Groundnut Virus Diseases in Africa

International Crops Research Institute for the Semi-Arid Tropics
Belgian Administration for Development Cooperation

Citation: Reddy, D.V.R., Delfosse, P., Lenne, J.M., and Subrahmanyam, P. (eds.) 1997. Groundnut virus diseases in Africa: summary and recommendations of the Sixth Meeting of the International Working Group, 18-19 Mar 1996, Agricultural Research Council, Plant Protection Research Institute, Pretoria, South Africa. (In En. Summaries in En, Fr, Pt) Patancheru 502 324, Andhra Pradesh, India: International Crops Research Institute for the Semi-Arid Tropics; and 1000 Brussels, Belgium: Belgian Administration for Development Cooperation. 64 pp. ISBN 92-9066-358-8. Order code: CPE 109.

Abstract

The International Working Group Meeting on groundnut viruses in Africa reviewed progress made on the detection, identification, characterization, and management of groundnut viruses in Africa, with special emphasis on rosette and clump viruses. Country representatives summarized the status of research on groundnut viruses in their countries. In order to accomplish integrated management of rosette and clump virus diseases, it was agreed that consolidated efforts should be made to understand their epidemiology. Among the important aspects discussed were the provision of diagnostic aids and training in the identification and detection of viruses for the national agricultural research systems in Africa, and strengthening of laboratory facilities.

Scientists from Burkina Faso, Kenya, Malawi, Nigeria, South Africa, and Zimbabwe, and from Belgium, Germany, India, UK, and USA attended the meeting, which was the first gathering of so many plant virologists in South Africa.

Résumé

Maladies virales de l'arachide en Afrique: compte rendu et recommandations de la Sixième réunion du Groupe de travail international, 18–19 mars 1996, Pretoria, Afrique du Sud. Le Groupe de travail international sur les maladies virales de l'arachide en Afrique s'est réuni a Pretoria en Afrique du Sud pour discuter des progrès récents dans les domaines de la détection, l'identification, la caractérisation et le contrôle des maladies virales qui affectent l'arachide en Afrique et plus particulièrement la rosette et le rabougrissement. Les participants africains ont fait part de la situation de la recherche sur les maladies virales de l'arachide dans leurs pays respectifs. En vue de développer un contrôle intégré de la rosette et du rabougrissement de l'arachide, le Groupe a convenu de l'importance de la recherche pour une meilleure compréhension de l'épidémiologie de ces deux maladies. Parmi les recommandations importantes du Groupe on peut citer la mise à disponibilité des outils de détection, la formation pour les scientifiques africains dans le domaine de l'identification et de la détection des virus, et l'aménagement des laboratoires dans le secteur de la virologie.

Des chercheurs du Burkina Faso, Kenya, Malawi, Nigeria, Afrique du Sud et Zimbabwe et de Belgique, Allemagne, Royaume-Uni et Etats-Unis d'Amérique ont participé à la réunion du Groupe. Il s'agissait du plus grand rassemblement de phyto-virologues que l'Afrique du Sud ait connu.

Sumário

Doenças virais do amendoim em África: actas sumário duma Reunião de Grupo de Trabalho, 18-19 de Março de 1996. Pretória, África do Sul. A Reunião do Grupo de Trabalho Internacional sobre viroses do amendoim em África, passou em revista os progressos feitos na detecção, identificação, caracterização, e maneio de doenças virais do amendoim em África, com especial ênfase na roseta e doença da moita. Os representantes dos diversos países sumarizaram o presente estado da investigação sobre o virus do amendoim nos seus países. De forma a conseguir um maneio integrado da roseta e doença do virus da moita, foi acordado que um esforço conjunto deve ser feito de forma a compreender a sua epidemiologia. De entre os importantes temas discutidos, salientaram-se as necessidades em meios de diagnóstico e treinamento na identificação e detecção de virus dos Sistemas Nacionais de Investigação Agrícola em África, e o reforço dos meios laboratoriais.

Cientistas de Burkina Faso, Quénia, Maláwi, Nigéria, África do Sul e Zimbabwe, em África, e da Bélgica, Alemanha, Reino Unido e Estados Unidos da América, participaram na reunião, que foi o primeiro encontro de tão largo número de virologistas de plantas na África do Sul.

Groundnut Virus Diseases in Africa

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

18-19 Mar 1996
Agricultural Research Council
Plant Protection Research Institute, Pretoria, South Africa

Edited by

D V R Reddy
P Delfosse
J M Lenne
P Subrahmanyam

ICRISAT
International Crops Research Institute for the Semi-Arid Tropics

Belgian Administration for Development Cooperation
1997

Organized by

Agricultural Research Council, Plant Protection Research Institute, Pretoria, South Africa

Southern African Development Community (SADC)/ICRISAT Groundnut Project, Malawi

ICRISAT Western and Central Africa Region

ICRISAT Asia Region

Co-sponsors

Belgian Administration for Development Cooperation, Belgium

Natural Resources Institute, UK

Overseas Development Administration, UK

Peanut Collaborative Research Support Program, USA

Contents

Opening Remarks

J M Lenne[1]

Virus diseases are among the most important biotic constraints to groundnut crops, especially in Africa. Groundnut rosette virus and peanut clump virus diseases continue to cause significant losses to groundnut in Africa.

Considerable effort has been focused on characterizing the causal agents and identifying the vectors; on understanding the biology and epidemiology of the agents and diseases caused; and in developing and applying management techniques. Representatives of institutions from Australia, Europe, and USA have joined with those from Africa and Asia, and with ICRISAT scientists, to achieve common goals. Three Working Groups have been formed to facilitate and enhance collaboration and communication.

Working Group meetings have been held frequently since 1983, when the First International Working Group on Groundnut Viruses met to coordinate collaborative research. A quick scan of the summary papers in the proceedings of the Fourth Meeting of the International Working Group on Groundnut Viruses in the Asia-Pacific region, held in Khon Kaen, Thailand in March 1995, clearly shows the substantial progress made in understanding groundnut viruses and in working together. This meeting is a landmark in continuing progress towards resolving serious problems caused by groundnut virus diseases in Africa.

International networking in research is not new. Scientists have long cultivated informal networks of contacts for exchange of ideas and information. Sometimes these informal groups develop a more formal organization with time, to facilitate technological exchange. This groundnut virus working group could best be described as a semiformal network. International networking in agricultural research has been comprehensively reviewed by Plucknett and Smith (1984). Some of the main points of this paper are summarized below.

International cooperation in agricultural research is rapidly increasing, as funding becomes scarce and the benefits of collaboration are realized. Most partnerships are forged on a regional or global scale to cut costs, avoid duplication, optimize resources, and accelerate transfer of technology.

Networks can assume various forms:

- Exchange of information among participants and a central hub.
- A design that allows participants to interact with one another and with the central hub.
- A complex system that involves subnetworks set up to focus on specific problems. Some networks pass through all three stages as they grow and evolve. The basic

1. Director, Crop Protection Division, ICRISAT Asia Center, Patancheru 502 324, Andhra Pradesh, India.

components of all are a two-way flow of information and materials, and a commitment by participants.

Successful networks are based on seven main principles:

- Clear definition of the problem/s and a realistic research agenda.
- The problem/s must be important and widely shared.
- Strong self-interest underpins productive networks: effective networking cannot be mandated.
- **Participants must be willing to commit such resources as personnel and facilities.** Goodwill and the desire to cooperate are important, but the acid test for a network is whether collaborators are prepared to make resources available.
- **Outside funding should be available to facilitate the birth of networks and keep them functioning.** Representatives from the national agricultural research systems (NARS) in developing countries often cannot attend meetings due to lack of funding, even though their desire to attend is great. Donors thus play an important role in starting and sustaining many international networks.
- **Participants must have sufficient training and expertise to make a contribution.** It is rare that all participants in a network will initially have the same level of expertise or sufficient expertise to effectively perform their part of the research effort.
- **Guidance by strong and efficient leaders who have the confidence** of **the participants.** Dissatisfaction is less likely when participants elect the network coordinator for a specified period. At the same time, when the research capabilities of participating institutes vary markedly, collaboration may best be served by initially leaving the leadership post with the strongest participant.

Problems faced by networks include:

- Difficulties in matching needs with capabilities.
- Quarantine bottlenecks in exchange of plant material, and more recently, such molecular outputs as constructs/transgenic plants.
- Problems faced by NARS in getting the work done (manpower shortages, lack of equipment, energy shortage, transport problems, harsh working conditions).
- Communication -both due to functional problems and personal communication.
- Balance between firm leadership and democratic leadership.

However, networking has several advantages. Although it is difficult to measure the value of networking in monetary terms, judging from the recent proliferation of collaborative programs/projects, the advantages far outweigh the problems. These include saving of time and funds; reduced duplication of effort; use of existing facilities and personnel; and better use and exchange of information.

During the past 13 years, we have seen the birth of three Working Groups, one on Asia-Pacific Groundnut Viruses, another on African Groundnut Viruses, and the third on Transformation and Regeneration of Groundnut and Utilization of Viral Genes. These three Working Groups also share common members, and all met together in August 1993 in Dundee, Scotland, UK. I feel there is a justification to develop a formal network within which the groups and other virus-specific subgroups could form subnetworks.

The Working Groups have adopted the principles of problem definition, priorities, self-interest, and commitment; a series of training workshops has been closely linked to the meetings; ICRISAT has assumed the role of 'manager' rather than leader, an important factor to achieve the objectives.

The crucial need is to identify donor/s who will sustain the working groups. ICRISAT is most grateful for the funding from Peanut Collaborative Research Support Program, Noble Foundation, the Belgian Administration for Development Cooperation, Overseas Development Administration, Deutsche Gesellschaft fur Technische Zusammenarbeit, and others who have supported the participation of many virologists at these meetings, but we are having increasing difficulties in meeting the complement of the funding. This year, until 2 weeks before the invitations were sent, we were still unsure whether the funds required would be approved. We need to be proactive in identifying a more durable source of funding. The progress made to date should convince the right donor of the value of sustaining the working group activities on groundnut viruses.

Reference

Plucknett, D.L., and Smith, N.J.H. 1984. Networking in international agricultural research. Science 225:989-993.

Introduction and Objectives

D V R Reddy[1] and P Subrahmanyam[2]

The first meeting of the International Working Group on Groundnut Virus Diseases to coordinate research on virus disease problems in groundnut was held in 1983 at the University of Georgia, Griffin, Georgia, USA. The main emphasis was on groundnut rosette disease. The group met subsequently in 1985, 1987, 1990, and 1993. The group was formed to coordinate research on groundnut viruses in Africa, leading to:

- Characterization of economically important groundnut viruses occurring in Africa, with emphasis on rosette and clump viruses.
- Development of both narrowly and broadly specific diagnostic tools for virus identification.
- Training and supply of diagnostic aids for scientists in national agricultural research systems (NARS) in Africa.

This group activity resulted in:

- Identification of causal viruses of groundnut rosette disease and development of versatile diagnostic tools.
- Location of resistance sources to rosette disease in germplasm accessions other than those collected in Africa.
- Information on precise distribution of peanut clump virus in Africa, and biodiversity among its isolates.
- Occurrence of peanut stripe virus and the need to follow rigorous quarantine procedures to prevent its entry.
- Utilization of virology laboratories in advanced countries in western Europe to improve skills of the African NARS in virus identification and detection.

The major objectives of this meeting are to:

- Discuss progress made in the diagnosis and management of economically important groundnut viruses, especially groundnut rosette and peanut clump viruses.
- Discuss how diagnostic tools can be used for virus detection and to study epidemiology, especially of groundnut rosette and peanut clump virus diseases.
- Seek the views of the NARS scientists on current problems of virus identification and potential areas for future collaboration, keeping in mind recent developments in molecular biology.
- Formulate recommendations and work plans to form a basis for continued international cooperative research on rosette, clump, and seedborne viruses of groundnut.

1. Crop Protection Division, ICRISAT Asia Center, Patancheru 502 324, Andhra Pradesh, India.
2. Crop Protection Division, SADC/ICRISAT Groundnut Project, Chitedze Agricultural Research Station, P O Box 1096, Lilongwe, Malawi.

Current Research on the Causal Agents of Groundnut Rosette Disease and their Diagnosis

A F Murant, D J Robinson, and M E Taliansky

Work on groundnut rosette disease at the Scottish Crop Research Institute (SCRI), funded by the UK Overseas Development Administration/has three primary objectives. The first is to unravel the aetiology of the disease, and the second, to develop diagnostic methods for the causal agents. The third objective, described in this paper, is to identify virus genes that might confer virus resistance if introduced into the groundnut genome.

Groundnut rosette disease in its various forms (green, chlorotic, mosaic, and mottle), is of major importance in sub-Saharan Africa, but is not known to occur in other parts of the world.

Groundnut rosette disease is caused by a complex of two viruses and a satellite RNA. One component, groundnut rosette virus (GRV), is a mechanically transmissible virus, which depends for transmission by *Aphis craccivora* Koch on the presence of the second component, groundnut rosette assistor virus (GRAV). This is aphid transmitted, and by itself, causes no symptoms in groundnut. The satellite RNA depends on GRV for its multiplication, is mainly responsible for rosette disease symptoms, and is also needed for aphid transmission. Groundnut rosette virus is a member of the new plant virus genus *Umbravirus.* Groundnut rosette assistor virus is *a Luteovirus,* with isometric particles, 25 nm in diameter. It is transmitted by aphids in a persistent, circulative, nonpropagative manner.

Diseased plants also contain double-stranded (ds) RNA that produces three major electrophoretic bands, estimated at >4000 bp (dsRNA 1), 1300 bp (dsRNA 2), and 900bp (dsRNA3). DsRNA 1 is probably the replicative form (RF) of the GRV genomic RNA, and dsRNA 2 is thought to correspond to a subgenomic RNA; dsRNA 3 is the RF of a satellite RNA, so called because it can be eliminated from GRV cultures, and is not necessary for the multiplication of GRV in plants. This satellite is a key molecule in the aetiology of rosette disease because it is primarily responsible for the symptoms of rosette disease; different forms of rosette being caused by different variants of the

1. Virology Department, Scottish Crop Research Institute, Invergowrie, Dundee DD2 5 DA, Scotland, UK.

Murant, A.F., Robinson, D.J., and Taliansky, M.E. 1997. Current research on the causal agents of groundnut rosette disease and their diagnosis. Pages 5-6 *in* Groundnut virus diseases in Africa: summary and recommendations of the Sixth Meeting of the International Working Group, 18-19 Mar 1996, Agricultural Research Council, Plant Protection Research Institute, Pretoria, South Africa (Reddy, D.V.R., Delfosse, P., Lenne, J.M., and Subrahmanyam, P., eds.). Patancheru 502 324, Andhra Pradesh, India: International Crops Research Institute for the Semi-Arid Tropics; and 1000 Brussels, Belgium: Belgian Administration for Development Cooperation.

satellite. Another reason is that it plays an essential, though unexplained, role in mediating the GRAV-dependent aphid transmission of GRV. The latter property presumably explains why GRV has never been found in nature without the satellite RNA. All satellite-free cultures have only been produced experimentally.

