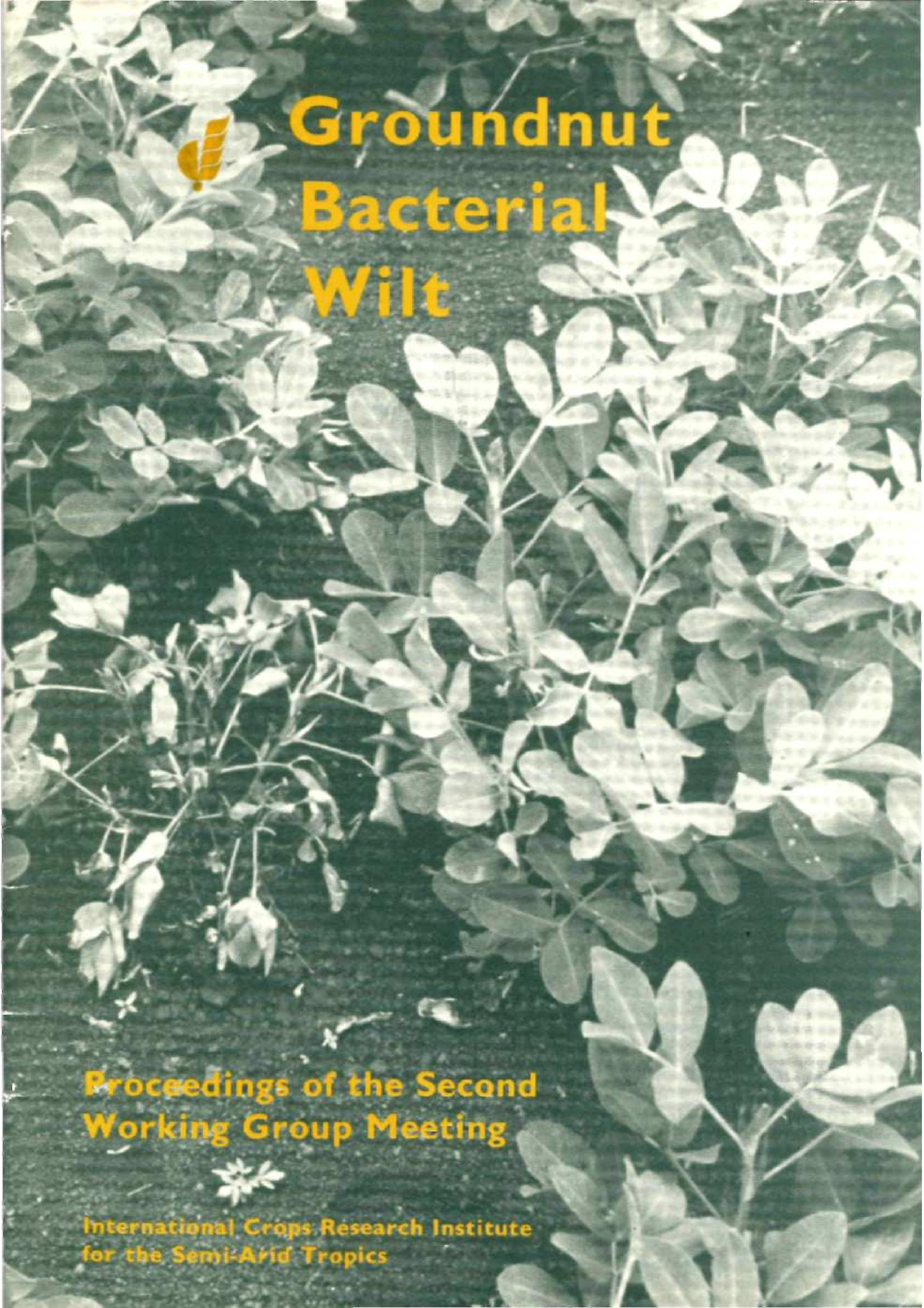
Groundnut Bacterial Wilt



Proceedings of the Second Working Group Meeting

International Crops Research Institute for the Semi-Arid Tropics

Abstract

Citation: 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.

The problem of bacterial wilt of groundnut has caused much concern in many countries of Asia and Africa. The second meeting of the Groundnut Bacterial Wilt Working Group (held in Taiwan on 2 Nov 1992) focused on the status of groundnut bacterial wilt research in Asia and Africa, and ways to improve disease management through collaborative research projects. This publication provides a background to the meeting, a record of discussions on research strategies and priorities, and recommendations for future research. Three papers on the status of bacterial wilt research in Malaysia, Uganda, and Indonesia are also included.

Résumé

Flétrissement bactérien de l'arachide—comptes rendus de la deuxième réunion du Groupe de travail, 2 nov 1992, AVRDC, Tainan, Taiwan. Le problème du flétrissement bactérien de l'arachide a causé beaucoup d'inquiétude dans plusieurs pays de l'Asie et de l'Afrique. La deuxième réunion du Groupe de travail sur le flétrissement bactérien de l'arachide (tenue au Taiwan le 2 nov 1992) a surtout porté sur le statut de la recherche sur le flétrissement bactérien de l'arachide en Asie et en Afrique ainsi que sur les moyens d'améliorer la gestion de la maladie à travers des projets de recherche collaborative. Cette publication fournit des informations relatives à cette réunion, un compte rendu des discussions sur les stratégies et les priorités de recherche et enfin des recommandations pour les travaux de recherche futurs. Trois communications sur le statut de la recherche sur le flétrissement bactérien en Malaysie, à l'Ouganda, et en Indonésie sont également présentées.

Resumen

Marchitamiento bacterial en maní: actas de la segunda reunión del Grupo de trabajo, 2 de noviembre, 1992, AVRDC, Tainan, Taiwan. El problema del marchitamiento bacterial del maní ha causado bastante preocupación en muchos países de Asia y Africa. La segunda reunión del Grupo de trabajo sobre marchitamiento bacterial en maní (celebrada en Taiwan, el 2 de noviembre de 1992) enfocó sobre el estado presente de investigación relacionada a marchitamiento bacterial en maní en Asia y Africa, así como sobre pasos para mejorar el manejo de enfermedades a través de proyectos de investigación colaborada. Esta publicación facilita un trasfondo a la reunión, un registro de discusiones sobre estrategias y prioridades de investigación así como recomendaciones para la investigación futura. También se incluyen tres ponencias relacionadas al estado de la investigación sobre marchitamiento bacterial en Malaysia, Uganda y Indonesia.

Groundnut Bacterial Wilt

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2 Nov 1992

Asian Vegetable Research and Development Center
Tainan, Taiwan

Edited by

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A.C. Hayward

ICRISAT

International Crops Research Institute for the Semi-Arid Tropics
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1993

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Contents

Preface

Bacterial wilt is an important constraint to groundnut production in several Asian and African countries. The Groundnut Bacterial Wilt Working Group (GBWWG) was formed in 1990, to coordinate research on the disease. The formation of the Group was recommended at the ACIAR/ICRISAT collaborative research planning meeting on bacterial wilt of groundnut, held in Malaysia, 18-19 Mar 1990. (For resolutions of the meeting, see ACIAR proceedings no. 31.) The Coordination Unit of the Asian Grain Legumes Network (now Cereals and Legumes Asia Network, or CLAN) was requested to provide administrative and logistic support to the Working Group.

