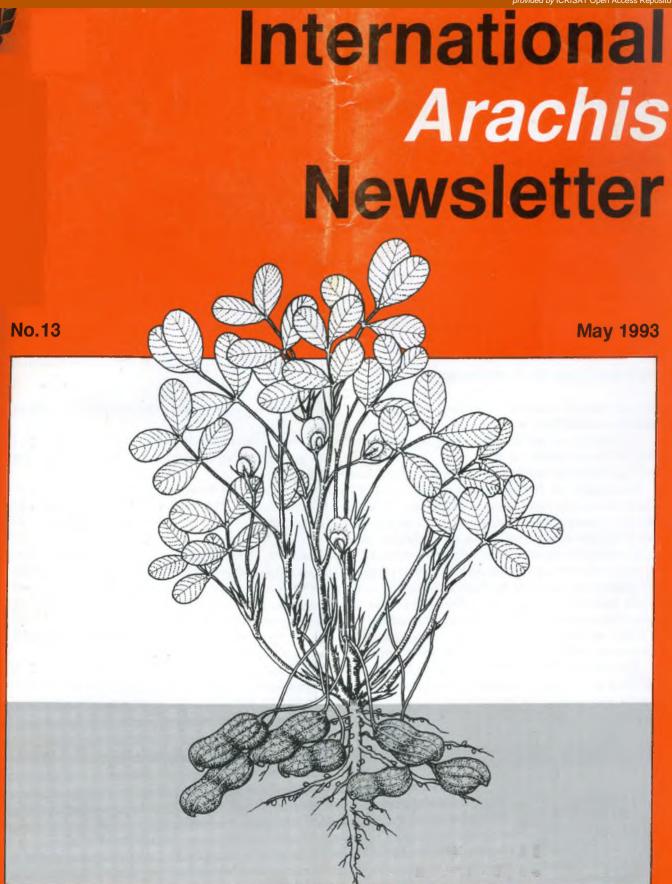
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Legumes Program, ICRISAT, Patancheru, Andhra Pradesh 502 324, India

# **International Arachis Newsletter**

Co-sponsors



Peanut Collaborative Research Support Program (Peanut CRSP)



### **Publishing Objectives**

The International Arachis Newsletter is issued twice a year by the Legumes Program, ICRISAT, in cooperation with the Peanut Collaborative Research Support Program, USA. It is intended as a communication link for workers throughout the world who are interested in the research and development of groundnut or peanut (*Arachis hypogaea* L.), and its wild relatives.

The Newsletter is therefore a vehicle for the publication of brief statements of advances in scientific research that are generally vetted within ICRISAT and have current-awareness value to peer scientists, particularly those working in developing countries. Contributions to the Newsletter are selected for both their news interest and their scientific content, in all expectation that the work reported may be further developed and formally published later in refereed journals. It is thus assumed that Newsletter contributions will not be cited unless no alternative reference is available.

### Style and Form for Contributions

We will carefully consider all submitted contributions and will include in the Newsletter those that are of acceptable scientific standard and conform to the requirements given below.

The language of the Newsletter is English, but we will do our best to translate articles submitted in other languages. Authors should closely follow the style of the reports in this issue. Contributions that deviate markedly from this style will be returned for revision, and could miss the publication date.

If necessary, we will edit communications so as to preserve a uniform style throughout the Newsletter. This may shorten some contributions, but particular care will be taken to ensure that the editing will not change the meaning and scientific content of the article. Wherever we consider that substantial editing is required, we will send a draft copy of the edited version to the contributor for approval before printing.

A communication should not exceed 600 words, but may, in addition, contain a maximum of two relevant and wellprepared tables (width not to exceed 17 cm or 85 characters in the typed version), or figures, or diagrams, or photographs. All photographs should be good quality black-and-white prints on matt (nonglossy) surface paper in 80 mm or 170 mm width (sent with negatives if possible). Color transparencies or color prints will not be accepted. Photos should be identified on a label attached to the back with author's name and figure number. They should not be folded, or written on. Captions or legends should be typed on separate sheets, also clearly identified. Electron micrographs or binocular microscope photographs should indicate the magnification in their caption.

Each communication should normally be confined to a single subject and should be of primary interest to scientists working on this crop. The references cited should be directly relevant and necessary to supplement the article's content (please see earlier issues for correct style in quoting references). SI units should be used. Yields should be reported in t ha<sup>-1</sup>.

All contributions should be typed in double spacing or preferably submitted on double-sided/high-density IBMcompatible diskettes, as Word Perfect 5.1 or ASCII files, together with a double-spaced printout.

All communications and requests for inclusion in the mailing list should be addressed to: •

The Editor International Arachis Newsletter Legumes Program ICRISAT, Patancheru Andhra Pradesh 502 324 INDIA

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# **News and Views**

# Editorial

This issue of the Newsletter comes to you with a new cover, and we hope our readers, especially those who have been receiving the Newsletter since its inception in 1987, will welcome this change. Also, we have brought in some changes in the cover verso, to reflect and appreciate the role of our cosponsors, the Peanut Collaborative Research Support Program, USA, in supporting this Newsletter.

In addition to research reports, this issue carries two reports on disease surveys, one in Nepal and the other in Swaziland. We have also included a report on 'Opportunities for increasing groundnut production in Pakistan' in this section. We invite our readers to submit reports of this nature, especially from those of you who have firsthand information on the countries where groundnut production is on the increase, or where there exists a great potential. We also solicit information on the problems affecting groundnut production and on the importance of the crop in the changing agricultural scenario of different agroclimatic regions.

We appreciate the efforts of K. Ramana Rao and A.R.R. Swamy of the Legumes Program for their assistance in the compilation and computer entry of the manuscripts for this Newsletter.

L.J. Reddy G.V. Ranga Rao

# About ICRISAT Groundnut Scientists and Research Fellows

- John A. Wightman, Principal Scientist (Entomology) and Groundnut Group Leader, returned to ICRISAT Center upon the conclusion of his sabbatical in Australia and the USA in December 1992.
- C.L.L. Gowda, formerly Senior Scientist (Plant Breeding), was appointed as Coordinator of the Cereals and Legumes Asia Network (CLAN) on 1 Jan 1993.
- D.V.R. Reddy, Principal Scientist (Virology), returned to ICRISAT after his study leave in the USA from May 1992 to May 1993, during which he worked with Dr R.J. Shepherd of the University of Kentucky and Dr J.M. Demski of the University of Georgia.

- **R.V. Satyanarayana Rao,** Postdoctoral Fellow in Groundnut Entomology, returned to the Indian Agricultural Research Institute, New Delhi, on 15 Jan 1993 after completing his final project report on 'Screening for resistance to groundnut leaf miner *Aproaerema modicella* (Deventer) in *Arachis hypogaea* and wild *Arachis* species.'
- Maria Luz J. Sison, Research Fellow in Groundnut Entomology, completed her research on the topic 'Groundnut leaf miner *Aproaerema modicella* (Deventer) and its natural enemies on eight groundnut genotypes' and left on 14 Mar 1993 to rejoin her parent organization, the Institute of Plant Breeding in Los Banõs, the Philippines.
- S.D. Golombek joined the Crop Physiology Unit as Postdoctoral Fellow on 10 Mar 1993. She is working on the topic 'Influence of soil temperature at root and pod zones on assimilate translocation to the pods in groundnut.'
- M. Satya Prasad completed his two-year assignment as Postdoctoral Fellow in the Cell Biology Unit on 28 Mar 1993. During his stay at ICRISAT, he worked on the topic 'Tissue culture and transformation studies in groundnut.'

# **Reports on Meetings/Tours**

# Study Tour of On-farm Research Trials in Nepal and Vietnam

The study tour was cosponsored by the FAO-RAS/89/040 Project and ICRISAT and hosted by the national programs of Nepal (Nepal Agricultural Research Council) and Vietnam (Ministry of Agriculture and Food Industry). The main objective was to visit the on-farm adaptive trials conducted under the Asian Grain Legumes On-farm Research (AGLOR) project. Eighteen participants from 10 member-countries of RAS, Food and Agriculture Organization (FAO), and ICRISAT visited AGLOR trials in Nepal (8-12 Feb 1993); and 28 participants from 12 countries, FAO, the Centre for Regional Coordination of Research and Development of Coarse Grains, Pulses, Roots, and Tuber Crops in Humid Tropics of Asia and the Pacific (CGPRT), the Asian Development Bank (ADB), and ICRISAT visited the AGLOR trials in Vietnam (15-17 Feb 1993). In both countries, the participants gained information on the procedures followed in the planning and conduct of on-farm trials. Discussions with farmers and extension staff from the country programs provided an insight into the progress of the

AGLOR project. Four study groups (on constraint identification, planning of on-farm trials, farmer participation, and dissemination of technology) were formed to collect information that could be used later by the discussion groups at the Regional Workshop on On-farm Adaptive Research, Ho Chi Minh City, Vietnam, held during 18– 20 Feb 1993.

> C.L.L. Gowda ICRISAT Center

# Regional Workshop on On-farm Adaptive Research (OFAR), Ho Chi Minh City, Vietnam

The workshop was cosponsored by the FAO-RAS/89/040 Project, CGPRT Centre, and ICRISAT in collaboration with the Ministry of Agriculture and Food Industry, Vietnam, and was held during 18-20 Feb 1993. Thirty-two scientists from the RAS project countries (Bangladesh, China, Indonesia, Laos, Myanmar, Nepal, Pakistan, the Philippines, South Korea, Sri Lanka, Thailand, and Vietnam) and from FAO, CGPRT Centre, ICRISAT, and ADB participated in the workshop. The AGLOR project countries (Indonesia, Nepal, Sri Lanka, and Vietnam) presented on-farm research case studies. This was followed by reports on the on-farm research program and methodologies followed by the different countries, and the experience of other organizations in Asia. The participants appreciated the progress made by the AGLOR projects in the four countries, and made several suggestions to improve the quality of the experiments, the conduct of trials, the involvement of extension staff in on-farm research, and the efforts needed to disseminate technology and assess impact. Group discussions were held on the observations of the monitoring tour, the issues raised at the workshop, and to formulate recommendations. The salient points made by each study group are given below.

#### **Constraint Identification and Diagnosis**

- Common/uniform terminology relating to on-farm research should be used by all countries to avoid confusion.
- Constraints relating to specific areas should be identified using standard data supported by information gathered through rapid rural appraisal methods.
- · Prioritization of constraints should be done on the

basis of spatial and temporal occurrence and yield loss estimates, taking into consideration economic advantages (or disadvantages) rather than yield alone.

- Complex problems identified by farmers should be broken down into components to find possible solutions.
- Reconnaissance and follow-up surveys should be conducted at different stages of crop growth, and feedback sought from farmers on the constraints identified.