Groundnut Rosette Assistor Virus

There are extensive serological relationships among luteoviruses, and many can be detected by antisera raised against others. Thus, GRAV can be detected by an antiserum to potato leafroll luteovirus (PLRV). However, GRAV reacts with only 3 of 10 monoclonal antibodies (MAbs) to PLRV raised at SCRI. A polyclonal antiserum raised to a Nigerian isolate of GRAV reacts with GRAV isolates from other parts of Africa (Malawi, Niger, South Africa, Uganda), and also with other luteoviruses, especially beet western yellows virus and PLRV. Thus, for unequivocal identification of GRAV, a panel of luteovirus MAbs must be used and additionally, MAbs should be developed for GRAV.

Groundnut Rosette Virus and its Satellite RNA

No serological test is available because GRV does not produce conventional virus particles. Diagnostic tests for GRV and its satellite RNA must be directed against the RNA molecules themselves.

The nucleotide sequences of 10 variants of the satellite RNA, associated with green, chlorotic, and mild, to symptomless forms of rosette from Nigeria and Malawi, were at least 87% identical, though differences associated with symptom type and geographical origin were found. A DNA probe complementary to a Malawian variant, has been used in dot blot tests (both radioactive and nonradioactive) to detect all the other variants. This probe effectively serves as a probe for GRV itself, because all naturally occurring cultures of GRV seem to contain the satellite RNA. The only GRV cultures that have not reacted with it have been those that have experimentally been deprived of the satellite.

The genomic RNA of GRV has also been sequenced, and the DNA complementary to this molecule has been used in dot blot tests to detect Malawian and Nigerian forms of GRV—though with less sensitivity than the satellite RNA probe. This is because the genomic RNA molecules of GRV are much fewer than the satellite RNA molecules.

Neither the GRV satellite probe nor the GRV genomic probe reacts with other umbraviruses tested to date, many of which contain satellite-like RNA molecules. These include one associated with groundnut streak necrosis and sunflower yellow blotch diseases, and two others associated with tobacco bushy top and tobacco rosette diseases. All three of these viruses occur in Africa. Although more such tests are required, these GRV probes seem to be highly specific at present.

Thus, there is now a range of diagnostic probes to detect and identify the three causal agents of groundnut rosette disease. These are currently being used in epidemiological studies, to identify alternate hosts of GRV and GRAY and should find application in facilitating the breeding of groundnuts for resistance to rosette.

Recent Advances in Understanding the Causal Agents of Groundnut Rosette Disease

D J Robinson, M E Taliansky, and A F Murant[1]

Recent work in Dundee, Scotland, UK, has aimed to explore ways in which sequences from the causal agents of groundnut rosette disease could be used to provide novel forms of pathogen-derived resistance. This work revealed new information about the molecular biology of the agents, in addition to providing very effective diagnostic tools.

The genome of groundnut rosette virus (GRV) consists of 4019 nucleotides (nts), and contains four open-reading frames (ORFs). The two ORFs at the 5' end of the RNA are expressed by a frameshift to give a single protein, that is probably an RNA-dependent RNA polymerase. The other two ORFs overlap each other in different reading frames. One of the ORFs apparently codes for a protein involved in cell-to-cell-movement of the virus, but the function of the other is unknown. Groundnut rosette virus does not possess a gene for a capsid protein. The organization of the GRV genome is very similar to that of another umbravirus, carrot mottle mimic virus, and to that of RNA 2 of pea enation mosaic virus (PEMV).

The satellite RNA of GRV consists of 895-903 nts, and contains several short ORFs. Clones of cDNA of several satellite variants were constructed, from which biologically active transcripts can be obtained. Deletion of nts 282-797, which destroyed all the ORFs, did not prevent the satellite from replicating in *Nicotiana benthamiana,* although it did so at a lower level than the wild-type satellite. Moreover, mutants in which the AUG initiation codons of each of the ORFs had been altered, replicated to normal levels. Thus, no satellite-coded proteins are required for satellite replication. However, deletion of nts 47-281 prevented satellite replication.

Satellite YB3 induces brilliant yellowing symptoms in *N. benthamiana,* and the production of these symptoms was not affected by mutations in the initiation codons of any of the ORFs. Deletion of either nts 282-474 or nts 629-797 abolished the symptoms, but they were produced in plants inoculated with a mixture of the two mutants. Full-length recombinant satellite RNA molecules could not be detected, and it seems therefore, that symptom production in *N. benthamiana* involves two domains

1. Virology Department, Scottish Crop Research Institute, Invergowrie, Dundee DD2 5DA, Scotland, UK.

Robinson, D.J., Taliansky, M.E., and Murant, A.F. 1997. Recent advances in understanding the causal agents of groundnut rosette disease. Pages 7-8 *in* Groundnut virus diseases in Africa: summary and recommendations of the Sixth Meeting of the International Working Group, 18-19 Mar 1996, Agricultural Research Council, Plant Protection Research Institute, Pretoria, South Africa (Reddy, D.V.R., Delfosse, P., Lenne, J.M., and Subrahmanyam, P., eds.). Patancheru 502 324, Andhra Pradesh, India: International Crops Research Institute for the Semi-Arid Tropics; and 1000 Brussels, Belgium: Belgian Administration for Development Cooperation.

of the satellite, which are likely to complement each other. A chimeric satellite, consisting of nts 282-474 from YB3 and nts 1-281 and 475-903 from the symptomless variant MC3, produced symptoms typical of YB3, but the converse construct did not. Thus, the key sequence for the production of yellow blotch symptoms ranges from nt 282 to nt 474.

Satellite NM3 produces few, if any, symptoms in groundnut, and unlike other satellite variants, decreases the replication of GRV genomic RNA in infected plants. Experiments with chimeric satellites comprising sequences from NM3 and YB3 showed that the ability to down-regulate GRV RNA synthesis is determined by nts 47-281. Infection of *N. benthamiana* with a GRV isolate containing either satellite MC3 or satellite NM3 prevents the appearance of yellow blotch symptoms when the plants are subsequently challenged by inoculation with GRV containing satellite YB3. However, two distinct mechanisms are involved. When the protecting satellite is MC3, GRV and satellite RNAs accumulate at normal levels, although no symptoms are produced. In contrast, when protection is by satellite NM3, accumulation of GRV and satellite RNAs is diminished.

Recent experiments (in collaboration with G de Zoeten and S Demler of Michigan State University, USA) have shown that PEMV can support replication of the GRV satellite. Moreover, a satellite naturally associated with some isolates of PEMV replicates when inoculated together with GRV, both in *N. benthamiana* and in groundnut. The combination does not produce symptoms in groundnut, which is not a host for PEMV

These findings suggest several possible routes to the production of novel resistance to rosette disease. Modified genes from the GRV genome, such as the RNA polymerase or the movement protein, could provide resistance to infection by GRV itself. Alternatively, it might be possible to use domains derived from the satellites to interfere with replication of GRV, and/or to ameliorate symptoms. It might even be possible to use sequences derived from the PEMV satellite.

Epidemiology of Groundnut Rosette Virus Disease: the Need for Additional Studies

J M Thresh and F M Kimmins[1]

Groundnut rosette virus (GRV) disease was first reported in 1907 and it has since been studied at different times and in several countries of sub-Saharan Africa including Malawi, Nigeria, South Africa, Tanzania, and Uganda. Considerable information has been obtained on the behavior and main features of the disease, on the biology of the aphid vector (*Aphis craccivora* Koch), and on the impact of cultural practices, notably spacing and date of sowing. However, the epidemiology of rosette is still not completely understood, and there is inadequate information on which to base forecasting and disease-control measures, or to explain the occasional sporadic epidemics that cause serious crop losses and sometimes, total crop failure.

There is a need for additional epidemiological information to facilitate control, whether or not virus-resistant varieties of groundnut (*Arachis hypogaea* L.) are developed and become generally available for use by farmers on a large scale. Moreover, the time is opportune for new initiatives, following advances made in recent years at the Scottish Crop Research Institute in UK, on modeling the spread of several tropical virus diseases including rice tungro, African cassava mosaic, and maize streak. This paper considers briefly, some of the main unresolved issues concerning the epidemiology of rosette, and outlines possible approaches which could be adopted in further studies.

Alternative and Perennating Hosts

Groundnut was introduced to Africa in recent centuries, yet the viruses causing rosette appear to be indigenous to Africa, as they have not been recorded elsewhere. This suggests that rosette viruses spread to groundnut from indigenous hosts, although such plants have not yet been identified. There is a need for additional information on this topic, and to determine whether alternative hosts are of continuing epidemiological importance as sources of infection to groundnut. This is a crucial issue to resolve because the seasonal cycle of infection has not been determined in any of the

1. Natural Resources Institute, University of Greenwich, Chatham Maritime, Kent ME4 4TB, UK.

Thresh J.M., and Kimmins, F.M. 1997. Epidemiology of groundnut rosette virus disease: the need for additional studies. Pages 9-12 in Groundnut virus diseases in Africa: summary and recommendations of the Sixth Meeting of the International Working Group, 18-19 Mar 1996, Agricultural Research Council, Plant Protection Research Institute, Pretoria, South Africa (Reddy, D.V.R., Delfosse, P., Lenne, J.M., and Subrahmanyam, P., eds.). Patancheru 502 324, Andhra Pradesh, India: International Crops Research Institute for the Semi-Arid Tropics; and 1000 Brussels, Belgium: Belgian Administration for Development Cooperation.

agroecosysterns in which groundnut is grown. Consequently, it remains unclear whether the main spread of rosette viruses is between groundnut crops or from other sources. Groundnut volunteers are known to be important in at least some areas where there is sufficient moisture for them to persist between growing seasons. Wild/weed species including trees or shrubs could also be significant as additional or perennating hosts, especially those that are able to survive during the sometimes-prolonged arid periods between seasons suitable for groundnut production.

Primary and Secondary Spread

As with Other vectorborne viruses, it is important to distinguish between primary spread into, and secondary spread within crops. In ecological terms, they represent the colonization of habitats and their subsequent exploitation. They present different problems and opportunities for those seeking to develop effective control measures.

With rosette, there is considerable uncertainty as to the relative importance of primary and secondary spread, and their contribution to the development of severe epidemics. The situation can only be resolved by detailed observations on the pattern and sequence of spread at a representative range of sites in different agroecological conditions, and over a period of several years. Experience with other arthropodborne viruses suggests that it is important to monitor the numbers and infectivity of the vectors reaching crops during the critical early stage of growth. Plants are then most vulnerable to infection, and most severely damaged if infection occurs. Moreover, early infection provides a good opportunity for secondary spread to occur within crops, and for repeated cycles of infection, before crops begin to mature and vector populations decline so that little further spread occurs.

Short- and Long-Range Dispersal

Experience with other arthropodborne luteoviruses suggests that the main spread of GRV into and between crops is over limited distances and not from afar. However, this has not been substantiated in the continuing absence of definitive information on the type, prevalence, and potency of sources of infection.

In areas where rosette can be a serious problem, because of the uncertainty and lack of obvious sources of infection, it has been suggested that the initial inoculum is introduced by migrant vectors from remote areas where growing conditions for groundnut crops and/or weed and wild hosts are relatively favorable. This view is consistent with the known behavior of *A. craccivora* in southeast Australia. There the aphid is regarded as a 'super migrant' because of its low tolerance of crowding, and ability to exploit a sequence of transient seasonal hosts as they become available in different parts of the region.

There is no direct evidence that *A. craccivora* behaves in this way in Africa, and such evidence would be difficult and expensive to obtain, as apparently from experience

with armyworm, locusts, and other migrant pests whose dispersal is influenced by prevailing winds and frontal systems. It remains to be determined whether such an effort is justified. A more immediate priority is to obtain additional information on the main sources of infection, and the extent to which the incidence and prevalence of rosette can be explained by conditions in the immediate vicinity of crops at risk.

Forecasting

In recent years, considerable attention has been given to the possibility of forecasting the incidence of crop pests and diseases, and the severity of the damage they cause. An ability to forecast with reasonable precision, and ideally before or soon after crops are sown, can lead to improved control measures and management practices.

Early studies of what would now be regarded as forecasting were made on rosette in South Africa during the early 1920s, when severe epidemics were associated with unusually high rainfall during the normally dry winter months before sowing began. Rainfall during this period facilitates the survival of Volunteer' (self-sown) groundnut seedlings that may support vector populations and become initial sources of virus inoculum.

Such studies have not been pursued in South Africa or elsewhere, although considerable progress has been made in forecasting the incidence of other vectorborne viruses, including several that persist in aphid vectors—potato leaf roll luteovirus, barley yellow dwarf luteovirus, and beet yellowing luteovirus. Some of the key parameters to emerge from such studies are the extent to which vectors and sources of infection survive during the off-season, and the number, timing, and infectivity of immigrants that enter the crop during the critical early stages of growth. Similar approaches are likely to be appropriate with groundnut rosette and merit high priority.

Resistance

The scope to use virus-resistant varieties has been apparent since the early work in what was then French West Africa. These studies were later continued in Nigeria, and more recently in Malawi, but the lack of virus-resistant, short-duration varieties has been a serious obstacle to progress. This limitation is now being overcome, and there are prospects of introducing virus-resistant varieties of different growth characteristics, and incorporating resistance to *A. craccivora.* Once such varieties are available, there will be a need to determine how they should be deployed to maximize their effectiveness, and to limit the chances of resistance 'breakdown'.

