with very low frequency of seed transmission.

In recent years, incidence of PBNV has been very high. Incidence of up to 20% was recorded in northeastern Thailand during the dry season in many farmers' fields. Occasionally, the incidence was as high as 90%. PBNV also occurred on other cash crops such as tomato (*Lycopersicon esculentum*), pepper (*Piper nigrum*), and cucurbits. Because of its wide host range and its severe effect on many crops, PBNV has become a major concern in Thailand. Serological tests performed recently have confirmed that the virus in Thailand is serologically identical to the Indian isolate of PBNV. Additionally, the Thai isolate did not react with tomato spotted wilt virus (TSWV-L) or impatiens necrotic spot virus antisera. The isolates collected from various crops differed considerably in host range and symptomatology, indicating the occurrence of various PBNV strains. Under field conditions, five weed species have been identified as possible reservoirs of PBNV. A study on seed transmission of PBNV has revealed that in groundnut and watermelon (*Citrullus vulgaris),* although the viral antigens were detected in the testa, none of these seed transmitted the virus. Nevertheless, PBNV was detected in 12% of the tomato seeds collected from infected fruits and 2.5% of the seedlings in grow-out tests showed symptoms of PBNV. The infected seedlings were malformed and had necrotic etching on primary leaves. All of

them withered and died prior to transplanting. Since plants that grew from infected seed did not survive beyond the seedling stage, they may not have acted as a source of inoculum under field conditions. Future research on PBNV will be directed towards identification of strains and methods for disease management.

Acknowledgement

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Current Research on Groundnut Virus Diseases in China

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Virus diseases of groundnut are economically important in China. Severe epidemics occurred in the major groundnut-growing provinces in the last two decades. Four plant viruses have now been reported to infect groundnut in China. Disease surveys conducted from 1990 to 1992 revealed that peanut stripe virus (PStV) was prevalent in all groundnut fields surveyed in six counties in Hebei and Shandong provinces. Severe epidemics due to peanut stunt virus (PSV) were recorded in Qianan and Luanxian counties and in Hebei in 1992. Cucumber mosaic virus (CMV) occurred in all six counties and reached epidemic proportions in Qixia, Penglai, and Muping counties and in Shandong in 1992.

Assessment of crop losses showed that early infection due to PStV reduced pod yields by 44.2% and 43.7% in the two commonly grown groundnut cultivars in China under field conditions. EC 36892, an aphid-resistant groundnut genotype, showed high resistance to aphid multiplication in field trials in Wuhan in 1990 and 1991. However, it failed to show resistance to nonpersistently transmitted PStV. Over 1300 groundnut genotypes were tested between 1989 and 1992 to determine the rate of seed transmission of PStV. All the genotypes transmitted PStV through seed. Seven genotypes showed less than 1% PStV seed transmission each in three consecutive tests. In the cooler regions of China, farmers cover the soil with plastic sheet prior to sowing. This practice has been shown to increase pod yields. Both silver colored and transparent plastic films were effective for repelling aphids in field trials conducted in 1990 and 1991. Aphids trapped in yellow pans were counted. Fewer aphids were trapped in mulched plots compared to those in control plots, especially during early stages of crop growth. Application of plastic film mulch reduced PStV incidence in the 2-year trials, particularly in 1991. In 1991, PStV incidence, estimated after flowering, was 17.8% in plots mulched with silver film and 27.4% in those with transparent film. In control plots, PStV incidence was 93.5%.

Seven PSV isolates that were characterized by host reaction included six PSV Chinese isolates, namely PSV-Mi, PSV 1, and PSV 13 from groundnut, PSV-P from bean, PSV-R from black locust (a tree legume), PSV-F from *Falso indigo* (a leguminous shrub), and one American isolate, PSV-E. All the six PSV Chinese isolates differed from PSV-E in that they induced systemic mosaic in *Chenopodium amaranticolor* and *C. quinoa.* Unlike the Chinese isolates, PSV-E produced systemic mosaic on *Trifolium*

pratense, Petunia hybrida, and *Zinnia elegans.* PSV 1, PSV 13, PSV-P, and PSV-E caused severe symptoms on groundnut, beans (*Phaseolus* spp), *Vigna vulgaris,* and peas (*Pisum sativum*). In contrast, PSV-Mi, PSV-R, and PSV-F caused mild mosaic on the leaves of groundnut, beans, and *Vigna vulgaris;* they did not infect peas.