This meeting, the second of the GBWWG, was organized on 2 Nov 1992 as a satellite meeting to the International Symposium on Bacterial Wilt (Taiwan, 28-31 Oct 1992). The objectives of the meeting were:

- To share information on the status of groundnut bacterial wilt research in Asia and Africa.
- To review the activities of the Working Group.
- To develop collaborative links for research and training, and for exchange of information, technology, and genetic material.

The meeting was attended by 21 scientists from 15 countries in Asia, Africa, Australia, Europe, and South America, including representatives of several international organizations.

Unfortunately, no scientist from the People's Republic of China was able to attend because of circumstances beyond the control of the organizers. Soon after the meeting, we visited the People's Republic of China to brief Chinese scientists on the deliberations at the GBWWG meeting, and to discuss with them in detail priorities and specific areas for future collaborative research.

The meeting was extremely successful, and the organizers are confident that it will be remembered as a milestone in progress towards the effective management of groundnut bacterial wilt.

V.K. Mehan
A.C. Hayward

Proposals and Recommendations

Several proposals were put forward for collaborative research on groundnut bacterial wilt among members of the Working Group. Discussions were held on major research areas, particularly disease management strategies, and on coordination of Working Group activities. A summary of the discussions and recommendations is given below:

Priorities for Future Research and Collaboration

Host-plant resistance

Genetic resistance was recognized as being the most effective disease management strategy, and the need for greater emphasis on host-plant resistance research was stressed.

In view of the narrow genetic base of the available wilt-resistant cultivars, it was suggested that scientists from the People's Republic of China, Indonesia, and Malaysia should continue screening germplasm to identify new and diverse sources of resistance to bacterial wilt. Efforts should be made to incorporate available resistance into diverse genetic backgrounds.

Landraces originating from humid areas in the centers of crop diversity should be evaluated for wilt resistance in wilt-sick plots in Indonesia and the People's Republic of China.

Incorporation of resistance to bacterial wilt, rust, and leaf spots into high-yielding cultivars adapted to specific environments should receive high priority. This is important as foliar fungal diseases are severe in many areas where bacterial wilt is also a constraint to groundnut production.

Lines that are resistant to rust and/or late leaf spot should be screened on a priority basis for resistance to bacterial wilt.

The Group stressed the need to evaluate available wilt resistance at different hot-spot locations over seasons in order to obtain reliable information on stability of resistance and the effects of environment on disease incidence/severity.

It was also suggested that selected resistant lines, and lines with differential reactions, be tested for their reaction to various aggressive groundnut-adapted strains/pathotypes of the wilt pathogen from different regions of the world. This work should be done under controlled conditions, especially in a country where groundnut is not grown, and where *Pseudomonas solanacearum* is not a problem.

Concerted efforts should be made to understand the mechanisms and components of wilt resistance.

The Group emphasized the need for more detailed studies on the genetics of wilt resistance, using a wide array of resistant material from different botanical types in combination with different strains/pathotypes. Data from such studies would help to accelerate resistance-breeding programs.

Disease management

More systematic research should be undertaken to understand the influence of different cropping systems and crop management practices on disease incidence and severity. Some cultural practices, such as flooding of fields and crop rotation with non-host crops [e.g., rice *(Oryza sativa),* sugarcane *(Saccharum officinarum),* and maize *(Zea mays)*], are effective in containing the disease. On-farm research is required to demonstrate the effectiveness of these approaches.

Crop sanitation is a major factor in disease management, and it is important to establish the impact of crop sanitation practices on the wilt pathogen and the disease.

Biological control was welcomed as an innovative approach that showed promise in the long term. Biological control systems based on antagonistic bacteria such as *P. fluorescens* and *Bacillus* spp, and on avirulent mutants of P. *solanacearum,* were discussed. The Group emphasized that mutants and avirulent strains must be used cautiously, since avirulent strains are reported to recover pathogenicity characters. It was also felt that interactions of *P. solanacearum* with *Rhizobium* in rice-based cropping systems should be investigated.

lt was suggested that soil solarization should be attempted, as this may prove to be effective in tropical countries.

It was agreed that integrated disease management, using wilt-resistant cultivars in Combination with appropriate cultural practices, could effectively control bacterial wilt. Some of these practices can influence both the pathogen and the host, as well as some beneficial organisms, and should provide low-cost disease control if chosen wisely. However, the effects of such management practices are poorly understood; their application on a large scale will depend upon a clear scientific understanding of the principles involved.

Studies on seed transmission of bacterial wilt of groundnut should receive more attention. Seed transmission is of quarantine significance, and reports should be validated. It is especially important in relation to the safe international movement of seed, and to integrated disease management.

Detection of *Pseudomonas solanacearum* and the diagnosis of wilt

It was agreed that priority should be given to determining the distribution of the strains of *P. solanacearum* that attack groundnut. For such studies, as also for disease diagnosis, it would be useful to have highly specific monoclonal antibodies to permit the detection of individual biovars/strains of the bacterium in soil, in the rhizosphere, and in root and seed tissues.

Efforts should be expanded to develop sensitive enzyme-linked immunosorbent assay (ELISA) techniques for pathogen detection.

International Groundnut Bacterial Wilt Nursery

The Group strongly recommended that an International Groundnut Bacterial Wilt Nursery (IGBWN) be established to determine the stability of wilt resistance through multilocational testing.

The disease nursery should be conducted at hot-spot locations in different regions where the crop and the disease are important, and where different strains of the wilt pathogen are suspected to occur.

The following locations were identified for the disease nursery:

People's Republic of China	Wuhan, Guangzhou
Indonesia	Bogor, Jambegede
Vietnam	Thanh Hoa and Long An Provinces
Malaysia	Serdang
Uganda	Bukalasa/Kawanda

Participants from Taiwan and Mauritius were eager to participate in the testing of nursery materials. The disease nursery could also be tested in the Philippines.

The following 36 resistant entries were identified for inclusion in the nursery, together with four controls: ICG 1326 and Chico as susceptible controls, and two local cultivars.

Schwarz 21	Bulundi
Gajah	EGPN 11
Kidang	GA 119-20
Pelanduk	Holland St. Runner
Xiekangqing	ICG 1703
Taishan Sanlirou	ICG 1704
Dingzixili	ICG 6280
Yue You 22	ICG 7887
Yue You 92	ICG 7894
Yue You 256	ICG 7895
Yue You 589	ICG 7898
El Hua 5	ICG 7900
Lu Hua 3	ICG 11306
Zhong Hua 2	ICG 11325
Guiyou 28	ICGV 88271
Taishan Zhenzhou	ICGV 88274
1005	ICGV 86606
Zhong Hua 112	ICGV 87206

It was recommended that ICRISAT should coordinate the IGBWN, and foster the exchange of wilt-resistant germplasm and breeding lines.

Specific wilt-resistant germplasm identified in the People's Republic of China, Indonesia, Uganda, and the Philippines should be supplied to ICRISAT; all such germplasm lines/cultivars should be genetically pure and virus-free. ICRISAT in turn should undertake multiplication, maintenance, and further distribution of such material for research.

Funding for collaborative research and training

The Group felt that national programs on groundnut bacterial wilt, particularly in the People's Republic of China, Indonesia, and Uganda, should receive support funding, if necessary, to conduct the disease nursery and assist research, especially on on-farm disease management.

Funds are essential for several other activities: exchange of germplasm and information between participating countries, research coordination/monitoring, and training programs. It was recommended that ICRISAT, ACIAR, and some national programs should prepare a joint proposal for submission to the World Bank/ACIAR/Food and Agriculture Organization (FAO) for funding of Working Group activities.