#### Planning of Experiments to Alleviate Constraints

- The success of OFAR depends on the soundness of planning and implementation.
- Researchers, extension staff, and farmers must work together throughout the whole process. However, OFAR may require major inputs from different sources depending on the stage of the developmental process.
- The number of treatment factors in OFAR should be kept to the barest minimum. Interaction among different factors should be accounted for. The combination of factors must be location-specific depending on the need to alleviate a constraint.
- Appropriate controls and replication procedures must be followed. Assessment must be made whether replications should be done on the same field or on a different one.

#### **Farmer Participation**

- The primary target group of OFAR are the farmers with small holdings who farm in less favorable environments, with limited resources. However, better-endowed farmers should not be excluded from any improved technology-transfer process.
- Due to the dual role of farmers as clients and informants, it is essential that they are involved in all stages of OFAR.
- Feedback from farmers must be ensured throughout the research and development process.
- In view of OFAR's essential contribution to agricultural development and increased production and productivity, it should be institutionalized and given a long-term perspective and support funding by national governments.
- Appropriate national policies relating to OFAR should be in place before the research process is started.
- Within the scope of the ongoing research programs, researchers and environmental specialists should ensure the long-term sustainability of the ecosystems.

• The workshop recognized the role of women in food legume and coarse grain production, and recommended that special efforts be made to involve women farmers actively in the OFAR process.

# Dissemination and Diffusion of Technology and Assessment

- The improved technology package should be flexible, with a range of options adaptable to specific situations. Appropriate feedback mechanisms should be incorporated into OFAR so that farmers' needs and perceptions can be continually monitored.
- The lack of inputs, especially good quality seeds and capital to buy inputs, are the major external constraints that should be addressed by the governments and NGOs.
- Efforts should be made to expand the role of extension workers so that they can reach a large number of farmers and involve them in technology adaptation.
- Concerted efforts should be made to disseminate information regarding research results, improved technology, and success and failure of new technology, etc. through the mass media and the extension network.
- Government policy support is needed to develop extension skills, supply inputs, and provide credit facilities.
- Technical and economic assessment should be done at each stage of the research and development process to monitor progress, refine technology, and to assess adoption and impact.
- There should be a conscious effort to identify and collect reliable data and information that will allow assessment of the impact of new technology.
- Economists should be involved at all stages of the research and development process so as to provide uniformity, standardization, and integration of all the information that is required for economic evaluation and impact assessment.

C.L.L. Gowda ICRISAT Center

# A Cooperative Project on Water-use Efficiency (WUE) in Grain Legumes

A collaborative research project on 'Selection for wateruse efficiency (WUE) in grain legumes,' involving the Australian Centre for International Agricultural Research (ACIAR), the Indian Council of Agricultural Research (ICAR), and ICRISAT began on 1 Jul 1993 in India. As part of this project, multilocational experiments on groundnut are being conducted at ICRISAT Center and six selected ICAR research institutes in India. The main aim of these experiments is to examine variability in groundnut for WUE and partitioning, and genotype  $\times$ environment interactions for these traits. Some basic studies on WUE in other grain legumes (chickpea and cowpea) are being conducted at the Crop Physiology Department, University of Agricultural Sciences, Bangalore, to examine the relationship between WUE and carbon isotope discrimination. The project's activities are being coordinated by the project leaders, Dr G.C. Wright (ACIAR), Dr M.S. Basu (ICAR), and Dr R.C. Nageswara Rao (ICRISAT).

As a prelude to this project, a methodology workshop on 'Selection for water-use efficiency and partitioning in groundnut' was held at ICRISAT Center during 5–7 May 1993. The objectives of the workshop were:

- To share information on drought research in groundnut with special reference to water-use efficiency; and
- To formulate a technical work plan on the conduct of the experiments, data collection, analysis, and reporting of results of the multilocational experiments.

More than 30 scientists from ACIAR, ICAR, and ICRISAT participated in the workshop and exchanged information on drought research in groundnut and wateruse efficiency. The utility of carbon isotope discrimination and specific leaf area as indirect measures of WUE in groundnut were discussed in detail. The collaborating scientists had hands-on experience on some methodologies, e.g., growth analysis, measurement of radiation interception, operation of portable rainout shelters and drip irrigation system, being used in the research project. During the workshop, the collaborators also formulated a technical work plan for the multilocational experiments.

#### R.C. Nageswara Rao ICRISAT Center

# **Recent ICRISAT Publications**

ICRISAT (International Crops Research Institute for the Semi-Arid Tropics). 1992. Groundnut elite germplasm ICGV 86564. Plant Material Description no. 38. Patancheru, A.P. 502 324, India: ICRISAT. 4 pp. ISBN 92-9066-260-3. Order code: PME-038. (Single copy free.)

ICRISAT (International Crops Research Institute for the Semi-Arid Tropics). 1992. Groundnut variety ICGS 35

(ICGV 87127). Plant Material Description no. 39. Patancheru, A.P. 502 324, India: ICRISAT. 4 pp. ISBN 92-9066-261-1. Order code: PME 039. (Single copy free.)

**ICRISAT** (International Crops Research Institute for the Semi-Arid Tropics). 1992. Groundnut variety ICGS 114. Plant Material Description no. 45. Patancheru, A.P. 502 324, India: ICRISAT. 4 pp. ISBN 92-9066-270-0. Order code: PME 045. (Single copy free.)

Mehan, V.K., and Hayward, A.C. (eds.) 1993. Groundnut bacterial wilt: proceedings of the Second Working Group Meeting, 2 Nov 1992, Asian Vegetable Research and Development Center, Tainan, Taiwan. (In En. Summaries in En, Fr, Es.) Patancheru, A.P. 502 324, India: International Crops Research Institute for the Semi-Arid Tropics. 32 pp. ISBN 92-9066-264-6. Order code: CPE 083.

Moss, J.P. (ed.) 1992. Biotechnology and crop improvement in Asia. (In En. Summaries in Fr, Es.) Patancheru, A.P. 502 324, India: International Crops Research Institute for the Semi-Arid Tropics. 396 pp. ISBN 92-9066-198-4. Order code: BOE 020.

Nigam, S.N. (ed.) 1992. Groundnut—a global perspective: proceedings of an International Workshop, 25–29 Nov 1991, ICRISAT Center, India. (In En. Abstracts in En, Fr, Es.) Patancheru, A.P. 502 324, India: International Crops Research Institute for the Semi-Arid Tropics. 548 pp. ISBN 92-9066-239-5. Order code: CPE 081. Price: India: Rs. 521.00, LDCs: US \$34.11, HDCs: US \$88.91.

Reddy, D.V.R., Moss, J.P., and McDonald, D. (eds.) 1992. Transformation and regeneration of groundnut, and utilization of viral genes to induce resistance to viral diseases: summary and recommendations of a meeting, 24–27 Apr 1992, Virology Department, Wageningen Agricultural University, Netherlands. (In En. Summaries in En, Fr.) Patancheru, A.P. 502 324, India: ICRISAT (International Crops Research Institute for the Semi-Arid Tropics). 24 pp. ISBN 92-9066-237-9. Order code: CPE 080.

Reddy, D.V.R., Wongkaew, S., Xu, Z.Y., Kuhn, C.W., Cassidy, B.G., Shukla, D.D., Saleh, N., Middleton, K.J., Sreenivasulu, P., Prasada Rao, R.D.V.J., Senboku, T., Dollet, M., and McDonald, D. 1993. Peanut stripe virus. Information Bulletin no. 38. (In En. Summaries in En, Fr, Es.) Patancheru, A.P. 502 324, India: International Crops Research Institute for the Semi-Arid Tropics, and Griffin, GA 30223, USA: Peanut Collaborative Research Support Program. 20 pp. ISBN 92-9066-262-X. Order code: IBE 038.

Waliyar, F., Ntare, B.R., and Williams, J.H. (eds.) 1993. Summary Proceedings of the Third ICRISAT Regional Groundnut Meeting for West Africa, 14–17 Sep 1992, Ouagadougou, Burkina Faso. (In En, Fr.) Patancheru, A.P. 502 324, India: International Crops Research Institute for the Semi-Arid Tropics. 224 pp. ISBN 92-9066-271-9. Order code: CPE/F 084.

# News from Peanut CRSP

# Workshops

A workshop on 'Improving production and quality of peanut in the Caribbean' was held at the Mandeville Hotel, Mandeville, Jamaica, during 12–14 Jan 1993. The workshop was sponsored by the Caribbean Agricultural Research and Development Institute (CARDI), the Jamaica Rural Agricultural Development Authority, and the Peanut CRSP. The 60 people who attended the workshop represented farmers, extension workers, NGO/PVO representatives, cooperative leaders, processors, researchers, administrators, and the Jamaican Minister of Agriculture. Ideas were exchanged that should help in the transfer of production and postharvest technologies in Jamaica, Belize, and other CARDI countries.

# **Publications**

A publication titled 'Thailand—Information on peanut virus diseases' generated from the Peanut CRSP-supported project is being published. The cost of publication will be borne by Peanut CRSP at the request of the Department of Agricultural Extension, Ministry of Agriculture and Cooperatives of Thailand. The book is intended for use by academics and extension workers and will concentrate on diagnosis, viral biology, and control measures. Although the text is in Thai, color illustrations will be self-explanatory (84 pictures). The book may be obtained from Dr Sopone Wongkaew, Department of Plant Pathology, Khon Kaen University, Khon Kaen, Thailand. The publication is free of cost.

The Peanut CRSP has published 'Technology transfer: development of peanut processing in Huay-Bong-Nua village, Thailand', Research Report 92-03. Copies may be obtained from the Peanut CRSP Management Office, The University of Georgia, Georgia, Georgia Station, Griffin, GA 30223-1797, USA.

# Personnel

- Barbara Donehoo, Administrative Secretary for the Peanut CRSP Management Office, retired on 30 Apr 1993 after 10 years of service to the CRSP and 20 years at The University of Georgia, Georgia Station. We will miss her valuable service to the CRSP.
- Belinda Purser, formerly Accountant in the Georgia Station Business Office and Secretary in the Department of Plant Pathology assumed charge as Administrative Secretary for the Peanut CRSP Management Office on 1 May 1993. We welcome her to Peanut CRSP.
- Keith Ingram has been selected for the new position of Assistant Program Director in the Peanut CRSP Management Office. He is presently Agronomist and Deputy Division Head, Agronomy, Plant Physiology & Agroecology Division, International Rice Research Institute, Los Banõs, the Philippines. His research has dealt with water relations and drought resistance of rice, and effects of global climatic change on rice production. He has also participated in the cooperative research conducted in Bangladesh, India, Indonesia, the Philippines, and Thailand. He will bring environmental and sustainability expertise to Peanut CRSP. He will assume charge on 15 Oct 1993. We welcome him to the Program.