The maximum seed transmission of PSV observed was 0.05%. Black locust may be acting as a perennial source of virus inoculum. Often, in northern China, PSV appeared first in groundnut plants located near black locust whereas plants located more than 500 m away from black locust trees showed very little PSV incidence in the early stages of crop growth.

A virulent strain of cucumber mosaic virus (CMV-CS) was identified. Groundnut plants infected with CMV-CS were scattered in the field. Extensive tests showed that seed transmission rates of CMV ranged from 0.4% to 4.2%, with an average of 1.5%, in groundnut seeds collected from the areas in which there had been an epidemic of the disease. *Aphis craccivora, A. robiniae, A. glycines, A. gossypii,* and *Myzus persicae* transmitted CMV with high efficiency. Sowing CMV-free seed and mulching with plastic film contributed to effective control of CMV disease in field trials conducted in two consecutive years.

Acknowledgement

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Virus Diseases of Groundnut in India with Particular Reference to Peanut Stripe Disease

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In India, many viruses have been reported to cause diseases in groundnut. The most important economically are those caused by peanut bud necrosis tospovirus (PBNV), peanut clump furovirus (PCV), and peanut stripe potyvirus (PStV). PBNV has been found to occur in almost all the major groundnut-growing areas of the country. PCV has been reported from Andhra Pradesh, Gujarat, Punjab, Rajasthan, and Tamil Nadu, and PStV has been reported from Andhra Pradesh, Gujarat, Karnataka, Maharashtra, and Tamil Nadu. So far PStV has not been found in Punjab and Rajasthan. The other seven viruses, namely cowpea mild mottle carlavirus, groundnut veinal chlorosis rhabdovirus, groundnut yellow mosaic geminivirus, peanut chlorotic leaf streak caulimovirus, peanut green mosaic potyvirus, peanut mottle potyvirus, and peanut yellow spot tospovirus, though widely distributed, are considered to be of minor importance.

PStV was first observed in 1987 (Prasada Rao et al. 1988) at ICR1SAT Asia Center, Hyderabad; Gujarat Agricultural University (GAU), Navsari; and agricultural research stations at Palem and Vikarabad in Andhra Pradesh and Raichur in Karnataka, simultaneously in one or more of the following 10 genotypes that were grown under a multilocational varietal trial of the All India Coordinated Research Project on Oilseeds (AICORPO) consisting of 49 entries: NRGS(E) 2, NRGS(E) 6, NRGS(FDRS) 1, NRGS(FDRS) 2, NRGS(FDRS) 3, NRGS(FDRS) 6, J 19, J 21, J 22, and J 24. At the National Research Center for Groundnut (NRCG), Junagadh, PStV was not found in any of the entries but was detected in one plant from a segregating material; the virus was also not found in these entries at six other locations. All the entries in which PStV was detected originated either from NRCG or GAU, in Junagadh. Of the 10 genotypes, in which the virus was detected in 1987, 8 were found infected at Raichur, 7 at ICRISAT, Hyderabad, 3 at Palem, and one each at Vikarabad and Navsari. The source of infection of these genotypes at different places is difficult to assign. As the virus is transmitted through the seeds, one would expect seed infection to be the main source. However, in that case, infection of different genotypes at different locations should have been more uniform, unless the seed derived from noninfected plants was mixed with that from infected plants inadvertently.

In Gujarat, which is a major groundnut-growing area in the country, negligible incidence of the disease has been observed in farmers' fields during the wet season *(kharif),* whereas high incidence is observed in the dry season (*rabi).* This difference in the incidence of PStV could be due to the differences in the movement of aphid population in these seasons. The dry season crop is sown in January when the aphid

population is high, whereas in the wet season negligible populations of aphids were observed in an experiment in 1992.

Surveys between 1987 and 1993 in farmers' fields and experimental plots around Junagadh and Navsari (Gujarat) indicated an increase in incidence of PStV, particularly in the dry season. In 1987/88, it was found only in traces in one breeding line in experimental plots. In 1991, 14 out of 80 farmers' fields had disease incidence up to 5% in the dry season. In 1992, 17 out of 18 farmers' fields showed 40% incidence. In 1993, up to 10% infection was observed in two fields. Attempts are being made to identify areas and seasons free of PStV for the production of virus-free seeds. For example, in the 1992 rainy season, no incidence of PStV was observed in Haryana, Rajasthan, and western Uttar Pradesh. Attempts are also being made to locate secondary hosts of the virus for developing appropriate management practices.