Training in wilt diagnosis and pathogen detection

The Group recognized that training is particularly needed in the areas of pathogen detection (using ELISA), disease diagnosis, and integrated disease management. In-country training courses should be organized for active researchers. Such training courses could be sponsored by ACIAR, ICRISAT, AVRDC, and International Potato Center (CIP).

The need to make information on groundnut bacterial wilt more readily available to researchers and extension workers was stressed. The Group suggested that ICRISAT should undertake to produce a comprehensive publication, covering various aspects of groundnut bacterial wilt including disease diagnosis, pathogen detection, screening and resistance-breeding, and disease management.

Coordination of Working Group activities

The Group suggested that ICRISAT should coordinate the exchange of resistant germplasm between GBWWG members, and distribution of IGBWN materials.

ACIAR and ICRISAT should assist in surveys for assessing disease incidence/severity and economic losses.

It is essential to provide researchers with standard inoculation and disease assessment procedures.

ACIAR should continue to support the publication of the Bacterial Wilt Newsletter. Scientists were encouraged to publish their research results/reports in the Bacterial Wilt and International Arachis Newsletters.

Next Working Group meeting

It was proposed to hold the next meeting of the Working Group in the People's Republic of China in July 1994, for two days. It was also suggested that a 4-day training workshop be held in conjunction with the meeting, and that ACIAR, ICRISAT, FAO, and the World Bank be requested to assist with funds.

Recommendations

- An International Groundnut Bacterial Wilt Nursery (IGBWN) should be established to determine the stability of wilt resistance. ICRISAT should coordinate the IGBWN, and foster the exchange of wilt-resistant germplasm and breeding lines.
- Priority should be given to breeding high-yielding cultivars combining resistance to bacterial wilt and major foliar fungal diseases, which are important constraints to groundnut production in warm, humid areas.
- Integrated disease management systems should be developed for different agroecological regions. Primary responsibility for research in this area lies with Chinese and Indonesian scientists.
- Immunochemical techniques should be developed to detect the wilt pathogen in seed, plant tissues, and soil samples. Specific monoclonal antibodies against biovars 3 and 4 of race 1 should be produced. Research along these lines at the University of Hawaii (USA) and the Rothamsted Experimental Station (UK) should be encouraged.
- Since seed transmission of the wilt pathogen is possible, it is necessary to restrict the movement of groundnut seed from areas where the disease has been reported.
- External funds should be sought to promote bacterial wilt research in the People's Republic of China, Indonesia, and Uganda. ICRISAT and ACIAR, in conjunction with national programs, should prepare proposals for submission to the World Bank/ACIAR/FAO for funding of Working Group activities.
- ACIAR and ICRISAT should assist national programs to conduct disease surveys and assess crop losses.
- In-country training courses should be organized for researchers on bacterial wilt, focusing on pathogen detection, and disease diagnosis and management. ACIAR, ICRISAT, AVRDC, and CIP should consider sponsoring such courses.
- ACIAR should continue to support the publication of the Bacterial Wilt Newsletter.
- ICRISAT should produce a manual on groundnut bacterial wilt, covering disease diagnosis and management, and techniques for resistance-screening and breeding.

Bacterial Wilt of Groundnut in Malaysia

S. Hamidah[1] and K.Y. Lum[2]

Abstract

Groundnut production in peninsular Malaysia has declined from 20 000 t in the 1980s to around 5 000 t today, primarily as a result of reduction in crop area. Bacterial wilt is another major constraint to production; wilt incidence is high in the two major production areas in the states of Kelantan and Terengganu. Information on the disease is being gathered, and isolates of Pseudomonas solanacearum *are being collected and identified. Results from a resistance screening program involving ICRISAT breeding lines and Indonesian varieties have shown that selected lines do not perform consistently in field trials at different locations. These observations, together with laboratory characterization of some wilt pathogen isolates, suggest the existence of different local pathogenic strains.*

Introduction

Bacterial wilt, caused by *Pseudomonas solanacearum* (Smith) Smith, is the only important bacterial disease affecting groundnut (Mehan et al. 1986). The disease is a serious threat to groundnut production in warm, humid regions in many parts of the world.

In Malaysia, the majority of groundnut farms are small holdings, in Kelantan (totalling 1219 ha), Terengganu (242 ha), Perak (252 ha), and Kedah and Perlis (82 ha). Area under groundnut declined from 5197 ha in 1980 to 1318 ha in 1986, and total production from 19 437 t to less than 5000 t within the same period. However, demand for groundnut is increasing. In 1986, 44 871 t of groundnuts, worth MR 29.7 million, were imported, mainly in the form of shelled nuts. Smaller amounts were imported as groundnut oil and oilcake residues for livestock feed. The major exporters to Malaysia are Vietnam, USA, the People's Republic of China, Thailand, Taiwan, and Hongkong. However, Malaysia also exports canned and uncanned nuts valued at around MR 5.8 million annually.

The decline in production is attributed to high production costs (particularly labor costs), low yields, and high incidence of bacterial wilt disease in the two major production areas in Kelantan and Terengganu. The wilt pathogen affects a wide range of crops in the country, but information on groundnut attack is scanty. On the basis of rotation trials with groundnut, tobacco *(Nicotiana tabacum),* potato (*Solanum tuberosum),* tomato (*Lycopersicon esculentum),* and eggplant (*Solanum melongena),* Schwarz (1926) attributed differing wilt disease incidence to strain differences in the pathogen. Various other reports

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(McClean 1930, Hopkins 1947, Dowson 1949, Kelman and Person 1961, He et al. 1983) suggest the existence of pathogenic strains. Pathotypes associated with particular geographic locations have also been suggested (Buddenhagen and Kelman 1964, Simbwa-Bunnya 1972). Tan and Liao (1990) reported that strains of groundnut bacterial wilt differ in their pathogenicity to the same host cultivar in different parts of the People's Republic of China.

Screening for wilt resistance

In collaboration with ICRISAT, various groundnut lines, including some known wilt-susceptible and resistant cultivars, were screened for resistance to bacterial wilt at three locations—Kelantan, Kedah, and Serdang. Twenty seeds of each line were sown at 10 cm x 50 cm spacing. Three replications were used. Response to natural infection was studied at Kelantan and Kedah, where disease incidence was recorded 4 weeks after sowing. Response to artificial inoculation was studied at Serdang. Leaves were inoculated (using the multi-pinprick method) with *P. solanacearum* isolates from groundnut-growing areas of Kelantan and Terengganu. The percentage of wilted plants was recorded 1 week after inoculation.

Screening results of the ICRISAT lines and local controls (Table 1) indicate that the lines show different responses to infection by the wilt pathogen. In general, wilt intensity

Table 1. Percentage wilt incidence in 22 groundnut lines in response to natural infection and artificial inoculation with *Pseudomonas solanacearum* at different locations in Malaysia.

	Wilt incidence (%)		
Cultivar/line	Kelantan[1]	Kedah[1]	Serdang[2]
Gajah	25	-	20
Kelinci	40	0	75
Macan	65	5	30
Matjam (Susceptible control)	100	30	65
MKT 1 (Susceptible control)	80	0	100
ICGV 86188		25	90
ICGV 86199		0	50
ICGV 86243		0	100
ICGV 86302		0	90
ICGV 86303		0	70
ICGV 86309		20	100
ICGV 86310		0	100
ICGV 86315		5	50
ICGV 86330		10	100
ICGV 86635		0	20
ICGV 86680		5	90
ICGV 86691		35	65
ICGV 86699		5	90
ICGV 86707		0	20
ICGV 86708		5	70
ICGV 86743		20	90
ICGV 86745		0	70
ICGV 87237		0	100
ICGV 87281		0	60

1. Natural infection.
2. Artificial inoculation.

was higher with artificial inoculation than with natural infection. However, the control cultivars responded differently to natural infection at different locations. These results emphasize the need to evaluate host resistance over time and across locations.