### Thailand

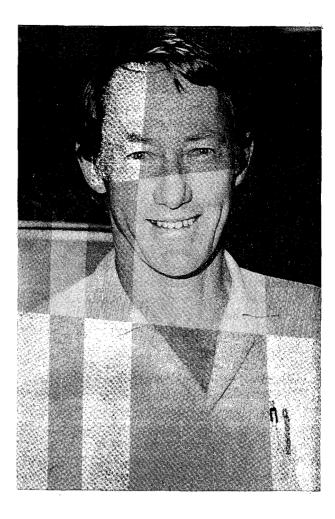
• Sopone Wongkaew, Peanut CRSP Virus Disease Collaborator at Khon Kaen University (KKU), Thailand, is now Head of the Department of Plant Pathology at KKU. He continues to perform his research duties. He will serve in this capacity until October 1995. The Department's staff has 12 members with Ph.D. degrees and one with an M.Sc. degree. It offers B.Sc. and M.Sc. degree programs.

# Obituary

## Keith John Middleton (1943-1993)

It is with great sadness that we announce the sudden death of Keith Middleton on 14 Jan 1993. Keith was a prominent groundnut pathologist and was highly regarded by groundnut scientists throughout the world.

Keith was born on 17 Oct 1943 in Toowoomba, Queensland, Australia. He spent his entire professional



career with the Queensland Department of Primary Industries (QDPI), which he joined in 1967, and was initially based in Toowoomba. In April 1975 he was transferred to Kingaroy, where his research and extension activities had an enormous impact on groundnut production in Queensland. His discovery of the close relationship between Sclerotium rolfsii damage and low levels of soil organic matter led to cultural recommendations with regard to stubble retention. The subsequent widespread adoption of stubble-retention practices by farmers in southern Queensland meant the disease ceased to cause further economic losses. A significant part of his research activity was directed towards the control of the foliar pathogens causing leaf spot and rust. He investigated aspects such as the biological spectrum of fungicides, pesticide application technology, economic injury levels, and genetic resistance. His work resulted in significant reductions in the quantity of fungicides used to control groundnut foliar diseases in Queensland, without resulting in yield losses. While this work had a significant impact on the profitability of the Queensland groundnut industry, it also gained recognition in the other groundnut-producing regions of the world. In 1980 he was invited to provide a consultancy to the University of Georgia, USA, on spraying technology in groundnut. Later that year he was invited to be the Australian delegate to an international workshop on groundnut at ICRI-SAT, where many aspects of his research on foliar diseases were presented.

When the Queensland groundnut industry was confronted with a serious aflatoxin contamination in harvested crops in the late 1970s, Keith, along with other industry representatives, investigated procedures to process contaminated stock. After an investigative visit to the USA where similar problems had been experienced, they formulated policies that have been successfully used to manage the high incidence of aflatoxin contamination in Australia's rainfed groundnut crop.

Keith's work on the international scene was as significant as his Australian endeavor. He was involved in an Australian Centre for International Agricultural Research (ACIAR) project in Indonesia, a component of which aimed to identify and develop control practices for the major groundnut diseases occurring there. Keith coordinated and managed much of this collaborative research which identified two major disease constraints, bacterial wilt (*Pseudomonas solanacearum*) and peanut stripe virus (PStV). He was actively involved in organizing a Bacterial Wilt Research Coordination Meeting in Malaysia in 1990, which brought scientists from many countries together to discuss methods of control.

He was a leading authority on PStV, and was instrumental in fostering collaboration between Indonesian and ICRISAT scientists which enabled the screening of more than 9000 entries out of the 11 800 in the world groundnut germplasm collection for resistance to PStV. Although the search for genetic resistance to PStV was unsuccessful, it did not take Keith long to initiate and coordinate another project with a multiaspect, biotechnical approach to the control of the virus. This project is now in progress. It seeks to generate transgenic groundnut plants with genetically engineered resistance to PStV infection due to accumulation of viral coat protein. Keith saw the threat posed by PStV to the Australian groundnut industry and knew that his efforts on the international scene may one day be valuable if the virus ever enters Australia.

Keith was the author or coauthor of over 50 scientific, conference, and newsletter papers, and coedited the AC-IAR proceedings on 'Bacterial Wilt of Groundnut' and 'Peanut Improvement: A Case Study in Indonesia.' His interest in food legumes research was also evident by his willingness to edit the ACIAR Food Legume Newsletter. His flair for managing research programs and getting people together to work on common problems was recognized by the QDPI in 1992 when he was appointed as the Field Crops Manager for southeastern Queensland. Keith had many lucrative offers to take up employment in other organizations around the world. He was, however, loyal to his employer and stayed with QDPI throughout his professional career. This sense of loyalty was an allencompassing feature of Keith's personality. He could always be relied upon to give good advice, assist in any problem, or to just have a good chat.

Keith Middleton had an immense practical knowledge of the whole spectrum of groundnut diseases and their control. His passing away is particularly tragic because this fund of knowledge has been prematurely lost. He had so much more to do. His impact on the people he worked with is still there however, and many of us are grateful for having had the opportunity to work with him and to learn from him. Keith Middleton is survived by his wife Betty, and two daughters, Kerri and Jillian.

# Reports

### **Diseases of Groundnut in Swaziland**

P. Subrahmanyam<sup>1</sup> and Z.J. Mamba<sup>2</sup>

(1. SADC/ICRISAT Groundnut Project,
 P.O. Box 1096, Lilongwe, Malawi;
 2. Malkerns Research Station, P.O. Box 4,
 Malkerns, Swaziland)

Groundnut (Arachis hypogaea L.) is an important leguminous crop in Swaziland. Though the crop is grown by farmers with small holdings in most parts of the country, production is largely concentrated in the *Middleveld* areas (mean elevation about 700 m, annual rainfall 650– 1150 mm). The average yield of groundnut has been very low, about 0.5 t ha<sup>-1</sup>, in marked contrast to yields of over 4.5 t ha<sup>-1</sup> obtained at research stations under good management conditions (Rao and Masina 1987). Diseases, among other factors, are one of the major constraints to groundnut production in Swaziland (Rao and Masina 1989, Rao and Mkhabela 1990).

In collaboration with the Ministry of Agriculture and Cooperatives, Swaziland, we conducted a survey in March 1993 to assess the relative importance of various diseases of groundnut in farmers' fields in the major groundnut-producing areas of Swaziland. Thirty farmers' fields were visited: 5 in the *Highveld* (mean elevation about 1300 m, annual rainfall 1000–2300 mm), 21 in the *Middleveld*; and 4 in the *Lowveld* (mean elevation about 200 m, annual rainfall 500–900 mm) areas. Three research farms were also visited (Fig. 1). The crop was at pod-filling to nearing maturity stages. In almost all the areas, groundnut was grown as a sole crop with or without rotation. The commonly grown types were spanish (cv Natal Common) and valencia (variety unknown) or a mixture of these two.

Rust (*Puccinia arachidis* Speg.) and late leaf spot [*Phaeoisariopsis personata* (Berk. & Curt.) van Arx = *Cercosporidium personatum* (Berk. & Curt.) Deighton] were the most predominant and destructive diseases of groundnut in the *Middleveld* (Empatheni, Debedebe, Mahlalini, Nhlangano, Luve, and Bhekinkosi) and the *Lowveld* (Sidvokodvo, near Mliba) areas, causing almost 100% damage to the foliage. These diseases were also very serious at the Malkerns, Luve, and Nhlangano research stations. Early leaf spot (*Cercospora arachidicola* Hori) and web blotch [*Didymella arachidicola* (Chock.) Taber, Pettit & Philley] were present in most of the fields surveyed but were especially serious in the *Highveld* in-

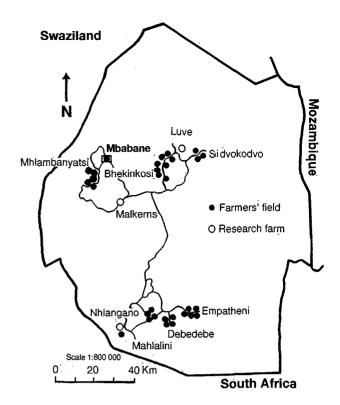


Figure 1. A map of Swaziland showing the route followed during the survey of groundnut diseases in March 1993.

cluding the areas of Siphocosini, Singangeni, and Mantabeni. The effect of crop rotation on the severity of both leaf spots and web blotch was spectacular. These foliar diseases were most severe in groundnut crops grown without any rotation. However, rust was severe in all fields irrespective of crop rotation.

Both chlorotic and green rosette were present in all the fields visited. However, disease incidence was very high (over 50%) only in late-sown (mid December 1992) groundnut. It was interesting to note that green rosette, which is the most common and destructive viral disease of groundnut in West Africa, was more predominant than chlorotic rosette in some fields near Sidvokodvo.

Other diseases observed during this survey included leaf scorch [Leptosphaerulina crassiasca (Sechet) Jackson & Bell], rhizoctonia leaf blight (Rhizoctonia solani Kühn), phyllosticta leaf spot (Phyllosticta arachidis-hypogaea Vasant Rao), collar rot (Aspergillus niger van Tieghem), stem and pod rots (Sclerotium rolfsii Sacc.), peanut mottle [peanut-mottle-virus-(PM-V)], groundnut streak necrosis [sunflower yellow blotch virus (SYBV)], and witch weed (Alectra vogelii Benth.). Plants showing typical symptoms of scab (Subrahmanyam et al. 1992) were observed at Luve, Sidvokodvo, and Malkerns. A microscopic examination of diseased samples at the Malkerns Research Station did not reveal the presence of any sporulating structures. Further investigations are required to confirm the etiology.

Although both early and late leaf spots, and web blotch diseases can be very effectively controlled by using fungicides like chlorothalonil, this practice may not be economically feasible for small farmers in Swaziland. The development of high-yielding disease-resistant genotypes appears to be the best means of containing these diseases. In the past, several high-yielding rust- and/or late leaf spot-resistant breeding lines developed at ICRI-SAT Center, India, were evaluated in Swaziland. Some of them, e.g., ICG (FDRS) 4 have performed well (Rao and Masina 1987). On-farm testing of these promising genotypes should be initiated. Early sowing and crop rotation should be beneficial in reducing the severity of leaf spots and web blotch.

High-yielding rosette-resistant breeding lines developed at the Southern African Development Community (SADC)/ICRISAT Groundnut Project, Malawi, should be evaluated and made available to farmers in Swaziland. Farmers should be advised to sow groundnut with the first spring rains (from October to early November) at optimum densities in order to minimize rosette disease incidence. Selection of good quality seed, seed treatment with a suitable chemical (e.g., thiram or captan), and sowing at optimum depth should be beneficial in achieving optimum plant densities.

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# First Report of Pepper Spot and Leaf Scorch on Groundnut in Nepal

S. Pande<sup>1</sup>, B.P. Sharma<sup>2</sup>, G.V. Ranga Rao<sup>1</sup>, J.V.D.K. Kumar Rao<sup>1</sup>, G. Koirala<sup>2</sup>, J. Narayana Rao<sup>1</sup>, D. McDonald<sup>1</sup>, and M. Joshi<sup>3</sup> [1. ICRISAT Center; 2. National Oilseed Research Program (NORP), Sarlahi, Nepal; 3. Nepal Agricultural Research Council (NARC), Kathmandu, Nepal]

Pepper spot and leaf scorch disease of groundnut caused by the fungus *Leptosphaerulina crassiasca* (Sechet) Jackson & Bell, has been reported from several groundnut-growing regions of the world (Jackson and Bell 1969, Subrahmanyam et al. 1992). The disease was observed for the first time both in farmers' fields and research stations in Nepal during the 1992 rainy season. Both pepper spot and leaf scorch symptoms of the disease were observed.