Modeling

Modeling is increasingly being adopted with various crop pests and diseases as a means of gaining a better understanding of the underlying biological processes that influence their prevalence, and to evaluate or simulate the effects of control strategies. Several

insectborne viruses have been studied in this way, including tropical examples such as African cassava mosaic geminiviruses, cocoa swollen shoot badnavirus, banana bunchy top virus, and rice tungro viruses.

Some models that have been developed are versatile and widely applicable, as they consider both primary spread into, and secondary spread within plant or crop stands, and the role of vectors and host-plant resistance. Such models could be adapted for use with groundnut rosette, and could soon lead to important advances.

Intercropping

In many parts of sub-Saharan Africa, groundnut is grown in association with one or more other crops. Some of the commonest of these are cassava, sweet potato, and cereals, including maize *(Zea mays),* sorghum [*Sorghum bicolor* (L.) Moench], and finger millet [*Eteusine coracana (L.)* Gaertn.]. Intercrops have marked effects on colonization by insect vectors, and on their movement and behavior within crops. There have been a few studies of these possibilities, but the results of preliminary trials in Malawi suggest that the spread of rosette can be decreased by an appropriate choice and spacing of the intercrops. However, studies need to be done on the effectiveness and feasibility of this approach before definitive recommendations can be made to farmers.

Outlook

The 1995 epidemic of groundnut rosette disease in Malawi, and the continuing losses experienced in other parts of sub-Saharan Africa, have again drawn attention to the problems posed by the disease, and the inadequacy of current approaches to control. This provided a stimulus to resistance breeding programs in Malawi and elsewhere in Africa, and led to renewed interest in field studies on *A. craccivora* and on the epidemiology and control of rosette. There are good prospects of substantial progress being made by developing a coordinated research program, and by making full use of the latest techniques, approaches, and experience gained with other insectborne viruses.

Screening Global Germplasm for Resistance to Groundnut Rosette Disease

P Subrahmanyam[1], L J Reddy[2], and A K Singh[3]

Rosette is the most destructive virus disease of groundnut in Africa. The disease is apparently restricted to the African continent, south of the Sahara, and to its offshore islands. Although disease epidemics are sporadic, yield losses approach 100% when an epidemic occurs.

Groundnut rosette is caused by a complex of three agents, groundnut rosette virus and its satellite RNA, and groundnut rosette assistor virus. The disease is transmitted by *Aphis craccivora* Koch.

During the 1994/95 growing season, groundnut rosette occurred in epidemic proportions in central Malawi, Lilongwe-Kasungu-Mchinje triangle, and in the Eastern Province of Zambia, which are the major groundnut-producing areas of these countries. Disease surveys conducted in over 140 farmers' fields in central Malawi revealed an average disease incidence of 50.7%. Rosette infected 100% of the plants in 13% of the surveyed fields. Farmers abandoned several of these fields, since nothing could be harvested from them. Rosette infection, which occurred before the onset of flowering/pegging, led to total destruction of the crop. This led to an acute shortage of groundnut seed for the 1995/96 growing season, as farmers experienced serious losses in many parts of the country. The 1995 rosette epidemic may have long-term implications on groundnut production in Malawi and Zambia, and a substantial reduction in groundnut acreage in 1995/96 is expected.

Although chemical control of the vector and cultural practices such as timely sowing and optimal plant densities are known to reduce the risk of rosette incidence, these practices are, for several reasons, seldom adopted. Therefore, host-plant resistance is the most effective control strategy. The SADC/ICRISAT Groundnut Project based at Chitedze Agricultural Research Station, Malawi, places major emphasis on the development of rosette-resistant varieties, particularly short-duration varieties that have not been available to date.

1. Crop Protection Division, SADC/ICRISAT Groundnut Project, Chitedze Agricultural Research Station, P O Box 1096, Lilongwe, Malawi.
2. Genetic Enhancement Division, ICRISAT Asia Center, Patancheru 502 324, Andhra Pradesh, India.
3. Genetic Resources Division, ICRISAT Asia Center, Patancheru 502 324, Andhra Pradesh, India.

Subrahmanyam, P., Reddy, L.J., and Singh, A.K. 1997. Screening global germplasm for resistance to groundnut rosette disease. Pages 13-14 *in* Groundnut virus diseases in Africa: summary and recommendations of the Sixth Meeting of the International Working Group, 18-19 Mar 1996, Agricultural Research Council, Plant Protection Research Institute, Pretoria, South Africa (Reddy, D.V.R., Delfosse, P., Lenne, J.M., and Subrahmanyam, P., eds). Patancheru 502 324, Andhra Pradesh, India: International Crops Research Institute for the Semi-Arid Tropics; and 1000 Brussels, Belgium: Belgian Administration for Development Cooperation.

Pioneering research on the development of groundnut varieties with resistance to rosette was carried out in West Africa. Sources of resistance to rosette were first discovered in Senegal in 1952. These sources formed the basis for rosette resistance breeding programs throughout Africa, and have contributed to the development of several high-yielding groundnut varieties. However, most of the rosette-resistant varieties released so far are medium and late maturing, and are not suitable for many production systems in Africa where the rainy season is short. In addition, the available sources of resistance were derived from a narrow genetic base.

In 1990, the SADC/ICRISAT Groundnut Project, in collaboration with the Genetic Resources Division, ICRISAT Asia Center (IAC), launched a program on screening global germplasm for resistance to groundnut rosette. During the 1990/91 to 1993/94 growing seasons, over 4600 germplasm lines from South America and Africa, and 160 interspecific hybrid derivatives were evaluated for rosette resistance. Over 100 rosette-resistant germplasm accessions, including 12 short-duration Spanish types, were identified. Resistance to groundnut rosette was also identified for the first time in genotypes other than those derived from West Africa.

During the 1994/95 growing season, over 2000 germplasm lines from Africa were screened for resistance to rosette using the infector-row technique. Each entry was grown in single-row field plots. Infector rows of a rosette-susceptible cultivar, Malimba, were included throughout the trial, one infector row flanking every two test rows. Potted spreader plants of Malimba, showing severe rosette symptoms and heavily infested with aphids, were raised in the greenhouse and transplanted to the infector rows (one plant per row), about 10 days after sowing. In addition, the viruliferous aphids reared in the greenhouse were also transferred to the infector rows to minimize any chances of escape.

Each entry was assessed for disease incidence at the pod-filling stage and at maturity. The disease incidence was 100% in the majority of genotypes. However, 13 germplasm lines from Asia showed low disease incidence (< 15%). These lines were further evaluated during the 1995/96 growing season, and their resistance confirmed. Of these 13 germplasm lines, ICGs 12988 and 12991 were short-duration Spanish types, and the rest were Virginia types.

During the 1995/96 growing season, an additional 1200 germplasm lines from Asia were tested for rosette resistance. Five germplasm lines, including one short-duration Spanish type, showed low disease incidence (< 15%), and have been selected for further testing during the 1996/97 growing season.

The short-duration rosette-resistant genotypes are currently being used to develop short-duration rosette-resistant cultivars suitable for various production systems in Africa.

Breeding for Rosette-Resistant Groundnut Varieties

L J Reddy[1] and P Subrahmanyam[2]

Groundnut rosette virus (GRV) is economically the most important among the groundnut viruses that occur on the African continent. Globally, rosette is estimated to cause annual yield losses worth US$ 156 million, and potential yield gains in alleviating this constraint through crop improvement are estimated at US$121 million. Although chemical control of the vector, *Aphis craccivora* Koch, and cultural practices such as early sowing and the use of optimum plant densities are known to control rosette, these are seldom practised by resource-poor farmers. So, the most practical way to alleviate this problem is through production of groundnut cultivars with rosette resistance.

Earlier, long-duration, rosette-resistant Virginia varieties, e.g., RMP 12, RMP 91, and RG 1, and Spanish varieties, e.g., KH 241D, were developed and released. However, their long maturity duration, their low yield potential, and/or poor agronomic traits (such as low shelling percentage as in RG 1) contributed to their low adoption. For most of the sub-Saharan Africa, characterized by short and erratic rainfall, short-duration resistant varieties are needed. Therefore, adoption of the long-duration, rosette-resistant varieties by farmers, has been rather poor.

One of the primary objectives of the SADC/ICRISAT Groundnut Project based in Chitedze, Malawi, has been to breed for high-yielding, short- and long- duration rosette-resistant cultivars, adapted to different agroecologies of the region. This paper presents progress made in developing such varieties, the issues involved, and future strategies for further development.

Resistance Sources

Apart from the 79 resistance sources which had their origin in the frontier regions between Burkina Faso and Cote d'Ivoire in western Africa, recently 28 additional

1. Genetic Enhancement Division, ICRISAT Asia Center, Patancheru 502 324, Andhra Pradesh, India.
2. Crop Protection Division, SADC/ICRISAT Groundnut Project, Chitedze Agricultural Research Station, P O Box 1096, Lilongwe, Malawi.

Reddy, L.J., and Subrahmanyam, P. 1997. Breeding for rosette-resistant groundnut varieties. Pages 15-19 *in* Groundnut virus diseases in Africa: summary and recommendations of the Sixth Meeting of the International Working Group, 18-19 Mar 1996, Agricultural Research Council, Plant Protection Research Institute, Pretoria, South Africa (Reddy, D.V.R., Delfosse, P., Lenne, J.M., and Subrahmanyam, P., eds.). Patancheru 502 324, Andhra Pradesh, India: International Crops Research Institute for the Semi-Arid Tropics; and 1000 Brussels, Belgium: Belgian Administration for Development Cooperation.

sources of resistance have been identified am the germplasm lines obtained from South America and Asia, and in interspecific derivatives. Of these, 95 are Virginia types and 12 are Spanish types (Subrahmanyam et al. 1994). These sources are expected to provide a sufficient genetic base for the development of broadbased resistant varieties suitable for different production systems in the region.

Utilization of Resistance Sources

Since the inception of the SADC/ICRISAT Groundnut Project in 1982, until 1993, 381 crosses have been made to incorporate rosette resistance. The Virginia resistance sources, RG 1, 48-36, RMP 40, RMP 91, and RMP 93, and the Spanish variety, KH 241D, have been extensively used in the crossing program. Out of these, 67 crosses were made with KH 241D. From these crosses, several resistant breeding lines were developed, that are in various stages of evaluation.

During the 1994/95 and 1995/96 cropping seasons, 69 more crosses were made, mostly using the newly identified short-duration resistance sources and advanced breeding lines.

We evaluated 33 resistant Virginia varieties in three station trials at Chitedze, Malawi, under high disease pressure during the 1994/95 cropping season. In addition, the regional and advanced trials were conducted under low disease pressure. In the regional trial, ICGV-SM 90704 (with 2.04 t ha^{-1} pod yield and 1.6% disease incidence) and ICGV-SM 91708 (1.40 t ha^{-1} pod yield and 6.1% disease) performed excellently, compared with the susceptible controls, CG 7 (0.03 t ha^{-1} and 94.9% disease) and Chalimbana (0.10 t ha^{-1} and 95.0% disease) under high disease pressure. These two varieties outyielded both CG 7 and Chalimbana under low disease pressure also. In the advanced yield trial, ICGV-SM 93718 outyielded the best control, CG 7, by 293% (1.06 t ha^{-1}) under high disease pressure, and by 89% higher yield (2.14 t ha^{-1}) under low disease pressure. Similarly, in the preliminary trials, two varieties, ICGV-SMs 94707 and 94703, produced 20% more pod yield (0.82 t ha^{-1}) than RG 1 under high disease pressure.

Thirty-three rosette-resistant, short-duration, Spanish breeding lines developed in the program were evaluated during the 1994/95 cropping season under high disease pressure. Eight lines, including ICGV-SMs 94584 (0.83 t ha^{-1}), 94581 (0.80 t ha^{-1}), 94587 (0.78 t ha^{-1}), and 94586 (0.77 t ha^{-1}) outyielded the controls, JL 24 (0.15 t ha^{-1}) and KH 241D (0.03 t ha^{-1}). All the eight promising varieties from this trial were multiplied at Masenjeri (Malawi), and promoted to regional trials. Most of these varieties mature in 100—110 days, and possess desirable pod and seed characters. During the 1995/96 cropping season, we identified nine short-duration resistant varieties from the crosses involving the short-duration resistance source, KH 24ID. All these varieties showed resistance in the disease nursery and yield data are awaited.

Issues Involved and Future Plans in Breeding for Rosette Resistance

Lack of Resistance to Groundnut Rosette Assistor Virus (GRAV)

Although GRAV does not produce any symptoms by itself, it plays an important role in rosette disease development (Murant et al. 1988 and Ansa et al. 1990). To date, the only known source of resistance to GRAV is the wild species, *Arachis chacoense,* reported to be immune to both GRV and GRAV (Murant et al. 1991). This suggests it is necessary to screen and evaluate segregating and fixed lines in the disease nursery, to capitalize on the possible tolerance to GRAV in these materials. Further, identification of resistance sources to GRAV will be useful to avoid further spread of the disease from infected groundnut crops.

Low Yield Potential of Resistant Varieties

Although most of the resistant varieties significantly outyielded the susceptible cultivars under high disease pressure, they were inferior to the susceptible cultivars under low or no disease pressure. So, to identify resistant varieties that give superior yield performances in all situations, yield evaluations of advanced breeding lines are being carried out under high, medium, and low/no disease pressures.

Low Recovery of Short-Duration Resistant Plants

A low recovery of sequentially branching (short duration) resistant plants from Virginia x Spanish crosses, observed by Dr C Harkness, has been confirmed by Hildebrand et al. 1994. This recovery was attributed to the duplicate recessive nature of inheritance of both resistance and sequential branching habit, and a possible linkage between these traits. To avoid this problem, limited backcrossing to the resistant Spanish donor, and triple crosses involving [Virginia x Spanish (high-yielding)] x resistant Spanish sources will be made.

Combining Resistance to the Virus and the Aphid Vector

Under high disease pressure, many of the stable resistant varieties such as RG 1 show some percentage of susceptible plants. So, to reinforce field resistance, attempts are in progress to combine vector and virus resistances. The aphid-resistant variety, EC 36892, is being used as a vector-resistant donor, and five Virginia and three Spanish resistant varieties as recipients.