Comparison of *Pseudomonas solanacearum* isolates

Isolates of *P. solanacearum* were identified using GN plates of the BIOLOG® microbial identification system (Biolog Inc., Hayward, California, USA). Four groundnut isolates—GN-1, GN-2, and GN-3 (all from Pasir Mas, Kelantan), and GN-4 (from Serdang, Selangor)—were compared with a tomato isolate T-1 from Serdang.

The groundnut cultivar MBT 91-2 was inoculated with these five isolates, and disease progression compared. Each isolate was used on 20 plants, each plant receiving a dose of 50 µL of the appropriate bacterial suspension (concentration 0.15 O.D. at 590 nm), delivered with a calibrated micropipette.

Of the five isolates, the tomato isolate was the most virulent on groundnut (Fig. 1). Observations on plant mortality over a 20-day period showed that plants wilted most rapidly when inoculated with the tomato isolate, although by day 12, mortality was similar (>88%) with T-1, GN-1, and GN-2. The isolates GN-3 and GN-4 seemed to be less virulent than the others.

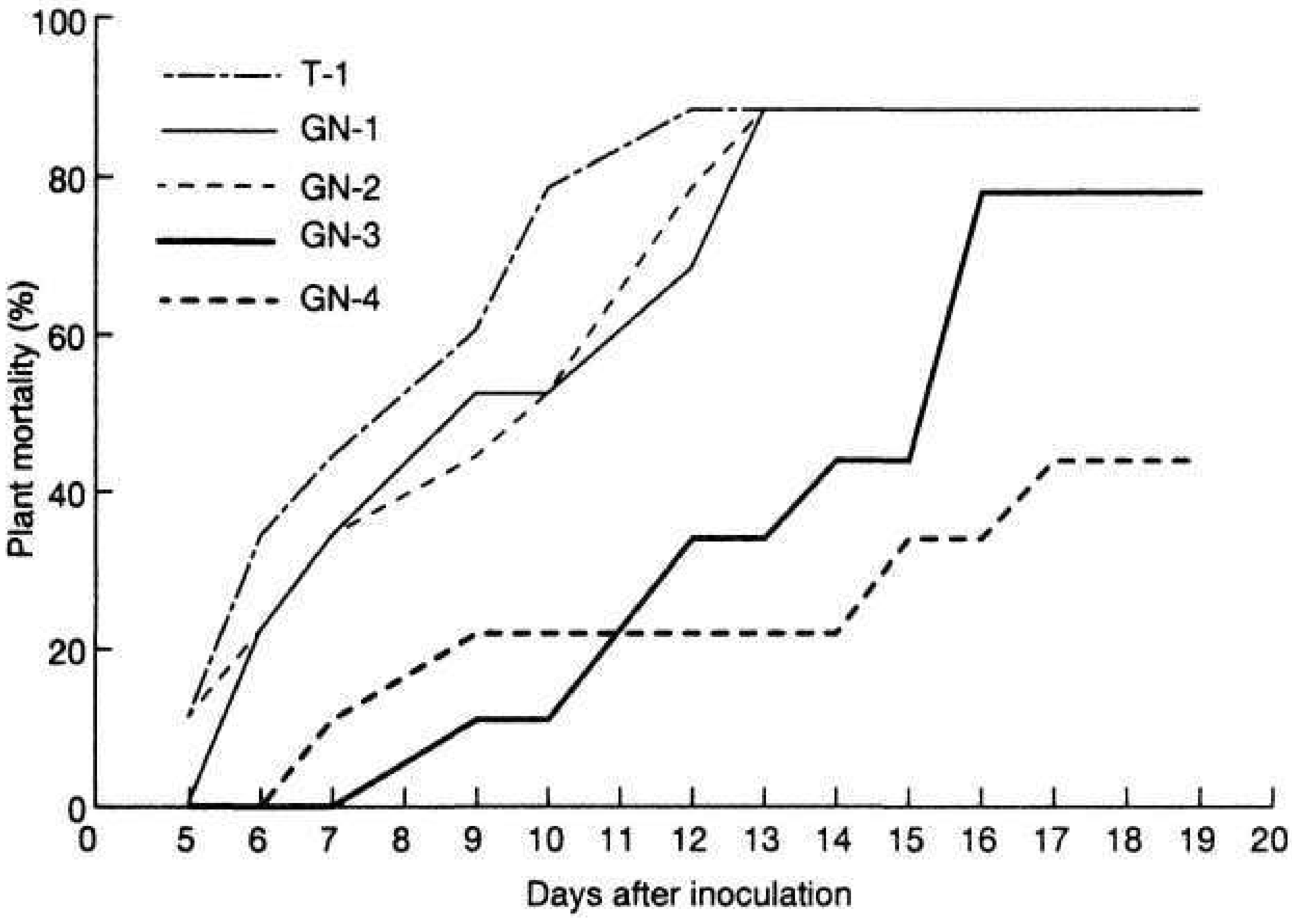

Figure 1. Percentage plant mortality following inoculation with one tomato and four groundnut isolates of Pseudomonas solanacearum *under greenhouse conditions, Malaysian Agricultural Research and Development Institute, Kuala Lumpur.*

Using BIOLOG® characterization, the four groundnut isolates were identified as *P. solanacearum* A, and the tomato isolate as *P. solanacearum* B. Comparison of results of the 95 tests which form the BIOLOG® GN Plate System indicated a number of key differences between the isolates in their utilization of substrates (Table 2).

Table 2. Differences between isolates in the utilization of substrates under the BIOLOG® GN Plate System.

	Reaction of isolate[1]				
Substrates	T-1	GN-1	GN-2	GN-3	GN-4
Dextrin	-	-	-	+	-
N-acetyl-D-glucosamine	-	-	-	+/-	+
D-galactose	-	-	-	+	-
m-inositol	-	-	+/-	-	-
Maltose	-	-	-	+	+/-
D-mannose	-	+	+	-	+
Psicose	-	+/-	-	+	+/-
D-sorbitol	-	-	-	+/-	-
Formic acid	-	-	-	+/-	-
β-hydroxybutyric acid	+	-	+	+	+
γ-hydroxybutyric acid	-	-	-	-	+
Propionic acid	+/-	+/-	-	+/-	-
Bromo-succinic acid	+/-	+	+	+	-
Succinamic acid	-	-	-	+/-	+
L-alanyl-glycine	-	-	-	+	+/-
D-serine	-	-	-	-	+/-
L-threonine	-	+	+	-	+
γ-amino-butyric acid	-	+	+	+	-
Urocanic acid	-	-	+/-	+	-
Glycerol	-	+/-	+	-	+/-
Mono-methyl succinate	-	+	+	+	+
Acetic acid	-	+	+/-	+	+/-
D-gluconic acid	-	+	+	+	+
D, L-lactic acid	-	+	+	+	+
Sebacic acid	-	+	+/-	+	+
Glucuronamide	-	+	+/-	+	+
Alaninamide	-	+	+	+	+
L-leucine	-	+	+	+	+
L-pyro-glutamic acid	-	+	+	+	+
Inosine	-	+	+	+	+

1. + = positive;- = negative.