The pepper spot phase was characterized by minute necrotic spots on the lower leaves, close to the soil surface. These spots were numerous, usually of pinhead size, in shades ranging from dark brown to black, and irregular to circular in shape. They were usually found on the upper surface of the leaflets but a few lesions were also seen on the lower surface. Leaf scorch was the most common symptom, and it was found developing usually from the tip of the leaflets. The wedge-shaped lesions had a bright yellow zone along the periphery of their advancing margins. The necrotic tissues were dark brown and tended to fragment along the leaflet margins.

Of the seven major groundnut-producing districts surveyed, the disease was found in Sarlahi, Chitwan, Rauthat, and Nawalparasi. The disease was not severe in farmers' fields except on a local groundnut cultivar at the research stations in Nawalpur, Sarlahi district, and in Rampur, Chitwan district. At these research stations, lo-

cal groundnut cultivars had up to 10% of the leaf area damaged. The disease was not found in the groundnutgrowing areas of Sunsari, Parsa, and Bara districts. Currently the disease is of only minor importance in Nepal, but its presence will have to be taken into consideration when introducing new cultivars.

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## **Opportunities for Increasing Groundnut Production in Pakistan**

Naazar Ali<sup>1</sup> and S.N. Nigam<sup>2</sup> [1. Barani Agricultural Research and Development Project (BARD), Pakistan; 2. ICRISAT Center]

In Pakistan Groundnut is grown over 80000 ha with approximately 84% of the crop area falling in the Punjab province, 11% in the Northwest Frontier Province, and 5% in the Sindh province. The average pod yield is 1.1 t ha<sup>-1</sup> but yields in the 'barani' (rainfed) areas, where the soils are sandy, are lower. Groundnut is grown mainly for direct consumption and confectionery use. There is potential for edible groundnut oil production in Pakistan given favorable market conditions.

Till recently, only two groundnut cultivars were available to farmers in Pakistan for rainy-season sowing. The spreading variety no. 334 is the oldest and most commonly grown cultivar in Pakistan. It matures in 180–200 days, has a relatively stable but low level of productivity, and does not respond to improved management practices. In 1973, a virginia bunch cultivar, Banki, was introduced in the country. It matures in 160–180 days. Though somewhat more responsive to improved management practices, it was not adopted by the farmers for large-scale cultivation.

Since 1984, Pakistan's Barani Agricultural Research and Development Project (BARD) has been evaluating introduced germplasm and breeding material, obtained mainly from ICRISAT, to identify the variety most suitable for cultivation in the country. In this effort, BARD has been assisted by the International Development Research Centre. These efforts have led to the identification and release of the following groundnut cultivars in Pakistan.

#### **BARD 699**

BARD 699, released in 1991, is a composite of ICGS 37 and ICGS 44 bulked in equal proportion. Both ICGS 37 and ICGS 44 originate from a natural hybrid population of Kadiri 3 and were developed at ICRISAT Center, India. They belong to the spanish group and have a semibunch growth habit. Both have two-seeded, mediumsized, smooth pods which are slightly beaked and constricted. Their tan-colored seeds have 52% oil and 27% protein, and weigh 41 g (100-seeds)<sup>-1</sup>.

As both ICGS 37 and ICGS 44 performed better than Banki and no. 334 in replicated yield trials during 1987– 1989 and looked phenotypically alike, they were bulked to form BARD 699 to achieve stability in production.

BARD 699 performs well in the medium-to-high rainfall zones of 'barani' areas and in the irrigated production system. It has consistently produced a 7 to 90% higher pod yield than Banki or no. 334. In on-farm trials, BARD 699 produced an average pod yield of 1.7 t ha<sup>-1</sup> compared to 1.3 t ha<sup>-1</sup> of no. 334. Its shelling percentage (70) is greater than that of the local cultivars. Its seeds can be used for both oil extraction and confectionery purposes. It matures in 150–160 days, which is about 3 to 4 weeks earlier than Banki.

#### **BARD 479**

BARD 479 was selected from a germplasm line, ICG 4989 (PI 270259, Natal Red), obtained from ICRISAT Center, India, in 1984. It is a semispreading groundnut variety maturing in 170–180 days under 'barani' conditions. Its mainly two-seeded rough pods have a moderate beak and moderate to deep constriction. Seeds of BARD 479 are large [60.5 g (100 seeds)<sup>-1</sup>] and are reddish brown. They contain 51% oil.

BARD 479 performs well under a wide range of 'barani' conditions. Increases in pod yield in BARD 479 have ranged from 31 to 71% over no. 334 and 20 to 42% over Banki in large-scale trials. In these trials BARD 479 produced an average yield of 2.1 t ha<sup>-1</sup> compared to 1.5 t ha<sup>-1</sup> of Banki and 1.2 t ha<sup>-1</sup> of no. 334. Its shelling percentage is 62, which is typical of large-seeded virginia types. Although released in 1993 mainly for confectionery use, it can also be grown for its oil.

### **BARD** 92

ICGS(E) 56, an early-maturing variety, included in the International Groundnut Early-maturing Cultivar Trial and received from ICRISAT Center, India, in 1985, has been redesignated as BARD 92. It was approved by the Varietal Evaluation Committee, Pakistan Agricultural Research Council (PARC), in 1993 and is awaiting final release by the National Seed Council, Ministry of Food, Agriculture, and Cooperatives, Government of Pakistan.

BARD 92 is a spanish variety with bunch growth habit. Its pods are mainly two-seeded, medium in size with slight reticulation and slight constriction. Its tan-colored seeds contain 48% oil and weigh 37.5 g (100-seeds)<sup>-1</sup>. The shelling percentage of BARD 92 is 68.5.

BARD 92 has produced an average of 17% higher pod yield than Banki in replicated yield trials conducted during 1985–1990. The average pod yield of BARD 92 in these trials was 1.6 t ha<sup>-1</sup> compared to 1.3 t ha<sup>-1</sup> of Banki. As BARD 92 matures in 120–130 days, it can be sown at the onset of the monsoon and harvested with crops down during May–June, thus avoiding the risk of drought during that period. It fits well in the existing cropping patterns and is suitable for double-cropping with wheat or other postrainy season crops. It is particularly adapted to the 'barani' conditions of the Pothwar area in the country.

With a modest start in 1949 in the Rawalpindi division, the groundnut crop in Pakistan now occupies the second largest area among the oilseed crops in the country. Currently, Pakistan is facing a severe deficit in edible oil. In 1989/90, the estimated deficit in edible oil was 931 000 t. Since then, it has increased further and is likely to grow more in the future. Groundnut offers a good opportunity to reduce this deficit. The area under groundnut in Pakistan increased progressively during the 1980s and has almost doubled since 1980/81. Production has also shown a corresponding increase but productivity has remained stagnant.

Groundnut is sown generally in mid April in fallow lands in 'barani' areas and in mid March in irrigated areas. After the harvest of wheat, poor establishment of the groundnut crop under residual/declining moisture conditions is a major problem. With the availability of short-duration varieties, the groundnut crop can be sown at the onset of the monsoon without affecting the sowing of the second crop in the postrainy season. Earlier, longduration varieties had to be sown in March/April to allow the growing of the wheat crop.

# **Research Reports**

# Influence of Sowing Dates on the Productivity of Three Groundnut Varieties in Punjab, India

J.N. Kaul (Department of Agronomy, Punjab Agricultural University, Ludhiana 141 004, India)

Groundnut (Arachis hypogaea L.), the most important oilseed crop of India, accounts for 55% of the country's total oilseeds production. However, in Punjab its importance has been dwindling ever since high-yielding varieties of rice and wheat were introduced, and the area under groundnut has declined sharply to 11 000 ha in 1991 as against 220 000 ha in 1970, 92 000 ha in 1981, and 58 000 ha in 1984.

Irrespective of whether the crop is grown rainfed or under irrigation, the popular cultivars comprising the spreading and semispreading plant types, mature late, are vulnerable to foliar diseases, and have obviously not brought about a breakthrough in raising productivity. The spanish bunch variety, SG 84 (a local selection from cv ICGS 1), was released in 1986 and recommended exclusively for sowing during the second fortnight of February as a spring-season crop. Two serious problems are experienced in the cultivation of this new plant type during the spring season in Punjab: (1) delayed and poor emergence of seedlings, and (2) the coincidence of harvesting with the rainy season.

On account of these problems, a comprehensive dateof-sowing trial was conducted with three varieties during 1989–91 at the research station of the Punjab Agricultural University, Ludhiana. Three groundnut varieties representing three plant types, i.e., spreading (M 145), semispreading (M 197) and spanish bunch (SG 84) were sown at fortnightly intervals from 8 Feb to 23 Jun and grown under seasonal variations (spanning spring, summer, and rainy seasons).

From the data on pod yield (mean of three years) presented in Table 1, it can be seen that SG 84 yielded more than the other two varieties. It recorded a mean yield of  $3.11 \text{ th}a^{-1}$  when sown on 23 Apr, 78% higher than the 1.74 t ha<sup>-1</sup> yielded in the recommended 23 Feb sowing. It also gave higher pod yields of 42% over M 145, and 9% over M 197. The spreading cultivar, M 145, gave the highest pod yield of 2.19 t ha<sup>-1</sup> in the 8 May sowing, whereas the semispreading cultivar, M 197, gave the highest yield of 2.86 t ha<sup>-1</sup> in the 23 May

Table 1. Pod yield of three groundnut cultivars under different sowing dates at Punjab Agricultural University, Ludhiana, Punjab, India, 1989–91.

	Po	d yield <sup>1</sup> (t ha-	1)
Sowing date	SG 84	M 145	M 197
8 February	1.78	1.35	1.66
23 February	1.74	1.41	1.75
8 March	2.07	1.56	1.98
23 March	2.47	1.61	2.05
8 April	2.74	1.60	2.30
23 April	3.11	1.76	2.31
8 May	2.92	2.19	2.61
23 May	2.53	1.83	2.86
8 June	1.92	1.45	2.13
23 June	1.57	1.40	1.88
LSD at 5%: D	ates × varieties	0.236	
1. Mean of 3 yea	rs.		

sowing. The spanish bunch variety, SG 84, gave higher pod yields than the other two cultivars in a majority of sowing dates.

Apart from vacating the land early, SG 84 can fit easily into the existing cropping systems. This innovation of sowing SG 84 in the summer (end of April to first week of May) has been approved for adoption by farmers

Table 2. Days taken for spanish bunch groundnut cultivar SG 84 to attain different growth stages<sup>1</sup> under different sowing dates at Ludhiana, Punjab, India.