Variations in Genetic Expression of Resistance to GRV (+ Satellite RNA)

Resistance to rosette is reported to be governed by two duplicate recessive genes (Berchoux 1960, Nigam and Bock 1990). This results in a ratio of 15 susceptible: 1 resistant plants in the F_2 generation. However, Olorunju et al. (1992) observed, in the F_2 populations of a cross between RMP 12 X M 1204.781 and its reciprocal, mostly a ratio of 3 resistant: 1 susceptible plants, both under field inoculation and mechanical grafting. Similarly, they observed more resistant plants among F_2 populations of certain cross combinations. More recently, a ratio of 225 susceptible: 31 resistant plants was observed (L J Reddy, unpublished) in the F_2 populations of crosses involving interspecific resistant derivatives, suggesting the possible involvement of two duplicate pairs of genes. These observations suggest the need to use a wide gene pool in a crossing program aimed at breeding for rosette resistance. This might result in identifying combinations, that could lead to a high recovery of the resistant plants and useful recombinants. Also, it is essential to establish the allelic relationships in crosses between the resistance genes available in different sources. A study has been initiated at Chitedze on allelic relationships between the resistant line from South America, ICG 11044, and six Virginia and Spanish lines from western Africa.

Adaptation and Stability Requirements in the Region

In most southern and eastern African countries, in addition to rosette resistance, a variety should have a combination of resistances to show wide adaptability and stability. Among the other constraints, foliar diseases (especially early leaf spot) are economically important. So, it is essential to breed varieties with combined resistance to rosette and foliar diseases. To meet this objective, 16 crosses to combine rosette and early leaf spot resistance, 10 crosses to combine rosette and late leaf spot resistance, and 5 crosses to combine rosette and rust resistance have been initiated at Chitedze.

References

Ansa, O.A., Kuhn, C.W., Misari, S.M., Demski, J.W., Casper, R., and Breyel, E. 1990. Single and mixed infections of groundnut (peanut) with groundnut rosette virus and groundnut rosette assistor virus. 1990 Proceedings of the American Peanut Research and Education Society 22:41. (Abstract)

Berchoux, D.C. 1960. La rosette de l'arachide en Haute-Volta: comportment des lignes resistantes. Oleagineux 15:229-233.

Hildebrand, G.L., Subrahmanyam, P., and Ndunguru, B.J. 1994. Pages 51-54 *in* African Crop Science Conference Proceedings, 14-18 Jun 1993, Kampala, Uganda. Vol. 1. Makerere University, Kampala, Uganda: African Crop Science Society.

Murant, A.F., Rajeswari, R., Robinson, D.J., and Raschke, J.H. 1988. A satellite RNA

of groundnut rosette virus that is largely responsible for the symptoms of groundnut rosette disease. Journal of General Virology 69:1479-1486.

Murant, A.F., Kumar, I.K., and Robinson, D.J. 1991. Current research on groundnut rosette at SCRI. Pages 7-8 *in* Proceedings of the Fourth Meeting of the Consultative Group on Collaborative Research on Groundnut Rosette Virus Disease, 18-20 Sep 1990, Montpellier, France. Patancheru 502 324, Andhra Pradesh, India: International Crops Research Institute for the Semi-Arid Tropics.

Nigam, S.N., and Bock, K.R, 1990. Inheritance of resistance to groundnut rosette virus in groundnut *(Arachis hypogaea* L.). Annals of Applied Biology 117:553-560.

Olorunju, P.E., Kuhn, C.W., and Demski, J.W. 1992. Inheritance of resistance in peanut to mixed infections of groundnut rosette virus (GRV) and groundnut rosette assistor virus and a single infection of GRV Plant Disease 76:95-100.

Subrahmanyam, P., Hildebrand, G.L., and Singh, A.K. 1994. Screening for resistance to groundnut rosette virus disease. Pages 32-33 *in* 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, Scotland, UK. Patancheru 502 324, Andhra Pradesh, India: International Crops Research Institute for the Semi-Arid Tropics.

Identification and On-Farm Evaluation of Rosette-Resistant Groundnut Genotypes in Malawi

A J Chiyembekeza[1], P Subrahmanyam[2], and G L Hildebrand[3]

Groundnut (*Arachis hypogaea* L.) is an important food legume in smallholder agriculture in Malawi, providing approximately 25% of the agricultural cash income. It contributes significantly to the dietary needs of the rural population, and is a major source of vegetable protein and fat. It is also a valuable component in crop rotation, and improves soil fertility. Groundnut is grown throughout the country almost exclusively by smallholder farmers. More than 63% of the crop is produced in the Central Region covered by the Lilongwe and Kasungu Agricultural Development Divisions (ADDs) at an average altitude of 1200 m.

Yields are generally low, averaging 700 kg ha^{-1}, in marked contrast to yields of over 4000 kg ha^{-1} obtained on research stations and in developed countries. In recent years, however, groundnut production and yield in Malawi have declined further. Among the factors that have contributed to this decline is groundnut rosette virus disease, present in all the major groundnut-producing areas of the country, and especially serious in the Phalombe plains of the Blantyre Agricultural Development Division. Both chlorotic and green rosette are present; chlorotic rosette is the most widespread form in Malawi, but green rosette was found to be serious in the Karonga Agricultural Development Division in surveys conducted in 1994 (Subrahmanyam and Chiyembekeza 1994).

Although adequate information is available on the beneficial effects of early sowing and optimal plant population on rosette disease incidence, unfortunately these cultural practices have not been implemented in Malawi due to certain difficulties. Farmers still sow groundnut late, i.e., after sowing maize, their staple food, and tobacco, the main cash crop. They sow the crops at low plant densities due to limited supply of seed. The only feasible solution is the use of genetic resistance to provide rosette-resistant groundnut varieties with desirable pod and seed quality characteristics.

Pioneering research on the development of groundnut cultivars with resistance to rosette was carried out in western Africa. Sources of resistance to rosette were first

1. Chitedze Agricultural Research Station, P O Box 158, Lilongwe, Malawi.
2. Crop Protection Division, SADC/ICRISAT Groundnut Project, Chitedze Agricultural Research Station, P O Box 1096, Lilongwe, Malawi.
3. Seed Co-op, P O Box CH 142, Chisipite, Harare, Zimbabwe.

Chiyembekeza, A.J., Subrahmanyam, P., and Hildebrand, G.L 1997. Identification and on-farm evaluation of rosette-resistant groundnut genotypes in Malawi. Pages 20-21 *in* Groundnut virus diseases in Africa: summary and recommendations of the Sixth Meeting of the International Working Group, 18-19 Mar 1996, Agricultural Research Council, Plant Protection Research Institute, Pretoria, South Africa (Reddy, D.V.R., Delfosse, P., Lenne, J.M., and Subrahmanyam, P., eds.). Patancheru 502 324, Andhra Pradesh, India: International Crops Research Institute for the Semi-Arid Tropics; and 1000 Brussels, Belgium: Belgian Administration for Development Cooperation.

discovered in 1952, when an epidemic destroyed a large collection of groundnut in Senegal (Sauger and Catherinet 1954). However, a few germplasm lines originating from the frontier region between Burkina Faso and Cote d.'Ivoire were able to withstand this epidemic. These sources formed the basis for rosette resistance breeding programs throughout Africa, including Malawi. The rosette-resistant cultivar RG 1 (Makulu Red X 48-14) was released for cultivation in southern Malawi, but was not readily accepted by the farmers due to its long duration, small seeds, and tough pod coat that makes it difficult to shell. Resistance was effective against both chlorotic and green rosette diseases, and is governed by two independent recessive genes (Nigam and Bock 1990). The SADC/ICRISAT Groundnut Project at Chitedze has developed several rosette-resistant groundnut genotypes with high yield potential. From preliminary evaluations carried out at Chitedze, five genotypes, ICGV-SMs 90704, 90706, 90707, 90708, and 90713 gave significantly higher yields ($P < 0.05$) under low, medium, and high disease pressures than the susceptible control variety CG 7, but gave similar yields to those of RG 1, the resistant control variety. At other locations in the southern, central, and northern regions of Malawi, these genotypes outyielded the control varieties when sown early or late. The selection of these genotypes was based on yield and such other quality aspects as pod size and shape, shell thickness, seed size, color, and shape. The objectives were to:

- Assess the yield potential of these five genotypes under farmer management.
- Ascertain adaptability of these genotypes in various agroclimatic conditions of the country.
- Identify high-yielding and well-adapted rosette-resistant groundnut genotypes to recommend for production to farmers in Malawi.

ICGV-SM 90704 (RG 1 x Mani Pintar) outyielded both RG 1 and CG 7 over the 2-year period of evaluation, i.e., 1993/94 and 1994/95, across 24 locations throughout the country. During the 1993/94 cropping season, ICGV-SM 90704 had a 5% seed yield advantage over CG 7, and a 10% seed yield advantage over RG 1. During the 1994/95 season, ICGV-SM 90704 had a 40% seed yield advantage over CG 7, and a 66% seed yield advantage over RG 1. Currently, ICGV-SM 90704 is being tested at 30 locations of the various Extension Planning Areas. This cultivar is expected to be released for production in 1996.

References

Nigam, S.N., and Bock, K.R. 1990. Inheritance of resistance to groundnut rosette virus in groundnut *(Arachis hypogaea).* Annals of Applied Biology 117:553-560.

Sauger, L., and Catherinet, M. 1954. La rosette chlorotique de l'arachide et les lignees selectionnees. L'Agronomie Tropicale 9:28-36.

Subrahmanyam, P., and Chiyembekeza, A.J. 1995. Survey of groundnut diseases in northern Malawi. International *Arachis* Newsletter 15:22-24.

Peanut Clump Disease: an Overview

M A Mayo[1] and R A Naidu[2]

Clump disease occurs in groundnut crops in several countries in western Africa, and in parts of the Indian subcontinent. The disease is induced by virus infection, which follows attack on the groundnut plant roots by viruliferous zoospores of the vector fungus, *Polymyxa* sp. Losses can be great, and the persistence of inoculum in the soil can lead to groundnut being abandoned as a crop. The virus which causes the disease in Africa (peanut clump virus, PCV) has been distinguished from that which causes the disease in India (Indian peanut clump virus, IPCV). Both viruses are serologically diverse, and have been reported to fall into distinct serotypes. Results of the molecular characterization of PCV and IPCV have shown that their genomes are similarly arranged; RNA 1 encodes three proteins, including the replicase, and RNA 2 encodes five proteins, including the coat protein. The extent of the sequence identity between corresponding proteins of PCV and IPCV is between 39% and 95%.

By using nucleotide sequences common to RNA from IPCV (H serotype) and PCV, a cDNA probe has been identified for use in hybridization assays, and pairs of oligonucleotide primers have been produced for use in reverse transcription/PCR assays. This probe could detect both PCV and various isolates belonging to IPCV (serotypes T and L) in groundnut.

No usable genes for resistance to IPCV have been found in groundnut germplasm. As an alternative approach, cDNA, encoding the coat protein, or part of the replicase of IPCV-H, was cloned into transformation vectors, for use once techniques for groundnut transformation become available. Some lines of *Nicotiana benthamiana* transformed with the coat protein gene showed virus resistance, suggesting that this approach could be useful.

Epidemiological work suggests that both PCV and IPCV are widespread, and infect a wide range of host species. Both viruses are also markedly variable. We now have molecular tools to detect these and related viruses, and the methodology to measure variation among isolates at the molecular level. The combination of these methods with a wide-ranging survey of biological variation should yield results of both virological and epidemiological interest.

1. Virology Department, Scottish Crop Research Institute, Invergowrie, Dundee DD2 5DA, Scotland, UK.
2. Crop Protection Division, ICRISAT Asia Center, Patancheru 502 324, Andhra Pradesh, India.

Mayo, M A , and Naidu, R.A. 1997. Peanut clump disease: an overview. Pages 22-23 *in* Groundnut virus diseases in Africa: summary and recommendations of the Sixth Meeting of the International Working Group, 18-19 Mar 1996, Agricultural Research Council, Plant Protection Research Institute, Pretoria, South Africa (Reddy, D.V.R., Delfosse, P., Lenne, J.M., and Subrahmanyam, P., eds.). Patancheru 502 324, Andhra Pradesh, India: International Crops Research Institute for the Semi-Arid Tropics; and 1000 Brussels, Belgium: Belgian Administration for Development Cooperation.

Acknowledgment

This work was funded in large part by the Overseas Development Administration Plant Sciences and Crop Protection Programmes. We acknowledge support from the Scottish Office of Agriculture, Environment and Fisheries Department.

Peanut Clump Virus in Western Africa

M Dollet[1] and M Ndiaye[2]

Studies carried out at the Laboratoire de phytovirologie des regions chaudes on groundnut viruses in western Africa for the last two decades demonstrated the extreme variability in peanut clump virus (PCV), and made available information on its distribution in western Africa (Manohar et al. 1995). Research over the past 3 years has concentrated on two aspects: a comparative study of new PCV isolates, collected from irrigated areas along the River Senegal (Richard Toll Region), and a study on seed transmission.

A serological study was carried out on 26 isolates from Senegal, including six from irrigated areas along the River Senegal, using one polyclonal and eight monoclonal antibodies produced for PCV in direct antigen coating or double antibody sandwich forms of ELISA. The samples could be grouped into six serotypes, four of which are already known (Manohar et al. 1995), and two new serotypes have been distinguished. All but one of the River Senegal isolates belonged to serotype 1. The odd isolate was serotype 5, which was previously represented by one isolate from Niger.

Research on transmission by seeds also revealed substantial variability depending on the isolate and/or the groundnut variety. The frequency of seed transmission ranged from 0.5 to nearly 40%. An isolate whose RNA 2 sequence revealed very substantial deletion in ORF II ("POUT" isolate from Senegal), had a low seed transmission frequency (7 out of 730 C. 1%). In isolate 'Thysie' from Senegal, where no deletion in ORF II was detected, seed transmission averaged to C. 11 % (25 out of 220). Although the function of the protein from ORF II is not known, it is assumed to play a role in regulating seed transmission.

Research on genome organization and expression of RNA 1 of PCV was done by Institut de biologie moleculaire des plantes du centre national de la recherche scientifique in Strasbourg, in cooperation with the Centre de cooperation internationale en recherche agronomique pour le developpement.

1. Laboratoire de phytovirologie des regions chaudes C1RAD-ORSTOM CIRAD-CP, BP 5035, 34032 Montpellier CEDEX 1, France.
2. Institut senegalais de recherches agricoles (ISRA), Centre national de recherches agronomiques, BP 53, Bambey, Senegal.