Although there were variations between the four groundnut isolates, the tomato isolate differed substantially from the groundnut isolates in the utilization pattern for a number of substrates.

Acknowledgements

The authors would like to thank the Director General, MARDI, for permission to present this paper. Thanks are also due to Siti Rohani, Abu Bakar, and Napsiah Abdul Rahim for technical assistance.

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Present Status of Groundnut Bacterial Wilt Research in Uganda

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Abstract

Bacterial wilt of groundnut was first reported in Uganda in the early 1940s, and severe yield losses have subsequently been reported in some areas of central and northwestern Uganda. Some introduced groundnut germplasm lines show resistance to the disease. A germplasm collection, consisting of Ugandan material and lines from ICRISAT, has been made for screening and subsequent hybridization work. There is a need for extensive disease surveys and to develop integrated disease control measures.

Scope of the problem

Bacterial wilt, caused by *Pseudomonas solanacearum* (Smith) Smith, has been observed in some areas of central and northwestern Uganda. This disease has undoubtedly been present on groundnut in the country for many years. Agriculture Department reports from the 1940s describe the symptoms quite clearly at Bukalasa, and subsequent studies have shown that the pathogen involved is of biotype 3 (Simbwa-Bunnya 1972).

Groundnut cultivars show different reactions to the disease. The red Valencia cultivars are highly susceptible and yet these are preferred by the great majority of people. In general, spreading types appear to be less susceptible than bunch types under Ugandan conditions. Bacterial wilt is particularly associated with partially-waterlogged soils and the causal bacterium is spread in surface water. Yield losses can be severe if the soil is heavily infested with the wilt pathogen.

It has been suggested that the disease may be seedborne in groundnuts and, for this reason, it has been Agriculture Department policy to discourage the distribution of any seed for sowing from areas where the disease is known to occur. In practice, however, there is free movement of groundnut seed by traders to various markets, and eventually into farmers' fields. The disease, therefore, could be more widely distributed in Uganda than is presently believed.

Several sources of resistance are available, e.g., Kanyoma, RMP 12, 708, ICGs 5313, 6417, 7393, and 7968. Some hybridization work was done in 1973 to incorporate resistance into popular but susceptible varieties. Progress was hindered by limitations on resources and research facilities, and eventually all the breeding materials were lost during the civil unrest during 1982-86.

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Present research status

In view of the seriousness of the disease, research work, which was discontinued in the mid 1980s, has recently been reactivated. Bacterial wilt is soilborne and therefore difficult—and prohibitively expensive—to control chemically. The only effective means is the development and use of resistant cultivars.

We have started screening some germplasm lines including those mentioned above. We plan to carry out hybridization work if the resistant parents do not satisfy Ugandan market requirements. Screening could best be done in the greenhouse, to eliminate the possibility of mistaking disease escape for disease resistance. However, due to lack of laboratory facilities and expertise, we will be able to do only field screening.

Another important research requirement is to establish the extent of occurrence of the disease outside the known endemic areas. This, too, will need supporting funds.

Currently, we are seeking collaboration with ICRISAT and ACIAR on the bacterial wilt disease problem. It is intended to establish more links with researchers, especially in Indonesia, Thailand, Malaysia, Sri Lanka, Australia, the Philippines, and UK.

Proposed Work Plan

Disease survey

The four field screening locations mentioned below as being infested with bacterial wilt were identified as early as the 1960s. Since then, there has been free movement of seed from infested areas to other areas, at least part of it for sowing. Furthermore, in northern Uganda, bacterial wilt has been reported on tobacco. It is necessary to establish through detailed surveys whether, and in which areas, the disease is now affecting groundnut, and the extent to which farmers' varieties are affected.

The survey would also enable us to establish whether there is pathogenic variation in the *P. solanacearum* population.

Field screening

Test fields will be selected at the Namulonge Research Station, the Kabanyolo University Farm, Kawanda Research Station, and the Bukalasa Variety Trial Centre. A number of lines, including 36 resistant lines obtained from ICRISAT, will be screened during the first and second growing seasons of each year. Promising lines will be selected on the basis of wilt resistance, yield, and other agronomic traits.

Greenhouse screening

Depending upon the availability of funds and training opportunities, greenhouse facilities will be established. Promising lines will be screened in the greenhouse. This is important in order to make sure that lines selected as resistant are genuinely so. Studies will be done using both infested soil and artificial inoculation.

Using infested soil. Soil collected from fields known to be infested with *P. solanacearum* will be used for host-resistance studies, as a source of inoculum. Medium-sized clay pots will be used in the experiments.

Artificial inoculation using a syringe. Isolates obtained from diseased plants will be injected into test plants of the appropriate age, using a hypodermic syringe. This will be done after identifying the virulent colonies using Kelman's method. This is intended to supplement the infested soil method described above.

Multilocational trials

After screening for resistance, the selected lines will be tested for yield and adaptability in various agroclimatological zones.

Hybridization

If the resistant lines cannot be utilized directly as new varieties due to undesirable agronomic or commercial attributes, it will be necessary to incorporate resistance into currently available high-yielding cultivars.

Epidemiological studies

It is planned to conduct some epidemiological investigations into bacterial wilt in Uganda. Research will concentrate on the following aspects:

- Sources of inoculum: weeds and alternate hosts; plant debris and soil; and seeds.
- The influence of sowing date on bacterial wilt.
- Crop mixtures.

Integration of control methods

An integrated approach to bacterial wilt control is advocated, with a combination of host resistance and cultural control practices. It would be desirable to study cropping systems, soil types, and cultural practices that affect persistence of the wilt pathogen.

Training

So far, our knowledge of *P. solanacearum* and wilt disease of groundnut has largely been acquired during field resistance screening trials/surveys. We need a greater understanding of several aspects: screening techniques, mechanisms of resistance, pathogen detection, disease diagnosis, and disease management. It is therefore felt that some form of training in the appropriate techniques is essential before researchers in Uganda can address these problems more effectively.

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Present Status of Groundnut Bacterial Wilt Research in Indonesia

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Abstract

Progress on groundnut bacterial wilt research in Indonesia from the 1984/85 wet season to the 1991 dry season is reviewed. Groundnut bacterial wilt is widely distributed in Indonesia, and is particularly important in West Java, Lampung, parts of Central and East Java, and South and North Sulawesi. Disease incidence in the field ranged from 15 to 35% on wilt-resistant cultivars and from 60 to 90% on susceptible ones. Two distinct symptoms of the disease were observed, the typical wilting symptom and a yellowing of foliage followed by retarded growth. Isolates of the wilt pathogen, Pseudomonas solanacearum, *varied in virulence and biochemical characteristics; isolates from different localities varied widely in their virulence on the wilt-susceptible cultivar Chico. Two hundred and fifty groundnut isolates were tested; all were of biovar 3, except for one, which was of biovar 4. The isolates have a wide host range, including both weeds and economically important crops. The bacterium could infect groundnut seeds and could be seed-transmitted. A number of local and introduced groundnut genotypes were found to be wilt-resistant. Cultural control methods, specifically rotation of groundnut with non-host crops, could reduce disease intensity in the field; growing groundnut after flooded rice was the most effective cropping pattern to control bacterial wilt.*

Introduction

Groundnut bacterial wilt, caused by *Pseudomonas solanacearum* (Smith) Smith, was first found in the Cirebon area of West Java by van Breda de Haan in 1905, and has since then become widespread in Indonesia, causing serious damage to groundnut. Considerable efforts were made by Dutch scientists in Indonesia (and after their departure in the 1950s, by Indonesian scientists) to control the disease by selection and development of resistant cultivars, and by propagating their use in conjunction with cultural control measures.