	Duration (days)					
Sowing date	Emergence	Vegeta- tive	Repro- ductive	Total		
8 February	36	41	67	144		
23 February	24	41	67	132		
8 March	18	42	72	132		
23 March	15	35	80	130		
8 April	8	34	100	142		
23 April	7	25	101	133		
8 May	7	26	95	128		
23 May	6	31	80	117		
8 June	6	29	79	114		
23 June	6	29	80	115		

1. Based on growth stages of peanut as described by Boote, K.J. (1982).

of the Punjab state and was included in the 'Package of Practices for Crops, Kharif (rainy season) 1992'.

A perusal of Table 2 on the phenological behavior of the bunch cultivar SG 84 indicates a reduction of 17 days in emergence, and 16 days in the vegetative growth stage and an increase of 34 days in the reproductive phase in the 23 Apr sowing compared to its normal 23 Feb sowing. This increase in the reproductive phase, without affecting the duration of the crop, may be the factor responsible for the higher productivity of SG 84 in the 23 Apr sowing.

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## **Groundnut Genotypes Tolerant** to Lime-induced Iron Chlorosis

# **A. Hadjichristodoulou** (Agricultural Research Institute, Nicosia, Cyprus)

Lime-induced iron chlorosis is a common problem in groundnut in most calcareous soils, which requires the application of iron chelates to overcome. Calcareous soils are very common in the Mediterranean countries and other parts of the world. In Cyprus, the relatively tolerant groundnut variety Cyprus Local expressed significant percentages of iron chlorosis in soils having more than 20 to 25% CaCO<sub>3</sub> and 10% active lime (Papastylianou 1989). Among the various Fe chelates, Fe-EDDHA was the most effective in correcting lime-induced iron chlorosis in groundnuts (Papastylianou 1990).

However, the use of iron chelates to correct iron chlorosis in groundnut adds to the cost of production, and therefore, some areas rich in  $CaCO_3$  may be excluded from groundnut cultivation. The most effective way to avoid iron chlorosis is to use tolerant varieties. In Israel, Hazera 234/73 was selected as a tolerant variety which could give high yields without the application of iron chelates unlike other varieties (Hartzook et al. 1974). In a trial conducted in Cyprus with five varieties in a representative groundnut field, the chlorosis in the control plots was an average of 43%, which was reduced to 13% by the application of iron chelates (Hadjichristodoulou 1990). Cyprus Local was the most tolerant variety fol-

lowed by Hazera 234/73, while the varieties affected most by chlorosis were GK 3 (a variety from the USA), and two ICRISAT lines.

ICRISAT groundnut breeders provided seeds of 102 promising confectionery groundnut varieties for screening for tolerance to chlorosis. Groundnut nurseries were established at two sites in Cyprus, Akhelia and Zyghi, with 4 m long plots of two rows at Akhelia and one row at Zyghi. The soil at the Akhelia station is a calcareous vertic Cambisol underlain by soft calcium carbonate at about 90 cm depth (Orphanos 1992). The calcium carbonate content was 26% at 0–30 cm, 32% at 30–60 cm, and 36% at 60–90 cm depth. The Zyghi field was of clayloam texture at the plow layer and clayey deeper down. The calcium carbonate content was 45–65% at all layers down to 1.5 m depth (Orphanos and Kokkinos 1983).

The nursery was replicated twice at each location. Spacing between rows was 0.45 m. Cyprus Local and Hazera 234/73, the relatively tolerant varieties, were used as controls at every 20th plot. The nurseries were sown on 29 Apr and 14 May, and were harvested on 15 and 22 Oct 1992 at Akhelia and Zyghi respectively. The nurseries were treated as normal groundnut crops, without the application of any chemicals. Chlorosis (in terms of the percentage of yellowed leaves in each plot) was recorded visually three times during the growing season, on 9 Jul, 27 Jul, and 12 Aug 1992 and the mean was taken as the variety score for the location.

Results on tolerance to chlorosis are given for the most promising varieties in Table 1. Pod yields for these varieties are also given, but they must be interpreted with caution as they were obtained from two replications of very small plots.

The mean iron chlorosis was generally higher at Zyghi (22.3%) than at Akhelia (18.2%), and mean pod yield was higher at Akhelia (5.1 t ha<sup>-1</sup>) than at Zyghi (3.6 t ha<sup>-1</sup>). There was no significant difference in mean chlorosis with regard to the dates of recording. There were, however, significant differences among the varieties. The range of the variety means over locations was 3.0-67.1% (2–80% at Zyghi and 0.8-71.7% at Akhelia). The mean chlorosis was 10.9% for Hazera 234/73 and 6.5% for Cyprus Local. ICRISAT varieties which recorded less than 6.5% iron chlorosis (equal to or less than Cyprus Local), and those with more than 50% chlorosis are given in Table 1. The mean pod yield of the 11 tolerant varieties was 5.1 t ha<sup>-1</sup> compared to 2.8 t ha<sup>-1</sup> for the susceptible varieties.

The varieties with less than 6% chlorosis at both locations were ICGVs 90185, 90186, 91093, 90305, and 90326. The following varieties had less than 6% chlorosis at Zyghi: ICGVs 90916, 90157, 90158, 90177,

Table 1. Mean iron chlorosis and pod yield of the most tolerant groundnut varieties (<6.5% chlorosis) and the most susceptible varieties (>50% chlorosis), Cyprus, 1992.<sup>1</sup>

Variety	Iron chlorosis (%)	Pod yield (t ha <sup>-1</sup> )
Tolerant varieties		(******)
ICGV 90162	5.6	5.6
ICGV 90182	3.8	3.8 4.7
ICGV 90185 ICGV 90186	3.0	4.7 5.3
ICGV 90180	5.4	5.5
ICGV 90201 ICGV 90285	5.6	5.2
ICGV 90285	6.2	5.2 5.4
ICGV 90288	2.8	5.4 4.8
ICGV 90303	6.3	4.0 5.5
ICGV 90326	5.8	5.1
ICGV 91092	5.3	5.0
ICGV 91092	5.1	5.0 4.7
1CU v 91095		
Mean	5.0	5.1
Susceptible varieties		
ICGV 90156	55.0	2.8
ICGV 90160	67.1	2.9
ICGV 90171	50.0	3.1
ICGV 90193	59.6	2.3
ICGV 91086	57.1	3.1
Mean	57.8	2.8
Controls		
Hazera 234/73	10.9	4.8
Cyprus Local	6.5	5.9

1. Observations are based on nurseries grown at two locations, Akhelia and Zyghi.

90186, 91093, 91099, 91100, 90305, and 90308 as compared to 10.8% for Cyprus Local. At Akhelia, the following varieties had less than 2.2% chlorosis: ICGVs 90201, 90174, 90184, 90185, and 91098, as against 2.2% for Cyprus Local.

It can be concluded that there is a significant genetic variation in groundnut varieties with regard to the absorption of soil iron in calcium-rich soils. Such ironefficient varieties could be profitably grown without incurring the cost of iron chelates. This material could be screened for yield and for direct use as varieties and also as parents in crosses for the development of material tolerant to lime-induced iron chlorosis.

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## Diseases of Groundnut in Groundnut/ Pigeonpea Intercropping System

S. Pande, J. Narayana Rao, D. McDonald, M.M. Anders, and L.M. Reddy (ICRISAT Center)

Groundnut is an important dryland crop that is commonly grown in intercropping systems by subsistence farmers who use traditional combinations often involving as many as five to six crops (Okigbo and Greenland 1976, Jodha 1981). Groundnut/pigeonpea is the most popular intercropping combination in central and southern India (Ali 1990). It has recently been shown that this combination can give a higher gross return per unit area than sole groundnut (Giri 1990, Kushwaha 1992).

Little is known about how the pests and diseases of groundnut are influenced by the intercropping system in which they occur. In general, an increased severity offoliar diseases has been observed when groundnut is grown in the shade of taller crops. This paper reports some observations on the severity of foliar fungal diseases and the incidence of soilborne diseases of groundnut in a groundnut/pigeonpea intercropping system.

An experiment was conducted in the 1992 rainy season on Alfisols and Vertisols at ICRISAT Center. The groundnut cultivar ICGS 44 was sown in Vertisols on 6 Jun 1992, and ICGS 11 was sown in Alfisols on 25 Jun 1992. The medium-duration pigeonpea cultivar ICP 1-6 (ICP 8858) was sown in a 2:1:2 ratio (groundnut:pigeonpea:groundnut) on broadbed and furrows (BBF) with an interrow spacing of 30 cm between groundnut rows. A sole crop of groundnut was also grown as a control. All the plots received a similar dosage of fertilizer comprising 18 kg N and 46 kg  $P_2O_5$  ha<sup>-1</sup>. The experiment was arranged in a randomized-block design with 54 m<sup>2</sup> plot size in four replications. The rainfall received during the cropping period was 557 mm.

Late leaf spot [*Phaeoisariopsis personata* (Berk. & M.A. Curtis) van Arx] and rust (*Puccinia arachidis* Speg.) were the dominant foliar fungal diseases in 1992. Disease severity was scored on a modified 1–9 point scale (Subba Rao et al. 1990) at 10-day intervals from the time of appearance of the first symptoms, [ $\geq$ 60 days after sowing (DAS)] to maturity. Soilborne diseases (mainly

crown rot caused by *Aspergillus niger* van Tieghem) were also monitored at 10-day intervals from seedling emergence until crop maturity, and the percentage of incidence was recorded.

Late leaf spot and rust developed less rapidly in Alfisols than in Vertisols. Both these diseases were significantly ( $P \le 1\%$ ) less in the sole crop than in the groundnut intercropped with pigeonpea in Alfisols (Table 1). In Vertisols, late leaf spot and rust were equally severe in both the sole and intercropping systems. However, up to 80 DAS, the progress of rust and late leaf spot was slower and was restricted to the lower canopy of the sole crop even in Vertisols.

Soilborne diseases were significantly higher in Vertisols than in Alfisols and occurred in a higher proportion (up to 6%) in the sole crop than in the intercrop. Significantly higher pod and haulm yields were obtained in sole groundnut than in the intercropped plots in Alfisols.

This study suggests that intercropping pigeonpea with groundnut enhances the buildup of foliar fungal diseases in groundnut grown in Alfisols. This may be because of the complete canopy cover and shading of groundnut by the taller companion crop which provides a more congenial microclimate in the intercropped plots than in the sole plots for the proliferation of the pathogen and the disease.

Treatment	Late leaf spot <sup>1</sup>	Rust <sup>1</sup>	Total mortality <sup>2</sup> (%)	Pod yield (t ha-1)	Haulm yield (t ha <sup>-1</sup> )	100-seed mass (g)	Shelling (%)
Alfisols		<u> </u>					
Sole groundnut	7.7	5.6	0.0	0.78	1.90	36.6	71.10
Groundnut/pigeonpea <sup>3</sup>	9.0	9.0	0.0	0.42	1.43	36.9	70.00
SE	±0.04	±0.06	±0.0	±0.006	±0.028	±0.863	±0.550
CV (%)	1.0	1.7	0.0	1.9	3.3	4.7	1.6
Vertisols							
Sole groundnut	9.0	9.0	5.60	0.75	1.19	37.00	<b>69.1</b> 0
Groundnut/pigeonpea	9.0	<b>9</b> .0	3.60	0.30	1.21	36.90	69.40
SE	±0.0	±0.0	±0.706	±0.012	±0.017	±0.508	±0.393
CV (%)	0	0	30.7	4.5	2.9	2.6	1.20

Table 1. Effect of groundnut/pigeonpea intercropping system on groundnut diseases and yield components at ICRISAT Center, rainy season 1992.