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General Discussions and Recommendations

Chairpersons: **R W Gibbons and D G Cummins**

Rapporteurs: **D V R Reddy and K K Sharma**

General Discussions and Recommendations

Following discussion, both general and specific recommendations were made for global cooperative research on groundnut viruses.

General recommendations

- Collaboration between members of the three working groups should be continued as group meetings are considered to be valuable.
- NARS should be encouraged to set up facilities for producing virus-free groundnut seed for use in seed multiplication systems and by growers.
- A diagnostic service should be set up for all known viruses of groundnut whereby ELISA plates processed with plant samples could be posted to a central laboratory, ideally the ICRISAT Asia Center in India. This service should be available to scientists in NARS in developing countries. For viruses such as groundnut rosette assistor luteovirus, double antibody sandwich ELISA would be necessary. Those requiring this service should write to the Principal Scientist (Virology), ICRISAT Asia Center, for procedures to prepare the samples and for shipping the ELISA plates.

  Diagnostic reagents should be supplied free of charge to ICRISAT for use in this service laboratory.

  ICRISAT should prepare guidelines, for distribution to all interested scientists in developing countries, on simple procedures for preparing and coating ELISA plates. The guidance should include instructions to make adequate records of date, locality, and symptoms (with color photographs, if possible) and for preservation of samples for later access if required.
- Studies on the epidemiology of groundnut viruses are encouraged.
- Participants should provide to Dr J W Demski, University of Georgia, Griffin, a list of all diagnostic tools available with them for identification of groundnut viruses. He will compile the entire list and submit it for publication in the *International Arachis Newsletter.*
- A formal network should be established for exchange of information by e-mail (CGNET, VITNET), fax, etc. The *International Arachis Newsletter* could also be used as a forum for dissemination of information by the Working Groups.

Specific recommendations

By the working group on transformation and regeneration of groundnut, and utilization of viral genes to induce resistance to virus diseases

- The feasibility and efficiency of a range of protocols for groundnut transformation should continue to be explored.
- The genotype EC 5 should be included among those that are already being used, to provide a basis for meaningful comparison of results obtained with different protocols in different laboratories. Dr Demski will provide a small quantity of seed of EC 5 for distribution to the research teams.
- Regenerated plants must be rigorously tested for gene integration and expression, and for the inheritance and genetic stability of the transgenes.
- Economically important groundnut viruses against which natural resistance is not currently available should be given high priority for obtaining transgenic resistance. Peanut stripe and peanut clump viruses, therefore, should be given preference.
- Careful thought and planning should be given to the components and detailed design of DNA constructs to be used for transformation. Simplified test systems such as those utilizing *Nicotiana* spp, should be used for preliminary evaluation of various constructs.
- In August 1994, each group attempting groundnut transformation should send a brief progress report to Dr D V R Reddy, who will consolidate the reports and send them to all the collaborating laboratories.