As early as 1910, a resistant cultivar Raja was developed, and grown by farmers. The resistance of this cultivar, however, lasted for only 10 years. Further selections were made, and in 1926 another resistant cultivar, Schwarz 21, was introduced for commercial cultivation. This cultivar has since been widely grown in the country and used as a resistant parent to develop improved cultivars. In 1953 it was replaced by Gajah, a Schwarz 21

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derivative with more stable resistance and higher productivity. Since then, other wilt-resistant cultivars have been bred, but despite the availability of several resistant cultivars and the development of other control measures, the disease is still a problem (Machmud 1986).

Research on groundnut bacterial wilt in Indonesia, which was more or less discontinued in the late 1950s, was reactivated in 1985. Collaborative research work between the Agency for Agricultural Research and Development (AARD), Indonesia, and the Australian Centre for International Agricultural Research (ACIAR), Australia, began that year, and has played an important role in the progress of bacterial wilt research in Indonesia.

A status paper on bacterial wilt research in Indonesia was published in 1992 (Machmud and Hayward 1992). This paper gives an overview of progress on groundnut bacterial wilt research in Indonesia.

Disease distribution and economic importance

In the past, most of Indonesia's groundnut crop was grown in Java. At present about two-thirds (approximately 400 000 ha) of the groundnut area is still in Java, but the crop is also grown on other islands, such as Sumatera, Bali, West Nusa Tenggara, Sulawesi, and Irian Jaya. Annual surveys carried out since the 1984/85 wet season indicate that bacterial wilt is still widespread in Indonesia (Fig. 1). High disease incidence was common in West Sumatera, Lampung, West Java, parts of Central and East Java, and South and North Sulawesi. Lower incidence was found in North Sumatera, Bali, Lombok, and Irian Jaya. Wilt intensity varied with season, locality, and cultivar. Disease intensity generally ranged

Figure 1. Distribution of groundnut bacterial wilt in Indonesia (• indicates the presence of bacterial wilt).

from 5 to 35% on resistant cultivars such as Gajah, Kidang, Pelanduk, and Tupai, and from 60 to 90% on susceptible cultivars such as Kelinci and Landak. In some cases the disease caused total crop failure.

Symptoms

The bacterium attacks groundnut at different growth stages. Infection in young plants may cause sudden wilting of the plants, though the leaves remain green. On a resistant cultivar, or in older plants, the wilting symptoms usually develop slowly, starting with the lateral branches, enabling the plants to produce pods and seeds. Under these conditions, however, the plants may produce infected seeds. In some situations infected plants do not show significant wilting symptoms, but growth is retarded. These plants show yellowing of leaves, and usually produce few pods.

Strains

More than 250 isolates of P. *solanacearum* have been collected since 1985 from groundnut and other hosts commonly found in groundnut-growing areas. Strain identification based on biochemical characteristics of the bacterium suggested that there were two biovars of *P. solanacearum* namely, biovars 3 and 4, that attack groundnut in Indonesia. All but one of the isolates collected were of biovar 3. The exception was a biovar 4 isolate from Manokwari, Irian Jaya. Hayward (1990) reported that there were three biovars namely, 1, 3, and 4 of race 1, that attack groundnut, of which biovar 1 was indigenous to the Americas. Some of the isolates, their hosts, and origins are shown in Table 1.

It is noteworthy that an isolate from a groundnut field in a potato-growing area (Pangalengan, West Java, 1400 m asl) was of biovar 3, race 1. Potato-growing areas at altitudes above 1000 m asl usually have common infestations of biovar 2, race 3; and this was the case at Pangalengan as well.

Variation in virulence

The results of a 1987 study indicated that groundnut isolates tested on the susceptible cultivar Chico varied greatly in virulence. Isolates from Lampung and South Sumatera were comparable in virulence to those from Cikeumeuh and Citayam (Bogor, West Java). These isolates were more virulent than isolates from Kuningan (West Java) or Malang (East Java). Two separate greenhouse trials were conducted on the wilt-susceptible cultivars Chico and Kelinci, in which the virulence of different groundnut isolates was evaluated using the infectivity titration technique. Virulence levels were significantly different in the two cultivars, but there were no interactions between the isolates and the groundnut cultivars. Table 2 shows wilt intensityµin cultivar Kelinci in response to inoculation with different isolates of *P. solanacearum.* The isolate from Ngale, East Java, was the most virulent, while the biovar 4 isolate from Manokwari seemed to be less virulent than the biovar 3 isolates.

Table 1. Race and biovars of some *Pseudomonas solanacearum* isolates collected from different localities in Indonesia during 1985-91.

Isolate no.	Host	Origin	Altitude (m)	Race/ biovar
B01-B05	Groundnut	Bogor, West Java	250	1/3
PS 8963	Groundnut	Kuningan, West Java	400	1/3
PS 8970	Groundnut	Tangerang, West Java	50	1/3
PS 9101	Groundnut	Sukabumi, West Java	350	1/3
PS 9102	Eggplant	Cirebon, West Java	20	1/3
B079	Tobacco	Kutoarjo, Central Java	35	1/3
PS 8520	Groundnut	Tegal, Centra] Java	20	1/3
PS 9031	Groundnut	Pati, Central Java	50	1/3
PS 9032	Groundnut	Jepara, Central Java	40	1/3
PS 9110	Groundnut	Kebumen, Central Java	10	1/3
B106	Groundnut	Jambegede, East Java	335	1/3
PS 9125	Groundnut	Malang, East Java	180	1/3
PS 9126	Groundnut	Blitar, East Java	200	1/3
B098	*Croton hirtus*	Sulusuban, Lampung	20	1/3
PS 9130	Groundnut	Tamanbogo, Lampung	40	1/3
PS 8945	Groundnut	Pasaman, West Sumatera	75	1/3
PS 8946	*Croton hirtus*	Pasaman, West Sumatera	75	1/3
PS 8721	Groundnut	Maros, South Sulawesi	120	1/3
PS 8722	Groundnut	Bonotbili, South Sulawesi	100	1/3
PS 8974	Groundnut	Manokwari, Irian Jaya	50	1/4
PS 9103	Groundnut	Wonosari, Yogyakarta	200	1/3

Source: Machmud and Hayward (1992).

Host range

Studies showed that the groundnut isolates of the bacterium have a wide host range, including both weeds and economically important crops. This indicates that the isolates are of race 1. Apart from groundnut, nilam, an economically important industrial crop which is frequently intercropped with groundnut, eggplant *(Solanum melongena),* pepper *{Piper* spp), and rubber *(Hevea* spp) in West Sumatera were heavily infected with the bacterium.

Various weeds are commonly found in groundnut-growing lands, both under upland and irrigated lowland conditions. These weeds not only compete with groundnut plants, but may also act as hosts for the bacterium or for other interacting pathogens such as nematodes. Several wilt-resistant and susceptible weed species were identified in greenhouse experiments (Table 3). Field surveys have also found several naturally-infected weed hosts.

Table 2. Wilt intensity in groundnut cultivar Kelinci following inoculation with different isolates of *Pseudomonas solanacearum,* Bogor.