1. Severity of late leaf spot and rust were scored at crop maturity (105 days after emergence) on a 1–9 point scale, where 1 = 0%, 5 = 21-30%, and 9 = 81-100% leaf area affected or defoliated.

2. Total mortality based on the number of groundnut plants infected with Aspergillus niger and/or Macrophomina phaseolina.

3. Two rows groundnut : one row pigeonpea : two rows groundnut on broadbed and furrow.

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## Preliminary Investigations on Variability in *Cercospora arachidicola* Hori

P.V. Subba Rao<sup>1</sup>, J.L. Renard<sup>2</sup>, and
D. McDonald<sup>1</sup> (1. ICRISAT Center;
2. CIRAD-CP, P.O. Box 5035, 34032
Montpellier, France)

A study was conducted to investigate variability in conidial length, number of septa, and germination in 16 isolates of *Cercospora arachidicola*, the causal agent of early leaf spot. Components of resistance to these isolates such as symptom type, incubation period, sporulation, lesion number, and defoliation were also studied in seven host genotypes. The isolates originated from 14 groundnut-producing countries from six continents. The genotypes included in the study comprised both cultivated groundnuts and wild *Arachis* spp.

The investigations were carried out as a joint project between ICRISAT, India, and the Centre de Cooperation Internationale en Recherche Agronomique pour le Developpement (CIRAD), France, from October 1990 to December 1992.

Considerable differences in conidial length and number of septa were observed among the isolates. The Nigerian isolate had the longest conidia and the Senegalese isolate the shortest. The Nigerian and Senegalese isolates also had the highest and lowest number of septa per conidium, respectively. Isolates of similar geographic origin did not fall in the same homogenous group. Differences in conidial germination were also observed. The isolate from Brazil had the highest germinability, and the Surinamese isolate the lowest. Temperature influenced conidial germination, and the percentage of conidia which germinated at extreme temperatures (10°C and 35°C) was very low for all isolates except that of Madagascar.

Cercospora arachidicola isolates showed a high degree of variability in symptom appearance. Genotype  $\times$  isolate interactions were observed in (a) days to appearance of the first visible symptoms, (b) intensity of sporulation, (c) total number of lesions per leaf, (d) infection frequency of sporulating lesions, (e) lesion diameter excluding yellow halo, (f) number of sporulating lesions per leaf, (g) percentage of defoliation, and (h) disease index (defoliation  $\times$ lesion diameter  $\times$  intensity of sporulation). The results of the study reveal that isolates of the early leaf spot pathogen *C. arachidicola* vary in their morphology and in their ability to produce disease on groundnut genotypes.

## Testing of Groundnut Genotypes against *Aspergillus niger* under Artificial Inoculation

**R.B. Gaur** and **R.D. Singh<sup>1</sup>** (Rajasthan Agricultural University, Agricultural Research Station, Sriganganagar, Rajasthan 335 001, India. 1. Present address: Professor and Head, Department of Plant Pathology, Agricultural Research Station, Durgapura, Jaipur, Rajasthan 302 018, India)

In Sriganganagar district of Rajasthan state in India, groundnut suffers heavily from collar or crown rot disease caused by *Aspergillus niger* van Tieghem (Gaur and Ahmed 1983). Since the development of resistant varieties is the most effective way of avoiding losses due to the disease, 28 groundnut genotypes procured from ICRISAT and the local breeder were evaluated for their reaction to collar rot disease under artificial inoculation at the Agricultural Research Substation, Hanumangarh, Rajasthan, India.

Seeds of each genotype, artificially inoculated with a thick mycelial and spore suspension of A. *niger* (5 g 100 mL<sup>-1</sup> water), were sown in the field in a 5 m row plot under a randomized-block design. Each entry was replicated four times with an interrow spacing of 50 cm and

intrarow spacing of 20 cm. Germination of test entries was recorded 21 days after sowing. Postemergence mortality was recorded periodically and the final plant count was taken before harvest.

In the entries evaluated, total mortality ranged from 30.15 to 89.62% (Table 1). The groundnut variety PG 1 showed the lowest mortality (pre- + postemergence rotting) with 70% healthy plants, and also gave good germination (78.50%). Other groundnut varieties which were promising against the disease were: C 331, M 13, and M 37. In these cultivars, mortality ranged from 32.75 to 39.31% and germination between 74 and 80%.

Table 1. Screening	of g	groundnut	varieties	against	Aspergillus	niger,	Rajasthan	Agricultural	University,
Agricultural Researc	h Su	ubstation, H	lanumanga	arh, Raja	asthan, India	• 2			

	Germination	Preemergence	Postemergence	Total mortality
Variety	(%)	rotting (%)	rotting (%)	(%)
J 11	52.00	48.00	19.28	67.28
Ah 7223	58.75	41.25	22.34	63.59
U 1-2-1	69.00	31.00	23.61	54.61
Var 27	59.00	41.00	16.96	57.96
Faizpur	64.00	36.00	24.39	60.39
PI 337394 F	62.00	38.00	25.38	63.38
UF 71513	67.50	33.50	38.04	71.54
EC 76446 (292)	43.00	57.00	32.62	89.62
TMV 2	66.00	34.00	25.61	59.61
55-437	61.00	39.00	22.66	61.66
ICG (FDRS) 3	53.50	46.50	42.93	89.43
ICG (FDRS) 4	46.00	54.00	24.15	78.15
ICG (FDRS) 5	30.00	70.00	18.09	88.09
ICG (FDRS) 10	45.00	55.00	26.19	81.19
ICG (FDRS) 11	34.00	66.00	16.44	82.44
ICG (FDRS) 17	31.00	69.00	19.84	88.84
ICG (FDRS) 18	29.50	70.50	14.36	84.86
ICG (FDRS) 19	31.00	<b>69</b> .00	5.78	74.78
ICG (FDRS) 20	53.00	47.00	25.63	72.63
ICG (FDRS) 21	39.00	61.00	24.23	85.23
RS 138	49.00	51.00	10.05	61.05
M 1	65.00	35.00	17.25	52.25
Ah 114	56.00	44.00	3.92	47.92
C 331	74.00	26.00	6.75	32.75
M 13	76.00	24.00	10.92	34.92
M 37	80.00	20.00	19.31	39.31
AK 12-24	58.50	41.50	17.21	58.71
PG 1	78.50	21.50	8.65	30.15
SEm	±1.33	±1.73	±1.06	±1.65
CV (%)	4.90	7.61	5.35	3.89

Collar rot incidence was much higher in the varieties supplied by ICRISAT. The highest mortality of 89.62% was recorded for EC 76446 (292). Only some entries, i.e., U 1-2-1, Faizpur, PI 337394 F, UF 71513, TMV 2, and 55-437 showed above 60% germination. The rest of the varieties supplied by ICRISAT had poor germination.

No correlation was seen between the number of plants which germinated and their ultimate survival. Data further revealed that in all the entries except UF 71513, preemergence rotting percentage was higher than postemergence rotting percentage.

Acknowledgement. The authors are grateful to Dr V.K. Mehan, Scientist, Legumes Pathology, ICRISAT, for providing seeds of 20 groundnut varieties.

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## Mycotoxin Contamination in Developing Groundnut

C.M. Usha<sup>1</sup>, K.L. Patkar<sup>1</sup>, H.S. Shetty<sup>1</sup>, R. Kennedy<sup>2,3</sup>, and J. Lacey<sup>2</sup>

 Department of Studies in Applied Botany, University of Mysore, Manasagangotri, Mysore 570 006, India;
 A.F.R.C. Institute of Arable Crops Research, Rothamsted Experimental Station, Harpenden, Herts AL5 2JQ, UK;
 Present address: Horticulture Research International, Wellesbourne, Warwickshire CV35 9EF, UK)

Groundnut is unique among the major crop plants because it requires minimal processing to produce a very attractive food. However, it was also the first crop shown to be heavily contaminated with aflatoxin and implicated in Turkey X disease in the 1960s. Since then, investigations have been directed toward the causal fungi, to determine how they colonize groundnut pods and their potential for mycotoxin production. Mycotoxin contamination of groundnut occurs not only during postharvest drying and storage but also before harvest. Preharvest aflatoxin contamination has been reported by several workers (Joffe 1970, Diener et al. 1987, Dorner et al. 1989), but there is little information on preharvest occurrence of other mycotoxins.

The objective of this study was to assess contamination of developing groundnut by aflatoxin  $B_1$  (AFB<sub>1</sub>), T-2 toxin (T2), and ochratoxin A (OA) from 60 to 65 days after sowing to harvest when grown under normal agronomic conditions in four farms located around Mysore (southern India) during the 1991 rainy season (June– September). The fields were at least 1 km apart and contained sandy loamy soil.

Developing pods were collected at random from each of the four farms by uprooting whole plants from growth stage (GS) 5 onward (Usha et al. 1991). The seeds were separated from the pods and plated in four replicates of 50 seeds each on DG 18 agar after surface sterilization with 2% sodium hypochlorite for 2 minutes. Seed samples were also plated on wet blotters without surface sterilization. The plates were incubated at  $27\pm2^{\circ}$ C for 7 days, and then colonization by mycotoxigenic fungi was assessed.

Contamination of AFB<sub>1</sub>, T2, and OA was assayed by indirect competitive ELISA (Ramakrishna et al. 1990). Samples (10 g) from each farm were blended with an extraction solvent (acetonitrile + 0.5% KCl + 6% H<sub>2</sub>SO<sub>4</sub>; 89:10:1), and the extracts were diluted to 1:20 in sample buffer solution before assay. Monoclonal antibodies for the three toxins were obtained from Rhone Poulenc Diagnostics, Glasgow, UK and IgG-HRP from Sigma Chemicals. Microtiter plates were read using a Dynatech MR5000<sup>®</sup> microtiter plate reader (Dynatech Ltd., UK) at 450 nm.

The incidence of Aspergillus flavus, Fusarium spp, and Penicillium spp, in developing groundnut from GS 5 to harvest and mycotoxin contamination are shown in Table 1. Incidence of A. flavus was already high at GS 6 and GS 7 but later it decreased before increasing again toward harvest. Fusarium spp generally contaminated fewer seeds at later growth stages (GS 9 onward) than the other fungi, and colonized, at the most, 37.6% of the seeds at GS 6. By harvest, their incidence had decreased to 1.3%. Penicillium spp were the most abundant fungi, colonizing 50% or more seeds between GS 5 and GS 11. But their incidence decreased to 11% by harvest. At GS 5, seeds were contaminated with 8.8 ng AFB<sub>1</sub>, 175 ng T2, and 30.8 ng OA g<sup>-1</sup> seed. Later, AFB<sub>1</sub> contamination decreased to 2.6 ng g<sup>-1</sup> seed but then increased to 12 ng g-1 seed at harvest.-T-2-toxin-increased-through seed development to a concentration of 462.5 ng g-1 seed at harvest. Ochratoxin A contamination also increased during seed development to 129.4 ng g<sup>-1</sup> seed at harvest.