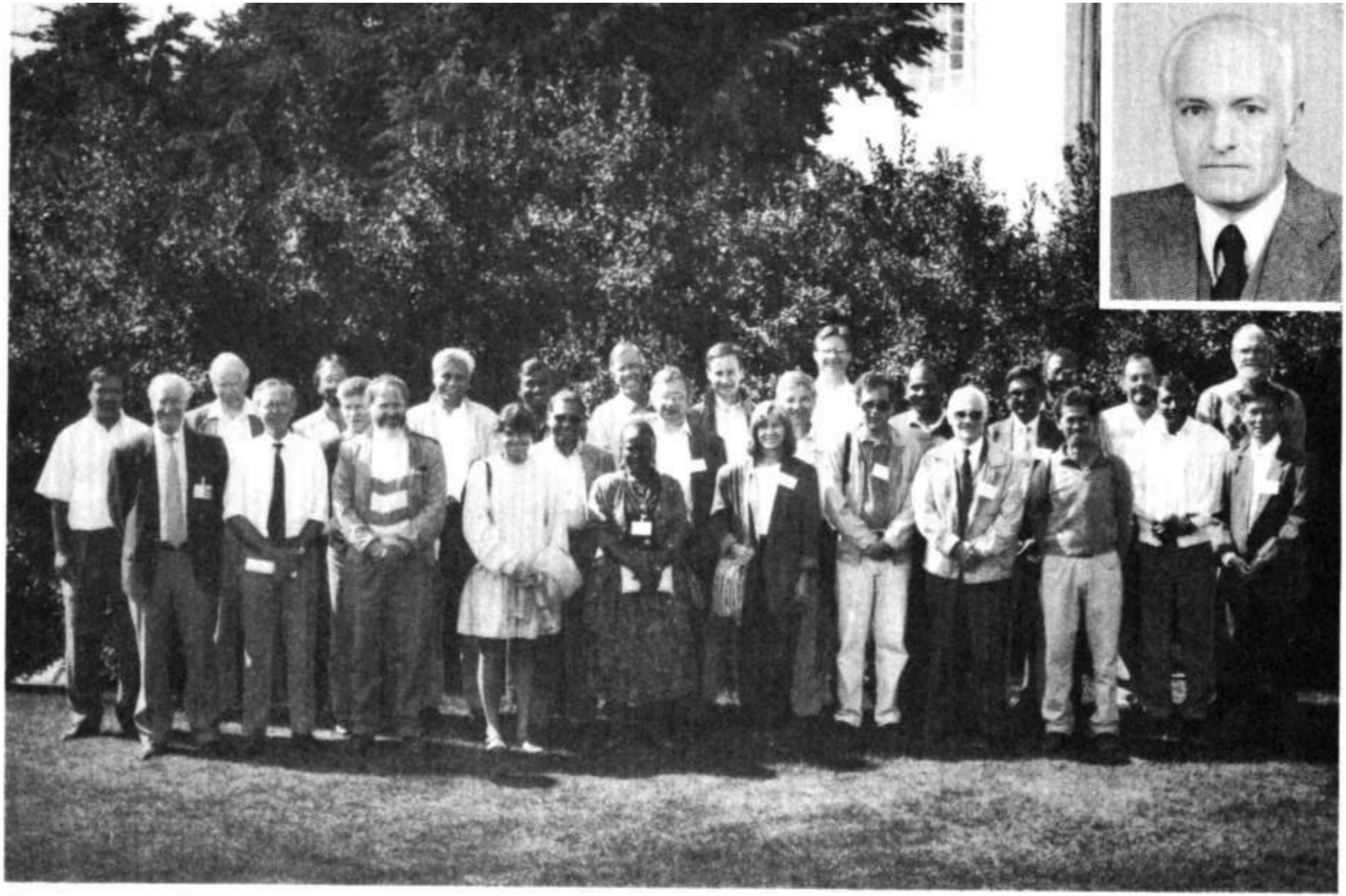
By the working group on groundnut viruses in Africa

- More accurate information is needed about the identity, occurrence, and distribution of groundnut viruses in Africa. This applies especially to tospoviruses, peanut clump furovirus, and cowpea mild mottle virus. There is also the problem of distinguishing between the symptoms of peanut clump and green rosette diseases. Therefore reports of occurrence of groundnut viruses on the basis of external symptoms alone should be discouraged.
- Funds should be sought for conducting surveys for peanut clump virus in West Africa. This survey would require the involvement of Dr M Dollet.
- An information bulletin on the groundnut rosette disease should be prepared by ICRISAT and Peanut CRSP. Dr A F Murant should coordinate the preparation of this bulletin.

By the working group on groundnut viruses in Asia

- Studies on the ecology of *Polymyxa graminis,* the vector of peanut clump virus, are encouraged.
- Groundnut seed, imported for research purposes, should be tested for all known seed-transmitted viruses, including peanut stripe and peanut clump viruses, by a grow-out test and/or by ELISA in quarantine facilities of the importing country.
- The next meeting of this working group is proposed to be held in Thailand in early 1995. Dr S Wongkaew would help with the choice of venue and arrangements. A training workshop on identification of economically important groundnut viruses in Asia should be held in Thailand just before or just after this working group meeting.

Participants



Participants in the international meeting of working groups on groundnut virus diseases, Scottish Crop Research Institute, Dundee, UK (15–19 Aug 1993). Inset: A F Murant, Convener, Local Organizing Committee

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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.

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