Isolate	Host/Origin of isolate	Wilt intensity[1] (%)
PS 9019	Groundnut/Ngale, East Java	86.00 d
PS 8954	Groundnut/Cikeumeuh, West Java	50.67 c
PS 9060	Groundnut/Malang, East Java	38.67 bc
PS 8974	Groundnut/Manokwari, Irian Jaya	38.17 bc
PS 9027	Groundnut/Muara, West Java	36.00 bc
PS 9006	Groundnut/Cikeumeuh, West Java	32.00 ab
PS 9028	Groundnut/Muara, West Java	30.67 ab
PS 9026	Pepper/Sumedang, West Java	21.33 a
PS 8962	Potato/Segunung, West Java	20.00 a
PS 9023	Potato/Lembang, West Java	20.00 a

1. Wilt intensity was recorded 2 weeks after inoculation. Numbers followed by a common letter are not significantly different at 5% level of Duncan Multiple Range Test.

Source: Machmud and Hayward (1992).

Table 3. Reactions to bacterial wilt of some weed species commonly found in groundnut-growing areas.

Family/species	Common name	Relative susceptibility to bacterial wilt[1]
Amaranthaceae		
Amaranthus spinosus	Bayam duri	R
Asteraceae		
Ageratum conyzoides	Goatweed, babadotan	R
Bidens pilosa	Spanish needle	R
Crassocephalum crepidioides	Thickhead	S
Sphilantes paniculata	Jotang	S
Euphorbiaccac		
Croton hirtus		S
Phyllanthus spp	Meniran	S
Physalis angulata	Ceplukan	S
Lcguminosae		
Crotalaria juncea	Crotalaria	S
Sesbania rostrata	Sesbania	S

1. R = resistant; S = susceptible.

Source: Machmud and Hayward (1992).

In areas surveyed in West Sumatera, crops and the weed *Crassocephalum crepidioides* that grew in proximity, were both severely infected. This suggests that intercropping using susceptible hosts could increase bacterial wilt severity in the field. Poor crop sanitation (for example, groundnut farmers usually neglect to remove weeds from their fields) may be another major cause of crop failure due to bacterial wilt.

Seed transmission

There has been controversy (mainly due to lack of experimental evidence) over the importance of groundnut seed infection and the role of infected seed in transmission of *P. solanacearum.* Palm (1922) first reported from Indonesia the transmission of the bacterium through groundnut seeds. He successfully isolated the bacterium from different parts of the seed, including the funiculus, pod shell, and seed coat, but not from the embryo. In the 1987/88 wet season, groundnut pods of eight cultivars were collected from plants showing wilting symptoms at the Cikeumeuh Experimental Farm, Bogor, an area known to be heavily infested with the wilt pathogen. The pods showed dark discoloration on the funiculus, pod shell, seed coat, and embryo. Bacterial isolates were obtained from different parts of the pods and seeds. Inoculation studies suggested that the isolates were of *P. solanacearum.* It was surprising that when the discolored seeds were sown in the green-house, wilted plants were observed 4 weeks after sowing at a rate of 5 to 8% in different cultivars (Table 4). These results (Machmud and Middleton 1990) confirm the report of Palm (1922) that the bacterium can be transmitted through groundnut seeds.

Table 4. Percentage of wilted plants from groundnut seeds harvested from eight cultivars infected with *Pseudomonas solanacearum.*

	Wilt intensity in the field (%)		
Cultivar	30 DAS[1]	100 DAS	Wilted seedlings from 100 seeds[2]
Gajah	8	12	5
Pelanduk	12	18	5
Kidang	10	16	6
Macan	6	16	5
Tupai	12	20	7
Kelinci	40	60	8
GH 467	18	42	7
GH 469	24	48	8

1. DAS - days after sowing.
2. 100 seeds of each cultivar were sampled from a bulk of seeds harvested from wilted plants. Seeds were grown in plastic trays containing sterilized soil from the Cikeumeuh farm. The incidence of wilted plants was recorded 2 and 3 weeks after sowing.

Source: Machmud and Hayward (1992).

Disease Control

No single control measure has proved effective in controlling bacterial wilt in groundnut. An integrated control program involving resistant cultivars and cultural practices such as crop rotation and crop sanitation can provide effective control of the disease (He 1990).

Host resistance

Several inoculation techniques were tested in order to develop a quantitative method to evaluate the resistance of groundnut germplasm in the greenhouse. All techniques tested were effective (Table 5). However, the hypodermic injection technique was considered the most appropriate.

Reactions of some Indonesian-bred cultivars to bacterial wilt are shown in Table 6.

Table 5. Bacterial wilt intensities on four groundnut cultivars inoculated with *Pseudomonas solanacearum* using five different inoculation techniques.

	Bacterial wilt intensity (%)			
Inoculation technique[1]	Gajah	Tupai	Red Spanish 119	Early Bunch
Leaf-axil wounding	8	4	80	84
Tooth-pick pricking	8	8	84	88
Hypodermic injection	12	8	88	88
Root dipping	28	20	100	100
Root drenching	16	20	80	80

1. Plants were inoculated 2 weeks after sowing. Cultivars Gajah and Tupai were resistant to bacterial wilt, while Red Spanish 119 and Early Bunch were susceptible.

Table 6. Reactions of 11 improved Indonesian groundnut cultivars to bacterial wilt, Cikeumeuh Experimental Farm, Bogor, 1988.

	Bacterial wilt	
Cultivar	Intensity (%)	Reaction[1]
Gajah	12	R
Pelanduk	18	R
Kidang	16	R
Macan	16	R
Tupai	20	R
Banteng	18	R
Anoa	18	R
Tapir	20	R
Kelinci	60	S
No. 467	48	S
Landak (No. 469)	42	S

1. R = resistant, S = susceptible.

Table 7. Groundnut cultivars and germplasm lines resistant and moderately resistant to bacterial wilt under natural and artificial conditions[1].

Genotype/identity	Country of origin	Genotype/identity	Country of origin
Resistant genotypes		Resistant genotypes	
Giza spread	Egypt	FESR 1	USA
Bola Blanco	Brazil	FESR 5	USA
Tatu (ICG 302)	Brazil	NC X-4	USA
Vera Cruz 2	Mexico	NC Ac 2145 (ICG 2308)	USA
Maria-B (ICG 243)	Mexico	Starr	USA
Cocalmete	Spain	PI 268653	Zimbabwe
Cocalmete do grand palmas	Spain	U 4-47-8 (ICG 232)	Sudan
		NG 2658 (ICG 235)	India
Chiba	Japan	SS 50 (ICG 2134)	India
Jaba 13	Japan	C 154 (ICG 2580)	India
Tachinasaki	Japan	VRR 407 (ICG 7594)	India
Argentina	Argentina	VRR 426 (ICG 7613)	India
EGPN 18	Unknown	Ah 7211 (ICG 539)	Sierra Leone
Brudul	Indonesia	S 7-1-7 (ICG 2667)	Tanzania
Lokal Tuban	Indonesia	286/63 (ICG 7502)	Uganda
Lokal Tasikmalaya	Indonesia	AH 7220 (ICG 3118)	Nigeria
Lokal Muneng	Indonesia	M 25-68(2)S (ICG 7635)	Nigeria
Deli Serdang	Indonesia	M471-75K (ICG 7639)	Nigeria
Kacang Brudul	Indonesia	2630-765 (ICG 7645)	Nigeria
Presi	Indonesia	M 979-75K (ICG 7563)	Nigeria
Macan	Indonesia		
Banteng	Indonesia	Moderately-resistant genotypes	
Kidang	Indonesia		
Ah 5/875/B-2-2	Indonesia	Giza 1	Egypt
Ah 5/875/38-8	Indonesia	Tainan No.7	Taiwan
790-61/26-36-B-1-2	Indonesia	Tainung	Taiwan
RR 6	Indonesia	Moket	Malaysia
RR 6/875-673-1875	Indonesia	E.G. Bunchag	India
GH 32/NC Ac 17090-4B-1	Indonesia	PI 19647	Unknown
		McCubbin	Australia
Tupai	Indonesia	Virginia Bunch	Australia
Tapir	Indonesia	Holland St. Runner	USA
Pelanduk	Indonesia	PI 393531	Peru
		NC Ac 17130	Peru

1. Under natural field conditions, seeds were grown in heavily infested soil at the Cikeumeuh Experimental Farm. Under artificial conditions, plants were inoculated using the leaf-axil injection technique in the greenhouse. ICG - ICRISAT Groundnut Accession Number.