Growth	S	Seed infected (%)		Mycoto	xin contaminati	ion (ng $g^{-1}$ )
stage <sup>1</sup>	Aspergillus flavus	Fusarium spp	Penicillium spp	Aflatoxin $B_1$	T-2 toxin	Ochratoxin A
5	0.8	24.5	75.3	8.8	175.0	30.8
6	7.0	37.6	89.0	4.9	225.0	48.3
7	7.7	18.7	84.0	4.0	218.5	77.5
8	1.5	4.3	61.5	3.5	238.0	25.0
9	2.3	1.3	57.5	2.8	250.0	42.5
10	2.3	1.3	59.5	2.6	250.0	30.6
11	2.5	1.0	51.3	3.6	506.2	51.9
12	3.8	1.3	20.6	8.3	466.7	82.5
13	4.5	1.3	11.0	12.0	462.5	129.4

Table 1. Incidence of major fungi and mycotoxin contamination in developing groundnut, Mysore, India, rainy season 1991.

Mycotoxin contamination could be detected from the early stages of groundnut pod development. More AFB<sub>1</sub> was observed in very young immature seeds and at harvest than at intermediate growth stages, supporting the opinion that mature groundnut seeds are more resistant to mycotoxin development than immature seeds (Dorner et al. 1989). The increase in AFB1 contamination toward harvest may be a consequence of decreasing soil and seed water content. Drought stress and temperature before harvest can increase the incidence of A. flavus and lead to increased aflatoxin contamination of seeds (Wilson and Stansell 1983, Sanders et al. 1985). Mean soil temperatures above 25°C favor the growth of A. flavus and decrease the competition from other soil organisms. The presence of T2 and OA in developing groundnut seeds is reported here for the first time. Concentrations of both these mycotoxins increased as the seeds matured, and both were present in their highest concentrations at harvest. There was a high incidence of Fusarium spp in immature seeds but fewer species of Fusarium could be isolated during the later stages of the crop although T2 was produced up to harvest. Ochratoxin A production in seeds must be attributed to Penicillium spp because no A. ochraceus was isolated. Several Penicillium spp are known to produce OA.

Health risks from mycotoxins are better controlled by avoiding contamination than by subsequent detoxification. Efforts must, therefore, be made to prevent toxin formation during crop growth. Many studies show that groundnut plants grown under adequate soil moisture are unlikely to become contaminated with aflatoxins (Mixon 1980, Cole et al. 1982, Hill et al. 1983) but further studies are needed to determine the effects of climate on the production of other mycotoxins. Development of cultivars resistant to fungal colonization or mycotoxin production should prove useful in minimizing mycotoxin contamination.

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# Occurrence of Mycophagous Midge on Late Leaf Spot and Rust of Groundnut

P.M. Reddy, G.V. Ranga Rao, D.H. Smith, J.A. Wightman, and P. Subrahmanyam (ICRISAT Center)

Late leaf spot [*Phaeoisariopsis personata* (Berk. & M.A. Curtis) van Arx] and rust (*Puccinia arachidis* Speg.) are economically important groundnut diseases (Subrahmanyam et al. 1984). A mycophagous midge was observed on late leaf spot- and rust-infected groundnut leaves in a fungicide trial conducted at ICRISAT Center, Patancheru (18°N 78°E), Andhra Pradesh, India, during the 1990 rainy season. It was noticed that the midge population was high on groundnut leaves wherever late leaf spot (LLS) and rust were severe. Microscopic examination revealed that the midge was feeding on the conidia of LLS and urediniospores of rust. Conidia and urediniospores were found in the gut of dissected midge larvae. The trial included fungicide treatments with carbendazim to control LLS, tridemorph to control rust, chlorothalonil to control LLS and rust, and water as a control (Smith and Littrell 1980, Subrahmanyam et al. 1984). The highest midge density (33 midges 10 leaflets<sup>-1</sup>) was found in carbendazim-sprayed plots, compared to 20 midges in chlorothalonil-treated plots, followed by 10 midges in water-treated plots, and 7 midges in tridemorph-treated plots (Table 1). Significant differences (P > 0.05) in midge population density in the fungicide trial could be due to the severity of the disease in different treatments. For example, in carbendazim-treated plots rust was severe compared to the other treatments. This may also indicate the preference of the midge for rust spores over LLS conidia (Table 1).

The midge larvae were reared through to the adult stage in the laboratory on rust- and LLS-infected groundnut leaves, and were identified at ICRISAT Center as *Micodiplosis* sp (Cecidomyidae) (Figs. 1 and 2). The association of this midge species with groundnut rust was earlier reported by Vaishnav and Kapadia (1972). This is the first known record of this species feeding on LLS. During 1991 and 1992; these midge species were noticed feeding on these pathogens which were particularly abundant during the rainy season. They occur regularly in both rainy and postrainy seasons and are commonly found feeding on these pathogens. Further studies are needed to determine their effectiveness as a disease control agent and the effect of insecticidal spray on midge incidence.

Table 1. Midge population observed on groundnut treated with different fungicides in a trial conducted at ICRISAT Center, rainy season 1990.

		Disea	Disease rating <sup>2</sup>			
Treatment	Midge population <sup>1</sup>	Rust	Late leaf spot			
Carbendazim	33	6.3	2.3			
Chlorothalonil	20	4.3	2.3			
Tridemorph	7	2.3	7.3			
Water	10	3.7	7.7			
SE	±1.1	±0.4	±0.4			
CV (%)	34.2	16.8	13.1			
LSD (0.05)	3.1	1.2	1.2			

1. Mean of five replications with 10 leaflets in each replication.

2. Disease severity was scored on a 1-9 point scale, where 1 = no disease and 9 = 80-100% damage by either of the two diseases.

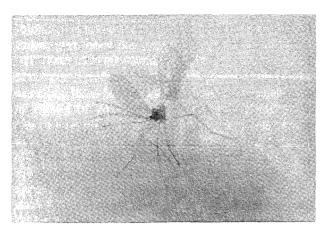


Figure 1. Micodiplosis sp adult.

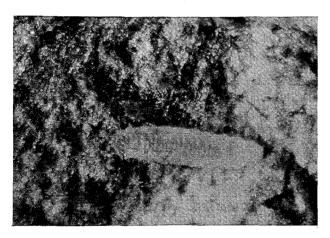


Figure 2. Micodiplosis larva feeding on fungal spores.

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# Leaf Toughness in Relation to Resistance to Jassid *Balclutha hortensis* Lindb. in Groundnut

V. Nandagopal, T.G.K. Murthy, T. Radhakrishnan, and P. Sen (National Research Centre for Groundnut, Junagadh, Gujarat, India)

The jassid *Balclutha hortensis* Lindb. was reported to be one of the major insect pests causing considerable yield loss in groundnut (Nandagopal and Reddy 1987). Due to concealed feeding, i.e., on the undersurface of the leaves, the damage is not seen unless the typical 'V'-shaped yellowing is expressed at the tip of the leaflet. Host-plant resistance is a suitable alternative to pesticide use in suppressing jassids (Dwivedi et al. 1986). The present investigation was aimed at evaluating the toughness of leaflets of selected groundnut genotypes and correlating it with jassid damage. The experimental materials for this study consisted of 3 released varieties, 4 germplasm accessions, and 11 advanced intra- and interspecific derivatives.

Field observations on the 'intensity of yellowing in leaves were made visually at the pod-filling stage. Five leaves sample<sup>-1</sup> and five samples plot<sup>-1</sup> (2.4 m  $\times$  5 m) were considered for the study. Leaf toughness was measured using an improvised top pan balance. The single top pan of an electric balance was removed and a plastic tube, fitted at one end with a 1.5 cm long steel needle, was fixed on the axis of the pan assembly of the balance. The diameter of the needle tip was 0.7 mm (Nandagopal and Radhakrishnan 1990). For measurement of leaf toughness, four predetermined sites were marked on both the sides of the midrib of the leaflet just above and below the center. Only the top two leaflets of the second fully opened leaves of a branch were used for measurement. All the plants were of the same age and soil moisture levels were similar at the time of sampling. The leaflet was held perpendicular to the needle with the abaxial surface facing it and pressed at the predetermined sites. The readings on the vernier were noted at the time of piercing and were expressed as toughness in mg mm<sup>-2</sup> (Table 1). Eighty such observations were made for each genotype.

There was a large variation in intensity of yellowing and leaf toughness among the genotypes tested. The intensity of yellowing was lowest in the interspecific derivative CS 17, and highest in V 6. Surprisingly, NC Ac 17090, a donor for jassid resistance, recorded 146.2 mg mm<sup>-2</sup> toughness, indicating that some other characters

Genotype	Description	Yellowing (%)	Leaf toughness (mg mm <sup>-2</sup> )
GAUG 10	Cultivar	8.29	129.4
Pol 1	Cultivar	8.34	112.4
Robut 33-1	Cultivar	2.55	181.4
NC Ac 17090	Germplasm accession	5.63	146.2
NC Ac 17484	Germplasm accession	4.78	130.0
ICG 5298	Germplasm accession	6.17	113.5
V 6	Germplasm accession	9.92	130.5
CS 8	Robut 33-1 × EC 76446(292)	8.38	128.6
CS 9	Robut 33-1 × EC 76446(292)	3.28	175.9
PS 50	Ah $50 \times JL 24$	4.20	169.4
16-18-1	Gujarat Narrow Leaf Mutant × Krinkle	7.19	99.1
17-22-1	Gujarat Narrow Leaf Mutant × Krinkle	8.29	129.4
12-11-2	Gujarat Narrow Leaf Mutant × Krinkle	6.17	118.1
8-4-1	Gujarat Narrow Leaf Mutant × Krinkle	7.17	142.2
44-2	ICG 4759 Krinkle × A. monticola	6.85	118.6
CS 11	M 13 $\times$ A. villosa	<b>3.9</b> 0	166.8
CS 17	M 13 $\times$ A. villosa	1.18	200.0
CS 19	M 13 $\times$ A. villosa	7.77	165.4
SE		±2.07	±2.30
CV (%)		14.42	17.55

#### Table 1. Leaf toughness and intensity of yellowing due to jassid attack in certain cultures of groundnut tested at NRCG, Junagadh, India.

may also be contributing to resistance. Correlation and regression between intensity of yellowing and leaf toughness are given in Table 2, indicating the negative relationship between percentage intensity of yellowing and toughness of different sites, different leaflets, and the cumulative leaf toughness. This observation suggests that leaf toughness is one of the reliable parameters to estimate damage due to jassids in groundnut.