Dollet, M., and Ndiaye, M. 1997. Peanut clump virus in western Africa. Pages 24-25 *in* Groundnut virus diseases in Africa: summary and recommendations of the Sixth Meeting of the International Working Group, 18-19 Mar 1996, Agricultural Research Council, Plant Protection Research Institute, Pretoria, South Africa (Reddy, D.V.R., Delfosse, P., Lenne, J.M., and Subrahmanyam, P., eds.). Patancheru 502 324, Andhra Pradesh, India: International Crops Research Institute for the Semi-Arid Tropics; and 1000 Brussels, Belgium: Belgian Administration for Development Cooperation.

References

Manohar, S.K., Dollet, M., Dubern, J., and Gardani, D. 1995. Studies on variability of peanut clump virus: symptomatology and serology. Journal of Phytopathology 143: 233-238.

Epidemiology of Peanut Clump Virus Disease

P Delfosse[1], A Legreve[2], P S Devi[1], A S Reddy[1], J Risopoulos[2], D Doucet[2], B Vanpee[2], H Maraite[2], and D V R Reddy[1]

The peanut clump virus disease caused by Indian peanut clump virus (IPCV) is widespread in the Indian subcontinent. It has recently been reported to also cause a disease on wheat *(Triticum aestivum).* The virus that causes clump disease in western Africa is referred to as peanut clump virus (PCV), and has been shown to affect sugarcane. The two viruses share several characteristics, but are serologically distinct. Indian peanut clump virus has been shown to be transmitted by the soil-inhabiting plasmodiophoromycete fungus *Polymyxa graminis,* and it is suspected that PCV is also transmitted by the same fungus. Both the viruses are transmitted through groundnut *(Arachis hypogaea* L.) seeds. Additionally, IPCV is transmitted through seeds of pearl millet [*Pennisetum glaucum* (L.) R. Br.], finger millet [*Eleusine coracana* (L.) Gaertn.], foxtail millet *(Setaria italica* Beauv.), maize *(Zea mays),* and wheat. The transmission through seeds of other hosts, particularly monocotyledonous weeds, is yet to be investigated. It is not known if seedborne inoculum can initiate the disease in areas where nonviruliferous populations *of Polymyoca* sp occur. Resistance could not be identified in nearly 9000 groundnut genotypes (both in A *hypogaea* and wild *Arachis* sp). Biocides, though effective in reducing disease incidence, are hazardous and uneconomical. The two main management options left are to devise cultural practices, or to induce host-plant resistance by nonconventional approaches. In a collaborative project between ICRISAT and Universite catholique de Louvain, Louvain-la-Neuve, Belgium, funded by the Belgian Administration for Development Cooperation, the epidemiology of the disease is currently being studied in order to develop appropriate strategies for smallholder farmers to manage the disease.

Work Done at the Universite Catholique de Louvain

The wide geographical distribution, and the large host range of both PCV and IPCV and the vector, increase the risk of disease extension. For those reasons, and to collect

1. Crop Protection Division, ICRISAT Asia Center, Patancheru 502 324, Andhra Pradesh, India.
2. Unite de phytopathologie, Faculte des sciences agronomiques, Universite catholique de Louvain, Place Croix du Sud 2 Bte 3, 1348 Louvain-la-Neuve, Belgium.

Delfosse, P., Legreve, A., Devi, P.S., Reddy, A.S., Risopoulos, J., Doucet, D., Vanpee, B., Maraite, H., and Reddy, D.V.R. 1997. Epidemiology of peanut clump virus disease. Pages 26-28 *in* Groundnut virus diseases in Africa: summary and recommendations of the Sixth Meeting of the International Working Group, 18-19 Mar 1996, Agricultural Research Council, Plant Protection Research Institute, Pretoria, South Africa (Reddy, D.V.R., Delfosse, P., Lenne, J.M., and Subrahmanyam, P., eds.). Patancheru 502 324, Andhra Pradesh, India: International Crops Research Institute for the Semi-Arid Tropics; and 1000 Brussels, Belgium: Belgian Administration for Development Cooperation.

basic information for the development of an integrated strategy to manage PCV, it is important to further characterize *P. graminis* isolates occurring in India and in western Africa. Indian *P. graminis* isolates were obtained in 1993 and 1994 by growing sorghum and pearl millet seedlings, on IPCV-infested soils diluted with sterile sand. The soil samples originated from Patancheru, Kartapalem, and Ganapavaram in Andhra Pradesh. From one of the isolates obtained from a soil sampled at Patancheru, three *P. graminis* single cystosorus strains were produced on sorghum. Those three single cystosorus strains grow in a narrow range of temperatures, between 23 and 30°C, and are favored by temperatures close to 30°C for primary infection, for multiplication through secondary zoospores, and for cystosori formation in the roots. The positive effect of dry and hot conditions (50°C for 7 days or 40°C for 4 weeks) to break down the dormancy of cystosori was demonstrated. Several species have been found to be naturally infected by the fungus. However, under controlled conditions with a defined inoculum produced *on Sorghum bicolor* (L.) Moench, studies on host range of the Indian *P. graminis* isolates or single cystosorus strains have shown that the hosts can be ranked on the basis of compatibility with the fungus. 'Favorable' hosts are those on which primary infection and multiplication are high, and can be easily detected, e.g., S. *bicolor, P. glaucum,* and *Z. mays.* In 'fortuitous' hosts, e.g., A. *hypogaea, Beta vulgaris,* and *T. aestivum,* primary infection does not result in the production of zoosporangia, and only the resting stage can be detected on rare occasions. Finger millet showed intermediate compatibility with the presence of low amounts of zoosporangia and resting spores. No infection was detected on *Dactyloctenium aegyptium, Digitaria ciliaris, Hordeum vulgare,* and rice (*Oryza sativa).* Despite the ability of *P. graminis* to infect a wide range of hosts in India, its multiplication, and consequently, its survival, seem to be supported by a more restricted number of hosts than previously assumed. A strategy of disease control based on the choice of 'fortuitous' host species in the cropping system can be justified, because this is expected to reduce the *P. graminis* inoculum in the soil. Recently, isolates were obtained from roots of *Sorghum arundinaceum, S. bicolor,* and *T. aestivum,* growing in soil sampled from Dudhial in the Punjab Province of Pakistan, and from *S. bicolor* and *P. glaucum* growing in soil sampled from several localities in the River Senegal, and from the Diourbel Regions of Senegal, Much diversity in host spectrum and temperature requirement exists among *P. graminis* from different places. These Pakistani and western African isolates are being further characterized to compare the ecological requirements of *P. graminis* isolates from different places, and to find out if control strategies can be extrapolated from one continent to the other. Determining whether western African and Asian *P. graminis* isolates can transmit PCV and IPCV isolates is essential, since the virus is seedborne and there is a risk of spreading the disease through germplasm exchange.

Work Done at ICRISAT Asia Center

The disease occurs mainly in the sandy soils of the Indian states of Andhra Pradesh, Gujarat, Punjab, Rajasthan, and Tamil Nadu, and in the Punjab Province of Pakistan. Disease incidence is high in areas where groundnut is rotated with wheat. A recent

survey conducted in western Africa indicated that it is widespread in Burkina Faso, Mali, Niger, and Senegal, and mostly restricted to sandy soils. Both the virus and its vector were found to have an extremely wide natural host range, including monocotyledonous and dicotyledonous plants of wild and cultivated species. Even though *Polymyxa* sp can transmit the virus to various dicotyledonous crops, it does not colonize these plants. On the contrary, the roots of various monocotyledonous weeds, sorghum, pearl millet, and maize were found to be heavily infected by the resting spores of the fungus, when grown in fields known to harbor IPCV Other monocotyledons such as finger millet and wheat showed only traces of infection. Dicotyledons and some monocotyledons are considered to be 'fortuitous' hosts of the fungal vector. Preliminary observations at IAC showed that it is possible to reduce disease incidence by growing a crop that does not support multiplication of *Polymyxa* sp in the postrainy season before a rainy-season groundnut crop. The disease incidence in plots where groundnut was grown in the preceding season, was much lower than in plots where sorghum was grown. Root exudates of monocotyledons are suspected to induce the germination of the fungal resting spores. Therefore, such crops as pearl millet or sorghum could be raised in clump-infested soils until they reach seedling stage, to induce germination of resting spores, and then destroyed before the fungus produced new resting spores. Such a practice may lead to reduction in disease incidence in the subsequent groundnut crop by reducing the inoculum potential. To test this hypothesis, pearl millet plants were grown in virus-infested plots for 15 days at the beginning of the rainy season, and then plowed into the soil before groundnut was sown. This technique gave very promising results. The disease incidence was reduced from 22-36% to 4-8% in experimental plots at IAC. The monocotyledonous weed, *Cynodon dactylon,* which reproduces vegetatively through rhizomes, is a host for both the virus and the fungus. Preliminary experiments showed that the rhizomes of *C. dactylon* carrying the virus could provide a primary source of inoculum in soil harboring nonviruliferous *Polymyxa* sp. Under similar conditions, seedborne inoculum from groundnut could not induce the disease. However, seedborne inoculum from 'favorable' hosts for the fungus, has a better chance of contributing to the primary source of inoculum. Experiments are now in progress to assess the risk of spread of the virus through seed. The infection by *Polymyxa* sp in India is influenced by such climatic conditions as rainfall distribution and soil temperature. A cumulative weekly rainfall of 14 mm and a soil temperature higher than 25°C were sufficient to induce infection of *Polymyxa* sp in seedlings of sorghum and pearl millet exposed for 1 week in the field.

Acknowledgment

The authors thank the Administration generale de la cooperation au developpement, Brussels, Belgium, for the funding program of collaborative research between Universite catholique de Louvain and the International Crops Research Institute for the Semi-Arid Tropics.

Agrobacterium-Mediated Transformation of Groundnut with Virus Coat Protein Genes

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

Several studies have demonstrated that resistance to viruses can be introduced into plants by utilizing virus coat protein genes. This remains one of the most promising approaches, especially in crops that lack natural resistance. Cultivated groundnut *(Arachis hypogaea* L.) has a relatively narrow genetic base, and lacks resistance to several viruses. In order to facilitate the introduction of virus-associated genes and other novel resistance characteristics into groundnut, a gene delivery system using *Agrobacterium-mediated* transformation (Cheng et al. 1996, Z Li, R L Jarret, M Cheng, and J W Demski 1996, personal communication) has been developed.

Leaf explants were collected from 10-day old in vitro grown groundnut seedlings of cv New Mexico Valencia A, and inoculated *with A. tumefaciens* strain EHA 105, carrying appropriate binary vectors preconditioned with tobacco leaf extract. Leaf explants were subjected to two rounds of selective growth in the presence of kanamycin and cefotaxime (100 mg L^{-1} each), after a 6-day period of co-cultivation. Transgenic groundnut shoots were subsequently identified using a reporter gene assay and its ability to root in kanamycin-containing medium. Transgenic groundnut plants were obtained within 3-4 months (Li et al. 1996), using this procedure. To date, over 70 plants from 22 transgenic lines containing various transgenes have been recovered. All T_o transgenic groundnut plants possessed normal agronomic traits, and produced viable T_1 seeds in the greenhouse. Molecular genetic analysis of transgene integration based on DNA hybridization and gene segregation data revealed, that among 10 independent lines analyzed, 6 lines contained a single copy, 3 lines contained two copies, and 1 line contained three copies, of an intact T-DNA insert. Extensive analysis using RNA blots, enzymatic activity assays, ELISA, and Western blots indicated that all the chromosomally integrated reporter and target genes were expressed as expected. To date, four virus (peanut bud necrosis virus, peanut chlorotic leaf streak, peanut stripe,

1. Department of Plant Pathology, University of Georgia, Georgia Experiment Station, Griffin GA 30223, USA.
2. USDA-ARS Plant Genetic Resources Conservation Unit, Georgia Experiment Station, Griffin GA 30223, USA.

Zhijian Li, Jarret, R.L., Ming Cheng, and Demski, J.W. 1997. *Agrobacterium-mediated* transformation of groundnut with virus coat protein genes. Pages 29-30 *in* Groundnut virus diseases in Africa: summary and recommendations of the Sixth Meeting of the International Working Group, 18-19 Mar 1996, Agricultural Research Council, Plant Protection Research Institute, Pretoria, South Africa (Reddy, D.V.R., Delfosse, P., Lenne, J.M., and Subrahmanyam, P., eds.). Patancheru 502 324, Andhra Pradesh, India: International Crops Research Institute for the Semi-Arid Tropics; and 1000 Brussels, Belgium: Belgian Administration for Development Cooperation.

and tomato spotted wilt virus) coat protein genes have been introduced into groundnut. Experiments are being conducted in the greenhouse to correlate the expression of virus coat protein genes with induced virus resistance in transgenic plants. Meanwhile, large amounts of viable seeds (up to T_3 generation) from transgenic plants, including homozygous progeny plants, have been obtained, and will be used in field trials to determine the effectiveness of transgene-induced virus resistance in a field environment. The high frequency (up to 60%) of single-copy transgene insertions with defined borders in transgenic groundnut plants produced could greatly facilitate genetic engineering of groundnut for crop improvement and molecular genetic studies.

Acknowledgment

This research was supported by the Peanut Collaborative Research Support Program (Peanut CRSP), USA.

References

Cheng, M., Jarret, R.L., Li, Z., Xing, A., and Demski, J.W. 1996. Production of fertile transgenic peanut *(Arachis hypogaea* L.) plants *using Agrobacterium tumefaciens.* Plant Cell Report 15: 653-657.

Seedborne Viruses of Groundnut: Epidemiology and Quarantine Implications

D V R Reddy, A S Reddy, and P Delfosse[1]

Of the 16 viruses currently known to infect groundnut *(Arachis hypogaea* L.) under natural conditions, peanut mottle virus (PMV), peanut stripe virus (PStV), peanut clump virus (PCV), Indian peanut clump virus (IPCV), cucumber mosaic virus (CMV), and peanut stunt virus (PSV) have been shown to be seed-transmitted in groundnut.

Of all the currently known seed-transmitted viruses of groundnut, PMV is the most widely distributed. It is endemic in most groundnut-growing countries. Since the seed transmission rate for PSV is often lower than 0.1 % in groundnut, it is not considered to pose any threat to germplasm exchange. Table 1 lists the groundnut viruses and their

Table 1. Seed transmission of viruses occurring naturally in groundnut.