Though wilt-resistant cultivars have been developed, they are usually susceptible to other diseases, and so their productivity is still low.

Field trials have been conducted since 1986 to evaluate the resistance of groundnut germplasm lines from Indonesia, and from ICRISAT, India. Several thousand germplasm accessions have been tested in wilt-sick plots at the Cikeumeuh Experimental Farm, Bogor, and the Jambegede Experimental Farm, Malang, using natural infection (Machmud and Middleton 1987, Machmud and Hayward 1992). Most of the germplasm lines tested were susceptible to bacterial wilt. Lines found resistant to the disease are listed in Table 7.

Cultural control

Organic amendments have been tried in the past for the control of bacterial wilt of groundnut, but the results are inconclusive. Pot trials were done in the greenhouse using green manure, animal manures, and compost. Compost and chicken manure proved more effective against the disease than green manure, cow dung, or goat manure.

A 3-year crop rotation trial was conducted at the Muara Experimental Farm. The wilt-susceptible cultivar Kelinci was used in rotation with different food crop combinations, including resistant groundnut cultivar Gajah, corn *(Zea mays),* soybean *(Glycine max),* irrigated rice, and sweet potato *(Ipomoea batatas).* The results showed that wilt intensity was generally reduced by rotation with resistant or non-host crops. The longer the rotation period, the more effective was the bacterial wilt control. Rotation with irrigated rice gave the best results, followed by combinations of corn-soybean, soybean-soybean, groundnut-corn, and sweet potato-sweet potato (Table 8). Crop sanitation by weed control and appro-

Table 8. Bacterial wilt intensity on susceptible groundnut cultivar Kelinci after 1 to 3 year rotations with resistant or non-host crops, Muara Experimental Farm, Bogor, 1987-1990.

	Wilt intensity[1] (%)		
Crop rotation combination	1	2	3
Rotation beginning wet season 1987/88			
Groundnut-groundnut	56	47	38
Groundnut-corn	48	40	28
Corn-soybean	44	31	23
Soybean-soybean	50	36	26
Rice-rice	33	21	12
Sweet potato-sweet potato	58	47	42
Control plots	66	62	65
Rotation beginning dry season 1988			
Groundnut-groundnut	58	46	40
Groundnut-corn	50	42	30
Corn-soybean	46	32	25
Soybean-soybean	51	38	27
Rice-rice	36	24	14
Sweet potato-sweet potato	54	44	38
Control plots	63	60	62

1. Assessments of bacterial will intensities were made before and after each rotation. 1, 2. and 3 are periods (in years) of rotation. Control plots were continuously grown with the susceptible groundnut cultivar Kelinci.

Source: Machmud and Hayward (1992).

priate water management (to prevent contaminated irrigation water from entering groundnut fields) are important to the success of crop rotation.

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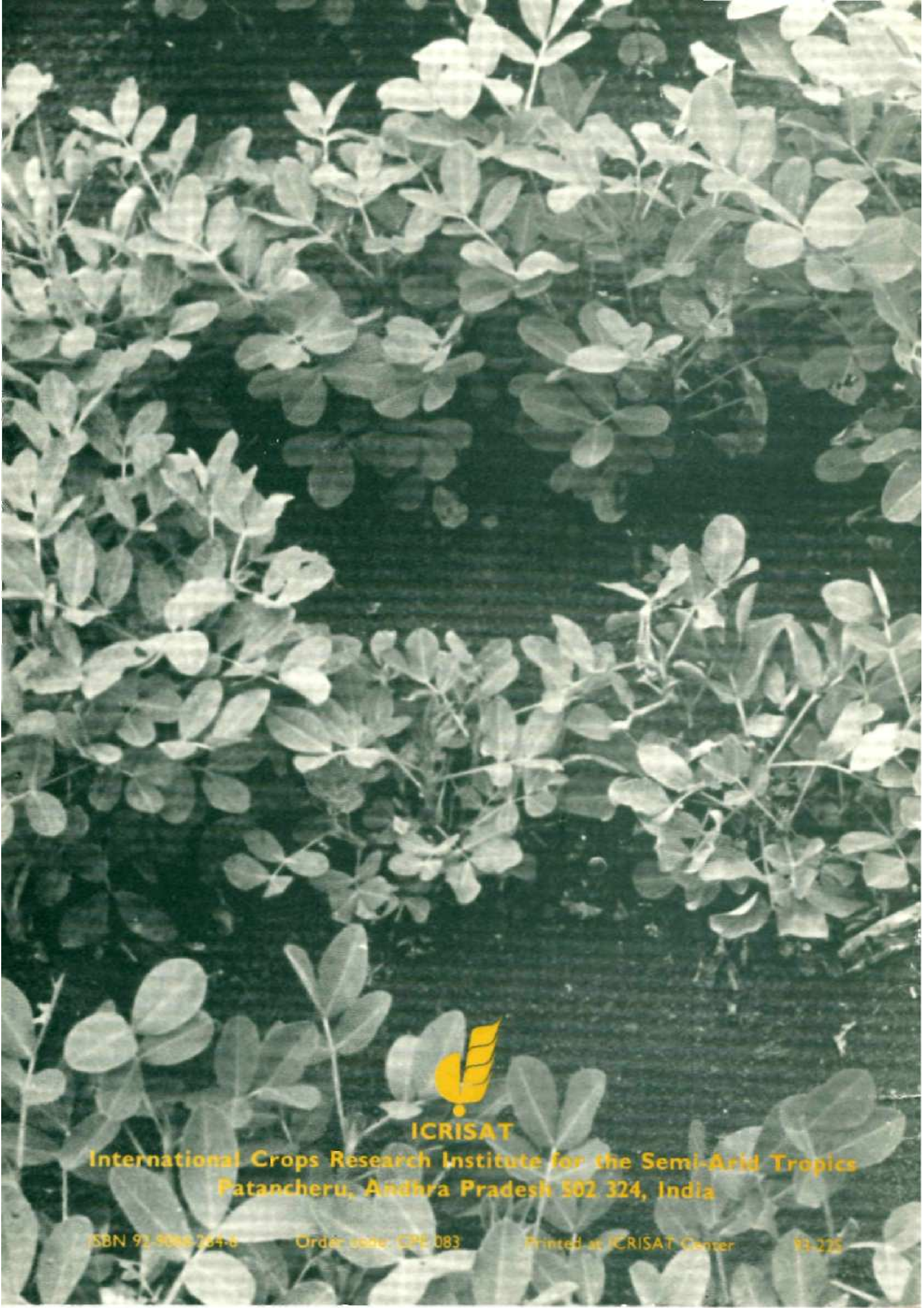

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ISBN 92-9066-264-8 Order code: CPE 083 Printed at ICRISAT Center 93-225