Table 2. Rejassid and le	g due to		
Variable	Slope	Intercept	ʻr'**
Leaflet 1	14.79	-0.16	-0.72
Leaflet 2	15.93	-0.18	-0.75
Leaflet 3	15.93	-0.17	-0.7 <b>6</b>
Leaflet 4	16.68	-0.18	-0.79

-0.18

-0.79

Leaflet 4 16.68 -0.18

16.63

Whole leaf

\*\* Significant at 1% level.

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## **Transfer of Resistance to Groundnut Rosette Disease from a Wild** *Arachis* **Species into Cultivated Groundnut**

J.P. Moss<sup>1</sup>, A.K. Singh<sup>1</sup>,

P. Subrahmanyam<sup>2</sup>, G.L. Hildebrand<sup>2</sup>, and A.F. Murant<sup>3</sup> [1. ICRISAT Center; 2. SADC/ICRISAT Groundnut Project, Chitedze Agriculture Research Station, Lilongwe, Malawi; 3. Scottish Crop Research Institute (SCRI), Dundee, Scotland, DD25A, UK]

A program to transfer genes from wild species of Arachis into tetraploid genotypes fully compatible with the cultivated groundnut was started at Reading University, UK, in 1973, in collaboration with scientists in USA and in Nigeria. Several hybrids were produced, and screened in Malawi, Nigeria, and at ICRISAT Center in India (Moss 1980). The initial emphasis was on developing techniques for transfer of resistance, using the wild diploid species, A. chacoense, A. cardenasii, and A. stenosperma. These species were chosen for their resistance or immunity to major fungal foliar pathogens (Stalker and Moss 1987). This program continued at ICRISAT Center from 1978, and expanded to include other species and to address a wider range of constraints (Moss 1985a, 1985b). Using an extensive crossing program, coupled with ploidy manipulations, it was possible to produce large numbers of tetraploid derivatives that contained genes from wild species but were fully compatible with the cultivated groundnut. These were made available to breeders for screening against a wide range of pathogens and for use in breeding programs.

Pioneering research on development of groundnut cultivars with resistance to rosette was carried out in West Africa, and these sources formed the basis for rosette resistance breeding programs throughout Africa. But they represent only a narrow genetic base. The resistance seems to be effective against both chlorotic rosette and green rosette and is governed by two independent recessive genes (Nigam and Bock 1990).

Groundnut rosette is caused by a complex of three agents, groundnut rosette virus (GRV) and its satellite RNA, and groundnut rosette assistor virus (GRAV) (Reddy et al. 1985, Murant et al. 1988). The disease is transmitted by *Aphis craccivora* Koch. Research on groundnut rosette at the Scottish Crop Research Institute (Murant et al. 1991) has shown that the GRV-resistant lines are fully susceptible to GRAV, but that *Arachis chacoense* appears immune to both viruses. A range of derivatives in which A. chacoense was a parent were therefore sent to the SADC/ICRISAT Groundnut Project in Malawi for field screening for resistance to GRV.

During the 1990/91 crop season, 1127 groundnut genotypes including the interspecific hybrid derivatives from ICRISAT Center, India, were screened against groundnut rosette. Seeds of each entry were sown in unreplicated single-row plots of 6 m length. Infector rows of a rosette-susceptible cultivar, Malimba, were sown after every two test rows. Potted groundnut plants infected with groundnut rosette and severely infested with viruliferous aphids were raised in the greenhouse and were transplanted into infector rows some two weeks after sowing. Approximately two weeks later, each infector row was examined and the plants that were free from rosette symptoms were infested with viruliferous aphids to minimize the chances of any escape. Disease incidence in susceptible test cultivars Malimba, Chalimbana, and Mani Pintar was 100%. Infected plants were severely stunted and chlorotic. Most of these plants died toward the end of the season. Disease incidence was almost 100% in most of the test entries.

Only one entry, 83/372-2-22-B1, showed a high degree of resistance (though not immunity) to rosette. Plants remained healthy and vigorous for most of the growing season (Fig. 1). However, toward maturity some plants showed mild mosaic mottling symptoms on young quadrifoliolates in the terminal bud region. In graft inoculation tests in Scotland, many plants of 83/372-2-22-B1 became infected with GRAV and GRV, and further tests are needed to determine the nature of the field resistance to rosette disease in this genotype.

Entry 83/372-2-22-B1 originated from a cross A. hypogaea 'Samaru  $38' \times A$ . chacoense, resulting in a triploid hybrid which was colchicine treated to give a hexaploid. This was then backcrossed to A. hypogaea to

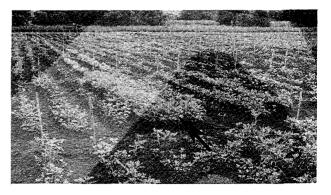


Figure 1. The groundnut rosette disease nursery at Chitedze, Malawi, showing the rosette-resistant entry, 83/372-2-22-B1 (arrow).

reduce the chromosome number to the tetraploid level, while selecting for fertility and desirable agronomic characters. Cultivars used as parents during this backcrossing program were 'Samaru 38', 'Samaru 61' ('Kano 50'), 'Makulu Red', and 'F334A-B-14'. All these cultivars are susceptible to both GRV and GRAV. Further studies are underway to confirm that the resistance has come from A. chacoense.

It is interesting to note that resistance to rosette disease has been transferred from a wild species originating from South America, where there are no reports of the occurrence of the disease, and that the gene or genes involved have led to resistance at the tetraploid level. The other species reported as showing some immunity (Murant et al 1991) is in section Erectoides, and is incompatible with *A. hypogaea*. Efforts are underway to overcome this incompatibility.

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# Production of Transgenic Plants of Groundnut by Agrobacterium-mediated Genetic Transformation

Kiran K. Sharma, V. Anjaiah, and J.P. Moss (ICRISAT Center)

There are several biotic constraints to the productivity of groundnut which result in heavy losses annually. These include various diseases caused by fungi, bacteria, viruses, insects, and nematodes. Sources of resistance to some of these have been identified in the germplasm of

Arachis hypogaea and its wild relatives, but for many constraints there are no known usable sources of resistance. Moreover, the agronomic improvement of A. hypogaea by integrating resistance to various traits from wild species is difficult due to sterility barriers and genomic incompatibilities associated with traditional breeding methods (Stalker and Moss 1987). Application of biotechnological methods for genetic transformation with a range of genes offers unique possibilities of developing groundnuts resistant to many of these constraints. Although genetic transformation of Arachis species has not been reported so far, reports on other crop species indicate the feasibility of employing useful genes for crop improvement by asexual means. Some of these include cross protection from viruses by introducing the viral coat protein gene (Hull and Davies 1992), and insect resistance by the introduction of Bacillus thuringiensis endotoxin gene (Feitelson et al. 1992) and protease inhibitor gene (Ryan 1990). Together with the advances in plant transformation and regeneration by disarmed Agrobacterium vectors (Zambryski et al. 1983; Fraley et al. 1985) and microprojectile bombardment (Klein et al. 1992), the techniques of advanced molecular biology offer new opportunities to alleviate various biotic constraints through the production of genetically engineered plants.

We have been developing efficient techniques for introducing economically important genes for the agronomic improvement of *Arachis* species by nonconventional means. Recent studies on the susceptibility of five Brazilian cultivars of groundnut to different wild strains of *Agrobacterium tumefaciens* showed variations in strain-cultivar compatibility (Lacorte et al. 1991). This study confirmed that groundnut is a permissive host for the acceptance of genes from specific gene vectors. Here we report our preliminary results on the optimization of a method for shoot regeneration in tissue cultures, and *Agrobacterium*-mediated genetic transformation of *Arachis hypogaea*.

Tissue culture and regeneration. To optimize the regeneration of adventitious shoot buds, various explants from mature seeds and seedlings of *Arachis hypogaea* var JL 24 were used. These included cotyledons and embryo axes from presoaked seeds, leaves, stem segments, and root segments from 7-day-old seedlings. Cotyledon explants produced a high frequency of multiple shoots (up to 90%) when cultured on MS medium containing an auxin and a cytokinin [shoot induction medium (SIM)]. On SIM the cotyledons produced multiple shoot buds at the cut end within two weeks. After four weeks on SIM, the explants with shoot buds were transferred to hormone-free medium for 3 passages of 4 weeks each until the shoot buds developed into shoots. During this process the elongated shoots were micropropagated by culturing nodal explants on MS medium containing N<sup>6</sup>-benzyladenine. The elongated shoots were subsequently rooted on MS medium containing  $\alpha$ -naphthalene acetic acid. After the formation of well-developed adventitious roots on the shoots, the plants were transplanted to pots containing sand:vermiculite (1:1) and maintained at 25°C in a growth cabinet with 80% relative humidity for 3 weeks prior to transfer to the greenhouse.

Genetic transformation. Two strains of disarmed A. tumefaciens, C 58 and LBA 4404, containing the binary vector p35S GUS INT harboring neomycin phosphotransferase II (npt II) and B-glucuronidase with an intron (GUS-INTRON) as marker genes were used. The cotyledon explants from presoaked seeds of var JL 24 were cocultured with the bacterial strains on SIM for 3 days and subsequently subcultured on the selection medium containing SIM + 125 mg L<sup>-1</sup> cefotaxime + 250 mg L<sup>-1</sup> carbenicillin. The medium was supplemented with 25 mg L-1 kanamycin as selection agent. After 4 weeks. 70% of the explants had produced numerous shoot buds which were then subcultured on the selection medium containing 50 mg L<sup>-1</sup> kanamycin. After 2 passages of 4 weeks each, the individual shoots were clonally propagated through nodal explants followed by rooting and transplantation.

Analysis of putative transformants. The leaves from in vitro growing shoots were histochemically analyzed for presence of the GUS gene (Jefferson 1987). Leaf pieces were incubated for 12-14 hours at 37°C in a reaction mixture containing X-gluc, which is a substrate for the enzyme B-glucuronidase, and cleared of chlorophyll by passing the leaf segments through 70-90% ethanol for at least 8 hours, mounted in glycerol, and observed under a light microscope. About 75% of the randomly selected plants exhibited a positive reaction suggesting activity of the introduced GUS gene. The plants were also analyzed for stable integration of the GUS gene by Southern hybridization. For this, the genomic DNA was isolated from the putative transformants and restricted with Hind III restriction endonuclease which liberated the 3.2 Kb GUS-INTRON gene. After running the restricted DNA on 0.8% agarose gel, the DNA was transferred to the nitrocellulose membrane by capillary blotting and the DNA was crosslinked to the membrane by exposing it to UV rays. The DNA on the blotted membrane was hybridized with the GUS probe by using the nonradioactive method utilizing digoxigenin. Preliminary observations

on the Southern hybridization have revealed the integration of the transferred gene. Work is underway to study the inheritance pattern of the introduced genes in the succeeding seed generations.

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# **About ICRISAT**

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

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

ICRISAT was established in 1972. It is one of 18 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 World Bank, and the United Nations Development Programme (UNDP).

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