Virus	Taxonomic group	Transmission (%)	
		Maximum	Mean
Peanut mottle	Poty	8.5	0.1-0.5
Peanut stripe	Poty	33	1-2
Peanut clump	Furo	28	10-15
Cucumber mosaic	Cucumo	2	1-2
Peanut stunt	Cucumo	0.1	0.05
Peanut marginal chlorosis[1]		To be confirmed	
Peanut bunchy top[2]		To be confirmed	
Peanut chlorosis[2]		To be confirmed	
Peanut ring spot[2]		To be confirmed	

1. The symptoms could be due to nutritional deficiency.
2. Causal agent could be peanut bud necrosis virus which is not known to be seed transmitted.

1. Crop Protection Division, ICRISAT Asia Center, Patancheru 502 324, Andhra Pradesh, India.

Reddy, D.V.R, Reddy, A.S., and Delfosse, P. 1997. Seedborne viruses of groundnut: epidemiology and quarantine implications. Pages 31-33 *in* Groundnut virus diseases in Africa: summary and recommendations of the Sixth Meeting of the International Working Group, 18-19 Mar 1996, Agricultural Research Council, Plant Protection Research Institute, Pretoria, South Africa (Reddy, D.V.R., Delfosse, P., Lenne, J.M., and Subrahmanyam, P., eds.). Patancheru 502 324, Andhra Pradesh, India: International Crops Research Institute for the Semi-Arid Tropics; and 1000 Brussels, Belgium: Belgian Administration for Development Cooperation.

Table 2. Seed transmission of groundnut viruses in natural hosts other than groundnut.

Virus	Host	Transmission (%)
Indian peanut clump	Wheat	1.0
	Italian millet	9.7
	Finger millet	5.2
	Pearl millet	0.9
	Maize	0.6
Peanut mottle	Lupin	4.0
	French bean	1.0
	Cowpea	<1.0

Table 3. Occurrence of seed-transmitted groundnut viruses.

Country/Region	Seedborne viruses recorded[1]
African countries	
West Africa	PMV, PCV
Southern Africa	PMV
East and Central Africa	PMV
Australia	PMV
China	PMV, PStV, CMY, PSV
Indian subcontinent	PMV, PStV, IPCV
South America	
Argentina	PMV
Bolivia	PMV
Brazil	PMY, PStV
Peru	PMV
Venezuela	PMV
Southeast Asia	
Indonesia	PStV, PMV
Malaysia	PStV, PMV
Myanmar	PStV, PMV
Philippines	PStV, PMV
Vietnam	PStV

1. PMV = peanut mottle virus, PCV = peanut clump virus, PStV = peanut stripe virus, IPCV = Indian peanut clump virus.

seed transmission frequency. Since IPCV is also seed-transmitted in cereal crops (Table 2), it adds a new dimension to the problem. Crops such as pearl millet [*Pennisetum glaucum* (L.) R. Br.], Italian millet *(Setaria italica* Beauv.), finger millet [*Eleusine coracana* (L.) Gaertn.], and maize (*Zea mays),* infected with IPCV, do not show any overt symptoms. As a result, there are very good chances of IPCV being disseminated to new areas through the seed of cereal crops.

The following measures are suggested, to minimize the risk of introduction of these viruses to new areas through germplasm exchange:

- Seed imports should be tested (individual seed, if sample is small, and several, if imports are in kilograms) for the presence of various seedborne viruses, depending on the country of origin (Table 3).
- Training in the detection of seedborne viruses for plant quarantine staff.
- Supply of diagnostic aids to those quarantine stations that have adequate facilities in developing countries.
- Supply of technical information, preferably with color photographs of symptoms, and options for diagnosis of all the viruses currently known to be seed-transmitted through groundnut seed.
- Deriving seed from disease-free plants.

Application of Homology-Dependent Gene Silencing for Production of Transgenic Virus-Resistant Groundnut

B G Cassidy, P Jayakumar, S Flasinski, D Huhman, Varsha Wesley, and D Post-Beittenmiller[1]

Peanut stripe potyvirus (PStV) can cause severe yield losses to groundnut crops. Conventional breeding strategies to introduce resistance to this virus have not been used, because no natural resistance has been found in the world collection of cultivated groundnut germplasm. Successful strategies have been developed to impart virus resistance where new genes are introduced into plants. The new genes expressed in the transgenic plant impart some character that prevents the establishment of viral infection, so the plant remains healthy. One such strategy, homology-based gene silencing, yields plants highly resistant to virus infection. By expressing a portion of the genome of the target virus, transgenic plants are resistant to infection by that virus.

This approach is being used to develop groundnut plants resistant to PStV Research at the Noble Foundation has been focused on three objectives:

- Transformation and regeneration of groundnuts *(Arachis hypogaea* L.).
- Construction of suitable vectors with a variety of gene sequences from PStV
- Elucidation of the yet-unknown mechanism of homology-based gene silencing using *Nicotiana benthamiana* as a model system.

Using immature embryonal axes from *A. hypogaea* cv Okrun, a highly efficient regeneration protocol, which results in several highly fertile regenerated plants, has been developed. A transformation protocol *using Agrobacterium* carrying the selectable markers, nptII or bar, and the coat protein (CP) gene from PStV is being evaluated. Screening protocols have been developed to detect the introduced gene expression and copy number in groundnut plants. Several putative transformed groundnut plants are currently being screened for the presence and expression of the selectable markers and PStV-CP gene.

1. Plant Biology Division, The Samuel Roberts Noble Foundation, P O Box 2180-2510, Highway 199 East, Ardmore, OK 73402, USA.

Cassidy, B.G., Jayakumar, P., Flasinski, S., Huhman, D., Varsha Wesley, and Post-Beittenmiller, D. 1997. Application of homology-dependent gene silencing for production of transgenic virus-resistant groundnut. Pages 34-35 *in* Groundnut virus diseases in Africa: summary and recommendations of the Sixth Meeting of the International Working Group, 18-19 Mar 1996, Agricultural Research Council, Plant Protection Research Institute, Pretoria, South Africa (Reddy, D.V.R., Delfosse, P., Lenne, J.M., and Subrahmanyarn, P., eds.). Patancheru 502 324, Andhra Pradesh, India: International Crops Research Institute for the Semi-Arid Tropics; and 1000 Brussels, Belgium: Belgian Administration for Development Cooperation.

To elucidate the mechanism of homology-based gene silencing and to determine how it results in virus resistance, *N. benthamiana* was transformed with PStV-CP and several other nonstructural protein genes. These plants have been analyzed for their resistance to strains of PStV, other potyviruses, and other unrelated viruses. The resistance was of two major types, constitutive and inducible, the latter being more common. Results demonstrated that some plants were highly resistant to PStV but not to potyviruses sharing less sequence similarity, or to viruses belonging to other families. These plants are currently being analyzed to determine transgene copy number, potential methylation patterns within the introduced genes, and other host or environmental elements that could be involved in transgenic resistance.

Collaborative Research on Groundnut Viruses Between University of Florida and ICRISAT

S Gowda[1], R A Naidu[2], T Satyanarayana[1], D V R Reddy[2], and W O Dawson[1]

Collaborative research between University of Florida at Lake Alfred, Florida, USA, and ICRISAT Asia Center has focused on developing biotechnological tools for the control of viral diseases of groundnut *(Arachis hypogaea* L.). The viruses include peanut bud necrosis tospovirus (PBNV) belonging to the genus *Tospovirus* of the family *Bunyaviridae,* cowpea mild mottle carlavirus (CMMV), and another carlavirus which has not yet been characterized. Peanut bud necrosis virus is considered by far the most economically important of all viruses infecting groundnut in the Indian subcontinent. In addition to bud necrosis disease in groundnut, this virus causes bud blight, curling, and necrosis in soybean (*Glycine max),* and a leaf curl disease in urd bean (black gram, *Vigna mungo),* two other important legume crops in the region. Peanut bud necrosis virus is an enveloped virus with three species of RNA. The S and M RNAs are ambisense in nature, whereas the L RNA is of negative sense polarity. The virus is transmitted in a persistent manner by *Thrips palmi* Karny. Identification of resistance to PBNV is essential in the short-duration groundnut cultivars, which are suitable under rainfed conditions by subsistence, small land-holding farmers in the Indian subcontinent. This can be achieved by the introduction of pathogen-derived resistance into these cultivars by biotechnological approaches. Towards this objective, the University of Florida and ICRISAT are collaborating to sequence the L RNA of PBNY to facilitate identification of regions in the L RNA that can be cloned into plant transformation vectors. The nucleotide sequence of S RNA (Satyanarayana et al. 1996a) and M RNA (Satyanarayana et al. 1996b) of PBNV are available. The cDNA for the L RNA of PBNV has been cloned into a modified pUC vector, and both the strands have been sequenced. The L RNA is nearly 8900 nucleotides long, with a single long open-reading frame (ORF) in the complementary strand. The coding region of PBNV exhibits 63% similarity, and 44% identity at the amino acid level to the L RNA of tomato spotted wilt virus. The homology is higher in the conserved core polymerase region (73% similarity

1. Virology Laboratory, Citrus Research and Education Center, 700 Experiment Station Road, University of Florida, Lake Alfred, FL 33850, USA.
2. Crop Protection Division, ICRISAT Asia Center, Patancheru 502 324, Andhra Pradesh, India.

Gowda S., Naidu R.A., Satyanarayana, T., Reddy, D.V.R., and Dawson, W.O. 1997. Collaborative research on groundnut viruses between University of Florida and ICRISAT. Pages 36-37 *in* Groundnut virus diseases in Africa: summary and recommendations of the Sixth Meeting of the International Working Group, 18-19 Mar 1996, Agricultural Research Council, Plant Protection Research Institute, Pretoria, South Africa (Reddy, D.V.R., Delfosse, P., Lenne, J.M., and Subrahmanyam, P., eds.). Patancheru 502 324, Andhra Pradesh, India: International Crops Research Institute for the Semi-Arid Tropics; and 1000 Brussels, Belgium: Belgian Administration for Development Cooperation.

and 57% identity) than in the 5' region (56% similarity and 44% identity) and 3' region (54% similarity and 33% identity) of the deduced protein sequence. Defective RNAs (D RNA) for the L RNA have been observed to occur when the virus is mechanically transmitted over many generations, and the nature of the defective RNAs is being investigated.

The nucleotide sequences of the 3' regions (approximately 3.0 kb) encompassing the triple gene block, p 25, p 12, and p7, the *C?* and the 3' ORF of the CMMV, and the unidentified carlavirus have been completed. These two viruses are very similar in their genome organization, and have 94% identity and 97% similarity at the amino acid level of their ORFs. Currently, sequencing of the polymerase regions of these two viruses is in progress.

Future research will focus on the identification of the regions of the S, M, and L RNAs of PBNV to develop pathogen-derived transgenic resistance in groundnut. Initially, various ORFs and modified regions of the ORFs will be transferred into plant transformation vector pKYLX 71, and used in the transformation of *Nicotiana benthamiana* to evaluate transgenic resistance against PBNV Based on these results, gene constructs will be used to transform short-duration groundnut cultivars. Efforts will also be made to obtain full length infectious cDNA clones of CMMV and another isolate of carlavirus in order to understand the biological functions of different genes.

References

Satyanarayana, T., Mitchell, S.E., Reddy, D.V.R., Brown, S., Kresovich, S., Jarret, R., Naidu, R.A., and Demski, J.W. 1996a. Peanut bud necrosis tospovirus S RNA: complete nucleotide sequence, genome organization and homology to other tospoviruses. Archives of Virology 141:85-98.

Satyanarayana, T., Mitchell, S.E., Reddy, D.V.R., Kresovich, S., Jarret, R., Naidu, R.A., Gowda, S., and Demski, J.W. 1996b. The complete nucleotide sequence and genome organization of the M RNA segment of peanut bud necrosis tospovirus and comparison with other tospoviruses. Journal of General Virology 77: 2347-2352.

Current Research on Groundnut Viruses in the University of Georgia

H R Pappu[1], A K Culbreath[1], and J W Todd[2]

Tomato spotted wilt virus (TSWV) continues to be the major virus disease constraint to groundnut *(Arachis hypogaea* L.) production in Georgia. This virus is also economically important on such other crops as tobacco, tomato, and pepper. Epidemics of spotted wilt have been regularly observed in the past several years in Georgia, and losses due to this disease run into several millions of dollars annually in Georgia alone.

Tomato spotted wilt virus belongs to the genus *Tospovirus* (serogroup I) in the family *Bunyaviridae.* Viruses belonging to this genus are classified as either distinct viruses, or strains of the same virus based on such widely accepted criteria as host range, serological relationships of the nucleocapsid protein, and nucleotide and amino acid sequences of the nucleocapsid protein gene (N gene). This virus has a wide host range, and is transmitted by several species of thrips in a persistent (circulative) manner. Epidemics in Georgia can be attributed to the presence of two vector species, *Frankliniella occidentalis* (Hinds) and *F. fusca* (Pergande). The fact that the virus multiplies in its insect vector makes managing the disease through vector control even more difficult. Therefore, there is a need to devise new and alternate methods to manage the disease.

A muitidisciplinary approach is being pursued in the University of Georgia's Coastal Plain Experiment Station at Tifton, Georgia, USA, in understanding the virus biology, molecular biology, epidemiology, and host resistance, with a view to developing a disease management program. Little or no molecular information is available on the various strains of the virus that infect groundnut, tobacco, and vegetables in Georgia. Groundnut leaf samples with virus symptoms were collected from various parts of the state and also from northern Florida and Alabama. Total nucleic acid extracts were prepared, and the nucleocapsid protein genes (N gene) of the TSWV isolates were obtained by reverse transcription-polymerase chain reaction. The N genes were either directly sequenced or cloned into pUC 118 vector before sequencing. The N gene was

1. Department of Plant Pathology, University of Georgia, Coastal Plain Experiment Station, Tifton, Georgia 31793-0748, USA.
2. Department of Entomology, University of Georgia, Coastal Plain Experiment Station, Tifton, Georgia 31793-0748, USA.

Pappu, H.R., Culbreath, A.K., and Todd, J.W. 1997. Current research on groundnut viruses in the University of Georgia. Pages 38-39 *in* Groundnut virus diseases in Africa: summary and recommendations of the Sixth Meeting of the International Working Group, 18-19 Mar 1996, Agricultural Research Council, Plant Protection Research Institute, Pretoria, South Africa (Reddy, D.V.R., Delfosse, P., Lenne, J.M., and Subrahmanyam, P., eds.). Patancheru 502 324, Andhra Pradesh, India: International Crops Research Institute for the Semi-Arid Tropics; and 1000 Brussels, Belgium: Belgian Administration for Development Cooperation.

775 nucleotides long, and can potentially code for a 258 amino acid protein. This finding is in agreement with previously reported TSWV isolates. Western blot analysis of TSWV-infected groundnut leaf tissue confirmed the size of the N-protein. Comparisons with the N genes of TSWV isolates from tobacco and tomato in Georgia, and those from other parts of the world showed a high degree of similarity (94-99%) at both nucleotide and amino acid levels.

Other projects that are in progress include understanding the epidemiology of TSWV epidemics on groundnut, and field-testing of transgenic groundnut lines expressing the N gene of TSWV. The former involves determination of viruliferous thrips populations during winter and cropping seasons, since only viruliferous thrips constitute the most critical component in the epidemiology of the disease. J Sherwood's laboratory at Oklahoma State University developed a monoclonal antibody (MAb) that is specific to one of the nonstructural gene products (NSs) coded by the small RNA of TSWV. Since NSs exists only in those thrips in which the virus had replicated, an ELISA test using the NSs-specific MAb can differentiate viruliferous thrips from those that have simply ingested the virus. These MAbs will be used to distinguish the viruliferous insects from nonviruliferous ones. Several transgenic groundnut lines developed in the laboratories of J Demski and B Jarrett at Georgia Experiment Station, University of Georgia, Griffin, are being evaluated in southern Georgia for their resistance to TSWV.

Acknowledgment

This research has been funded in part by the Georgia Agricultural Commodity Commission for Peanut. Support from the Office of the International Agriculture, University of Georgia, and ICRISAT is gratefully acknowledged.

Country Reports

Occurrence and Importance of Groundnut Viruses in South Africa, and Future Requirements for Integrated Disease Management

G Cook and G Pietersen[1]

Groundnut (*Arachis hypogaea* L.) production represents approximately 1% of the gross value of all cultivated crops in South Africa. The average production during 1994/95 was 105 000 t. The Plant Protection Research Institute has been surveying the crop for viral diseases since 1990. Before these investigations, few groundnut viruses had either been reported or characterized in South Africa, owing to limited virological studies. Apart from confirming certain previous reports, and identifying the economically important viruses, new and uncharacterized viruses have also been detected.

Previous reports that tomato spotted wilt virus and groundnut rosette viruses occur in South Africa have been confirmed. Groundnut ringspot tospovirus has also been identified in groundnut in South Africa. The only potyvirus detected in commercial groundnut fields is peanut mottle virus.

In 1995, peanut stripe virus was intercepted in an experimental plot of germplasm material at the Grain Crops Institute. The spread of the virus could be contained because of an early identification.

A potyvirus causing chlorotic ringspots was isolated from a groundnut plant found on an experimental plot at Potchefstroom in the North West Province. The potyvirus did not react with any of the antisera to groundnut viruses available, and host range studies indicated that it was a new groundnut potyvirus. Subsequent sequence data confirmed that the virus is a new one (unpublished results).

Just before this meeting, a field survey to establish the presence of peanut clump virus in South Africa was conducted in Potchefstroom, Lichtenburg, and Hartswater areas of the Transvaal Province. It was apparent from this survey that none of the known serotypes of PCV and Indian peanut clump virus occurred in those areas (P Delfosse and A S Reddy, ICRISAT Asia Center, personal communication).

Identification of an isometric virus isolated in 1992 from a plant displaying reduced young leaves with some malformation and chlorotic patches is still in progress.

1. Virology Section, Agricultural Research Council, Plant Protection Research Institute, Private Bag X134, Pretoria 0001, South Africa.

Cook, G., and Pietersen, G. 1997. Occurrence and importance of groundnut viruses in South Africa and future requirements for integrated disease management. Pages 43-44 *in* Groundnut virus diseases in Africa: summary and recommendations of the Sixth Meeting of the International Working Group, 18-19 Mar 1996, Agricultural Research Council, Plant Protection Research Institute, Pretoria, South Africa (Reddy, D.V.R., Delfosse, P., Lenne, J.M., and Subrahmanyam, P., eds.). Patancheru 502 324, Andhra Pradesh, India: International Crops Research Institute for the Semi-Arid Tropics; and 1000 Brussels, Belgium: Belgian Administration for Development Cooperation.

Future thrusts will be on disease surveys, characterization of new viruses, monitoring of germplasm, especially for seed-transmitted viruses, and production of suitable diagnostic aids.

Occurrence, Distribution, and Importance of Groundnut Viruses in Burkina Faso, and Future Requirements for Integrated Disease Management

G Konate[1]

Peanut clump virus (PCV) and groundnut rosette virus (GRV) are the two major viruses that infect groundnut *(Arachis hypogaea* L.) in Burkina Faso. Groundnut rosette virus is specific to the southern part of the country, which receives 900-1100 mm rainfall annually, and infects long-duration (120 days) varieties that are grown widely in this region. Varieties that have, in the past, been considered resistant to GRV have been affected by the disease over the last decade without any apparent reason. Disease incidence varies from year to year.

Peanut clump is more widely distributed than GRV Surveys conducted over the last few years indicate that the virus is present in all the groundnut-growing regions in Burkina Faso. Two forms of clump disease have been recorded. The PCV-Green strain causes typical 'clump' symptoms, whereas the PCV-Yellow strain causes only a small reduction in leaflet size and strong bright yellow symptoms on leaflets. Disease incidence varies from year to year, but can reach up to 50%. Observations recorded on the experiment station in Ougadougou indicate that drought apparently favors the expression of disease symptoms. Yield losses due to PCV can reach up to 60%. As a result, the disease is considered to be economically important.

Since 1988, research on PCV in Burkina Faso has focused on disease epidemiology, and includes:

- Screening for sources of resistance (400 varieties have been screened to date).
- Identification of alternative hosts of PCV.
- Role of contaminated seed in virus transmission.
- Effect of crop rotations on disease establishment.

1. Institut d'etudes et de recherches agricoles (INERA), BP 7192, Ougadougou, Burkina Faso.

Konate, G. 1997. Occurrence, distribution, and importance of groundnut viruses in Burkina Faso, and future requirements for integrated disease management. Pages 45-46 *in* Groundnut virus diseases in Africa: summary and recommendations of the Sixth Meeting of the International Working Group, 18-19 Mar 1996, Agricultural Research Council, Plant Protection Research Institute, Pretoria, South Africa (Reddy, D.VR., Delfosse, P., Lenne, J.M., and Subrahmanyam, P., eds.). Patancheru 502 324, Andhra Pradesh, India: International Crops Research Institute for the Semi-Arid Tropics; and 1000 Brussels, Belgium: Belgian Administration for Development Cooperation.

On the basis of the results obtained, two major recommendations have been made to prevent the disease from spreading. These are:

- Stop the production of groundnut seed in plots recognized as clump infested.
- Avoid sorghum (*Sorghum bicolor* (L.) Moench]/groundnut crop rotation, especially for long periods.

The second recommendation conflicts with the recommendation of the Department of Agriculture, and interferes with an age-old agricultural practice. Rotation of groundnut with sorghum was seen to benefit sorghum because of the ability of groundnut to fix nitrogen. Moreover, in western Africa, sorghum-groundnut rotation is a standard practice. It is therefore necessary to continue research on clump in order to develop alternate methods acceptable to farmers.

In Burkina Faso, the following areas of research have been identified to manage PCV:

- Develop economical methods for virus diagnosis, suitable for conditions in Africa.
- Re-evaluate the importance of PCV (distribution, incidence, etc.) using diagnostic tools.
- Continue studies on alternative hosts of PCV and assess their role in increasing the inoculum in soils.
- Study the serological and pathogenic variability of PCV in order to determine precisely the virus distribution, biodiversity, and host range.
- Utilize the information generated to develop integrated management practices for PCV.

The virology laboratory in Ougadougou has the basic equipment and expertise to conduct the proposed research activities.

Current Research on Groundnut Rosette Disease in Nigeria

M D Alegbejo[1], P E Olorunju[1], R A Naidu[2], and F Kimmins[3]

Rosette is an important virus disease of groundnut *(Arachis hypogaea* L.) in Nigeria. It is found in all the major groundnut-growing regions, particularly in northern Nigeria. Frequent epidemics have affected groundnut production in the country. Cultural practices such as early sowing and close spacing were recommended earlier, to minimize/ reduce crop losses. However, sporadic rainfall during the groundnut-growing season, and lack of good quality seed often preclude smallholder farmers from adopting these control measures. Therefore, alternative, low-input control strategies based on host-plant resistance are being pursued at the Institute for Agricultural Research (IAR), Zaria.

Previous research on rosette disease was supported by funds from the IAR, Peanut Collaborative Research Support Program (Peanut CRSP), and the European Communities. In recent years, the emphasis has been on developing short-duration rosette-resistant groundnut varieties with the agronomic characters that Nigerian farmers want. The short- and long-duration varieties developed by the Southern African Development Community(SADC)/ICRISAT Groundnut Project at Chitedze, Malawi, are being used for this purpose.

A survey of rosette disease was conducted during August 1995 in the four major groundnut-growing states (Kano, Kaduna, Katsina, and Bauchi) of northern Nigeria. High incidence of the disease (more than 50%) was observed in several of the farmers' fields surveyed, especially in late-sown crops with low plant populations. A majority of the plants showed chlorotic, rather than green rosette symptoms. In addition, variability in the symptoms caused by chlorotic rosette was observed. Samples collected during the survey were analyzed for GRAV by triple antibody sandwich-ELISA and for groundnut rosette virus and satellite RNA by dot-blot hybridization assays at the Scottish Crop Research Institute (SCRI), UK. This demonstrated the effectiveness of diagnostic tools to detect the three components of rosette disease, in spite of variability in

1. Institute for Agricultural Research, Ahmadu Bello University, PMB 1044, Zaria, Nigeria.
2. Crop Protection Division, ICRISAT Asia Center, Patancheru 502 324, Andhra Pradesh, India.
3. Natural Resources Institute (NRI), Central Avenue, Chatham Maritime, Kent ME4 4TB, UK.

Alegbejo, M.D., Olorunju, P.E., Naidu, RA., and Kimmins, F. 1997. Current research on groundnut rosette disease in Nigeria. Pages 47-48 *in* Groundnut virus diseases in Africa: summary and recommendations of the Sixth Meeting of the International Working Group, 18-19 Mar 1996, Agricultural Research Council, Plant Protection Research Institute, Pretoria, South Africa (Reddy, D.V.R., Delfosse, P, Lenne, J.M., and Subrahmanyam, P., eds.). Patancheru 502 324, Andhra Pradesh, India: International Crops Research Institute for the Semi-Arid Tropics; and 1000 Brussels, Belgium: Belgian Administration for Development Cooperation.

the symptoms. This survey also reemphasized the need for such low-input control measures as host-plant resistance, and demonstrated the strong linkages between national agricultural research systems (NARS), international agricultural research centers, and institutions such as the Natural Resources Institute (NRI) and SCRI for collaborative research and technology exchange. Such continued collaboration would help NARS undertake the studies listed below, to manage rosette disease.

- Information on disease incidence and yield losses for rosette and other groundnut virus diseases.
- Understand the variability in rosette symptoms.
- Identify alternative hosts of the rosette disease and the feasibility of their eradication.
- Information on influence of agroclimatic conditions on the severity and incidence of rosette disease in order to develop disease-forecasting systems.
- Develop integrated disease management practices.

Groundnut Rosette and Peanut Clump Diseases in Western Africa

R A Naidu[1], F Kimmins[2], P Delfosse[1], M D Alegbejo[3], and F Waliyar[4]

A survey was conducted of virus diseases of groundnut *(Arachis hypogaea* L), with special reference to groundnut rosette (GRV) and peanut clump viruses (PCV) in selected regions of Burkina Faso, Mali, Niger, northern Nigeria, and Senegal in August-September 1995. Surveys were conducted in farmers' fields situated along or near the roads, keeping as far as possible, a minimum distance of approximately 20 km between the fields. A total of 32 fields was surveyed in northern Nigeria, 5 fields in Niger, and 12 fields each in Mali and Burkina Faso. In Senegal, all groundnut and a few pearl millet fields on the Centre national de la recherche agronomique of Bambey farm were surveyed. Disease incidence in each field was assessed by taking random observations across the entire cross section of the field, and at the borders. Symptoms were recorded in each field and samples were analyzed at the Scottish Crop Research Institute (SCRI), UK, by serology and dot-blot hybridization assay for groundnut rosette assistor virus, GRY and satellite RNA, and by electron microscopy for PCV. In addition to groundnut, certain weeds and pearl millet samples collected in Senegal and Niger were tested for the presence of PCV, by ELISA, at the Universite catholique de Louvain, Louvain-la-Neuve, Belgium.

High incidences of the rosette diseases (chlorotic and green) were observed in several farmers' fields in northern Nigeria (mean of 20%). Very low incidences of rosette (average of 1%) were observed in farmers' fields in Burkina Faso, Mali, and Niger. Chlorotic rosette was more prevalent than green rosette in all the fields visited. A wide variation in symptoms of chlorotic rosette was observed in northern Nigeria. Peanut clump virus was observed in many fields in Burkina Faso, Mali, and Niger. The infected plants showed a wide range of symptoms - from extreme stunting to no apparent symptoms. In addition, it was difficult to distinguish between green rosette and peanut clump diseases based on symptoms alone, and consequently, it was difficult to assess the incidence and distribution of peanut clump disease on this basis. Therefore, to

1. Crop Protection Division, ICRISAT Asia Center, Patancheru 502 324, Andhra Pradesh, India.
2. Natural Resources Institute, Central Avenue, Chatham Maritime, Kent ME4 4TB, UK.
3. Institute for Agricultural Research, Ahmadu Bello University, PMB 1044, Zaria, Nigeria,
4. Crop Protection Division, ICRISAT-Western and Central Africa Region, BP 320, Bamako, Mali.

Naidu, R.A., Kimmins, F., Delfosse, P., Alegbejo, M.D., and Waliyar, F. 1997. Groundnut rosette and peanut clump diseases in western Africa. Pages 49-51 *in* Groundnut virus diseases in Africa: summary and recommendations of the Sixth Meeting of the International Working Group, 18-19 Mar 1996, Agricultural Research Council, Plant Protection Research Institute, Pretoria, South Africa (Reddy, D.V.R., Delfosse, P., Lenne, J.M., and Subrahmanyam, P., eds.). Patancheru 502 324, Andhra Pradesh, India: International Crops Research Institute for the Semi-Arid Tropics; and 1000 Brussels, Belgium: Belgian Administration for Development Cooperation.

identify PCV, it is essential to test plants by serology and/or dot-blot hybridization techniques.

Results of this survey revealed much wider variability in the symptoms of chlorotic rosette disease than reported earlier, and demonstrated the effectiveness of diagnostic tools developed at SCRI for the detection of the three components of rosette disease. These tools have also provided a greater opportunity to study the ecology and epidemiology of rosette disease than ever before, and to test groundnut cultivars, breeding materials, and germplasm lines for resistance to the three components of rosette disease. Based on the current survey and information available from earlier studies, some areas of future research on rosette and PCV are proposed. They are discussed below.

Groundnut Rosette Disease

For the effective deployment of rosette-resistant, short-duration groundnut varieties developed at the Southern African Development Community (SADC)/ICRISAT Project in Malawi, the following issues should be considered:

- Variability in the causal agents of groundnut rosette disease.
- Resistance mechanisms in rosette-resistant groundnut germplasm, and breeding lines in different agroclimatic zones of Africa.
- Identification of the dry-season hosts of the aphid vector, and the three components of rosette disease.
- Vector biotypes (collected from different agroecological zones), and their transmission efficiency of green and chlorotic forms of rosette on a range of groundnut genotypes, including those that are rosette resistant.
- Systematic disease surveys in different agroecological zones in western, eastern, and southern Africa, to assess the economic impact of the disease.
- A study of farmers' existing knowledge, management practices, and attitudes to adopt new potential control measures, including resistant varieties.
- Influence of environmental factors on aphid migration and disease outbreaks.

Peanut Clump Disease

Since natural sources of resistance are not available in the groundnut germplasm or in the wild *Arachis* species tested, alternate approaches need to be adopted for the control and/or management of this disease. The following aspects need to be addressed:

- Understand the range of symptom variability and genetic diversity in the PCV genome.
- Assess the role of intercropping/crop rotation with cereals and other crops on disease incidence.

- Optimize methods (serology and nucleic acid based) to detect PCV in groundnut plants.
- Seed transmission in groundnut cultivars/varieties grown in western African countries, and its impact on germplasm conservation.
- Develop transgenic resistance in groundnut, using viral genes.
- Conduct disease surveys to assess the distribution of PCV in African countries.

Current Status of Research on Groundnut Viruses in Kenya

A W Wangai[1]

Several legume crops are grown in Kenya for grain, fodder, forage, and oilseed. Some major legume food crops are phaseolus bean (*Phaseolus vulgaris),* garden pea *(Pisum sativum),* groundnut *(Arachis hypogaea* L.), and pigeonpea (*Cajanus cajan* (L.) Millsp.]. Soybean *(Glycine max)* is also grown as an oilseed crop.

The main groundnut-producing areas are in the western and coastal districts, where the crop is grown mainly by smallscale farmers, either as a Sole crop or intercropped with maize (*Zea mays),* sorghum (*Sorghum bicolor* (L.) Moench], or cassava *(Manihot esculenta* Crantz). Groundnut is valuable to farmers, both as a cash crop and as a dietary supplement to maize and cassava, the major staple foods in these regions.

The most common varieties are Red Valencia (a red, small-seeded erect bunch type) and Homabay (brown, large-seeded spreading bunch type). Average yields under farmers' conditions are 0.5-0.8 t ha^{-1}.

The production trend has been on the decline, with the cultivated area falling by 75% between 1990 and 1993. This drastic reduction has been attributed to several factors including the lack of improved high-yielding cultivars, high incidence of diseases [groundnut rosette (GRV) and leaf spot], poor agronomic practices, and the nonavailability of certified seeds.

Several virus diseases that are of economic importance have been reported in groundnut in Kenya. The occurrence of GRV was reported by Storey in 1935 in the western Kenya region. Peanut mottle virus was reported in the coastal and western regions. Cowpea mild mottle virus (CMMV) is widespread, and considered to be economically important. Yield losses due to CMMV in groundnut were found to be 20% in Natal Common, 36% in a local cultivar, and 100% in RG 1.

Information on the current status of distribution of groundnut viruses in Kenya is lacking. In order to generate information that can be used to develop integrated management strategies for groundnut viruses in Kenya, there is a need to initiate a multidisciplinary approach to the research.

1. Kenya Agricultural Research Institute, National Plant Breeding Research Centre, P O Njoro, Kenya.

Wangai, A.W. 1997. Current status of research on groundnut viruses in Kenya. Pages 52-53 *in* Groundnut virus diseases in Africa: summary and recommendations of the Sixth Meeting of the International Working Group, 18-19 Mar 1996, Agricultural Research Council, Plant Protection Research Institute, Pretoria, South Africa (Reddy, D.V.R., Delfosse, P., Lenne, J.M., and Subrahmanyam, P., eds.). Patancheru 502 324, Andhra Pradesh, India: International Crops Research Institute for the Semi-Arid Tropics; and 1000 Brussels, Belgium: Belgian Administration for Development Cooperation.

Aspects of research that need emphasis are:

- Surveys on the distribution of groundnut viruses, and precise identification of the widely distributed ones on the basis of biological, serological, and molecular methods.
- Identification of the virus vectors and determination of the epidemiology.
- Screening and development of cultivars with virus resistance.

To accomplish the above objectives, there is a need for collaboration with advanced virus laboratories in developed countries and international agricultural research centers. Training personnel in virus techniques for characterization and diagnosis of viruses should receive high priority.

Recommendations

The participants were divided into two groups according to their preference. Group 1, led by Dr D J Robinson, focused on 'Molecular Biology', and Group 2, led by Dr M Thresh, focused on 'Epidemiology and Management.' After discussion, the recommendations of each group were presented by the group leaders and discussed further. The emphasis was on groundnut rosette (GRV) and peanut clump viruses (PCV). Other recommendations are consolidated by category.

Groundnut Rosette

Causal Agent

- A panel of monoclonal bodies for luteoviruses should be assembled and used to investigate the variability of groundnut rosette assistor virus (GRAV).
- Tests based on polymerase chain reaction should be developed to detect GRAY GRV, and its satellite RNA.
- Variability of GRV should be assessed.

Epidemiology

- Large-scale surveys for GRV should be undertaken at a few selected locations in diverse agroecological zones to understand the biodiversity among the causal agents.
- Factors that contribute to rosette epidemics should be determined. These include the influence of environmental factors on the multiplication and migration of the vector, and the identification of dry-season hosts of both the disease agents and the vector.
- Molecular tools should be developed to discriminate between biotypes of *Aphis craccivora* Koch.
- Modeling should be applied, especially to predict epidemics.

Management

- The impact of cultural practices, with emphasis on intercropping, on the disease and on *A. craccivora,* should be studied.
- Advanced GRV-resistant breeding lines should be moved rapidly into on-farm evaluation. Provision should be made to produce adequate quantities of seed. This will

include streamlining of procedures for cultivar release and seed multiplication and distribution.

- Importance should be given to breeding short-duration rosette-resistant cultivars, using both conventional and nonconventional approaches.
- Since resistance to *A. craccivora* is available, efforts should be made to incorporate this resistance into existing advanced rosette-resistant breeding lines.
- Additional sources of resistance to GRAV, GRV, and the aphid vector need to be studied and used.

Peanut Clump Virus

Identification and Detection

- Efforts should be made to produce an antiserum that can detect as many isolates as possible. Until then, it is recommended that a 'cocktail' of all available antisera be used, especially to detect isolates in samples collected during surveys and in quarantine.
- A broadly specific nucleic acid probe, for sequences conserved in both the RNA species, is recommended for the detection of virus in samples collected during surveys and in plant quarantine.
- Assessment of variability in the virus and in its fungal vector, *Polymyxa* sp, should be given priority.

Epidemiology and Management

- The importance of cereal crops used as intercrops or in crop rotation for establishment, spread, and perpetuation of the clump disease on groundnut, should be assessed.
- The ecology of the fungal vector, *Polymyxa* sp, especially in relation to soil factors and climatic conditions, should be studied,
- Seed from plants raised in clump-infested soils (including those of cereal crops which host the virus) should not be used for sowing, and should not be supplied to any agencies. Infested areas, especially on research stations, should be treated to eliminate both the virus and the vector,
- Efforts to introduce PCV resistance by nonconventional approaches should be given high priority.
- Efforts should be made to identify virus immunity in cereal crops used in cropping systems involving groundnut.

- Available knowledge on integrated pest management (including cultural practices and treatment with chemicals) on Indian PCX should be used for PCV in Africa.
- Modeling should be explored, especially to predict the severity of disease, and frequency of seed transmission.
- All the new collections of ICRISAT's groundnut germplasm from western Africa should be tested for the presence of virus in the seed.

Other Groundnut Viruses

- Seed should be carefully monitored for the presence of peanut stripe, peanut mottle, and other seedborne viruses, especially in germplasm originating from countries where these viruses are known to be endemic.
- The occurrence of cowpea mild mottle or related viruses and various tospoviruses should be monitored.

General Points

- Efforts should be made to inform plant quarantine authorities about the danger of introducing seedborne groundnut viruses.
- Surveys on the occurrence and distribution of rosette and clump viruses will be necessary in future, but they should be need driven.

Development of Virus-Resistant Cultivars by Nonconventional Approaches

- The development of genetic map of groundnut, and use of molecular markers in breeding should be encouraged.
- High priority should be given to developing constructs based on polymerase gene or a combination of coat protein and polymerase genes to induce resistance to clump virus.
- Resistance to GRAV should be introduced into existing rosette-resistant cultivars by utilizing the coat protein gene.
- Consideration should be given to how and where transgenic groundnuts are likely to be released. There is a need to investigate the practicalities of getting necessary permission. This includes country regulations for growing transgenic plants and Intellectual Property Rights implications. ICRISAT should disseminate the necessary information.
- Biotechnologists and breeders should consider the implications of multiple transgenic resistance and/or conventional resistance genes.

Participants

Belgium

H Maraite
Head
Unite de phytopathologie
Faculte des sciences agronomiques
Universite catholique de Louvain
Place Croix du Sud 2 Bte 3
B-1348, Louvain-la-Neuve

Tel +32 10 473 749
Fax +32 10 478 697

Burkina Faso

G Konate
Institut d'etudes et de
recherches agricoles (INERA)
BP 7192
Ougadougou

France

M Dollet
Laboratoire de phytovirologie
des regions chaudes
CIRAD-ORSTOM
BP 5035
34032 Montpellier Cedex 1

Germany

G Adam
Professor and Department Head
Plant Protection Service FHH
Universitat Hamburg
Hamburg, Brd; Sitz Marseillerstr.7
D-20355 Hamburg

India

ICRISAT Asia Center
Patancheru 502 324
Andhra Pradesh

Tel +91 40 596161
Fax +91 40 241235
Telex 422203 ICRI IN
E-mail (Internet)
ICRISAT@CGNET.COM

P Delfosse
Visiting Scientist
Crop Protection Division

J M Lenne
Principal Scientist and
Research Division Director
Crop Protection Division

R A Naidu
Senior Scientist
Crop Protection Division

D V R Reddy
Principal Scientist
Crop Protection Division

L J Reddy
Senior Scientist
Genetic Enhancement Division

Kenya

A W Wangai
Kenya Agricultural Research Institute
National Plant Breeding Research Centre
P O Njoro

Malawi

A J Chiyembekeza
Chitedze Agricultural Research Station
P O Box 158, Lilongwe

P Subrahmanyam
Principal Scientist
Crop Protection Division
Southern African Development
Community (SADC)/ICRISAT
Groundnut Project
Chitedze Agricultural Research Station
P O Box 1096
Lilongwe

Tel +265 720968, 720906
Fax +265 741872
E-mail ICRISAT-
MALAWI@CGNET.COM

Nigeria

M D Alegbejo
Institute for Agricultural Research
Ahmadu Bello University
PMB 1044
Zaria

South Africa

Plant Protection Research Institute
Virology Section
Agricultural Research Council
Private Bag x134
Pretoria 0001
South Africa

Tel +27 12 329 3269
Fax +27 12 329 3278

G Cook
G Kassdorf
G Pietersen
T van Tonder
M Walters

United Kingdom

M A Mayo
A F Murant
D J Robinson
Scottish Crop Research Institute
Virology Department
Invergowrie, Dundee DD2 5DA
Scotland

Tel +44 1382 562 731
Fax +44 1382 562 426

F Kimmins
M Thresh
Natural Resources Institute
Central Avenue
University of Greenwich
Chatham Maritime
Kent ME4 4TB

Tel +44 634 880088
Fax +44 634 880066

United States of America

S Gowda
Virology Laboratory
Citrus Research and Education Center
700 Experiment Station Road
University of Florida
Lake Alfred
FL 33850

Tel +1 941 956 1151
Fax +1 941 956 4631

H R Pappu
Department of Plant Pathology
University of Georgia
Coastal Plain Experiment Station
Tifton, GA 31793-0748

Tel +1 912 386 3370
Fax +1 912 386 7285

J W Demski
Professor (Emeritus)
Department of Plant Pathology
University of Georgia
Georgia Experiment Station
Griffin, GA 30223.

Tel +1 770 412 4011
Fax +1 770 228 7305

B G Cassidy
Plant Biology Division
The Samuel Roberts Noble Foundation
P O Box 2180-2510
Highway 199 East
Ardmore, OK 73402

Tel +1 405 223 5810
Fax +1 405 221 7380
E-mail: BGCASSIDy@noble.org

RA 00308

About ICRISAT

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

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

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

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

Belgian Administration for
Development Cooperation
Rue du Trone, 4
1000 Brussels, Belgium

ISBN 92-9066-358-8 CPE 109 Printed at ICRISAT Asia Center 393-96