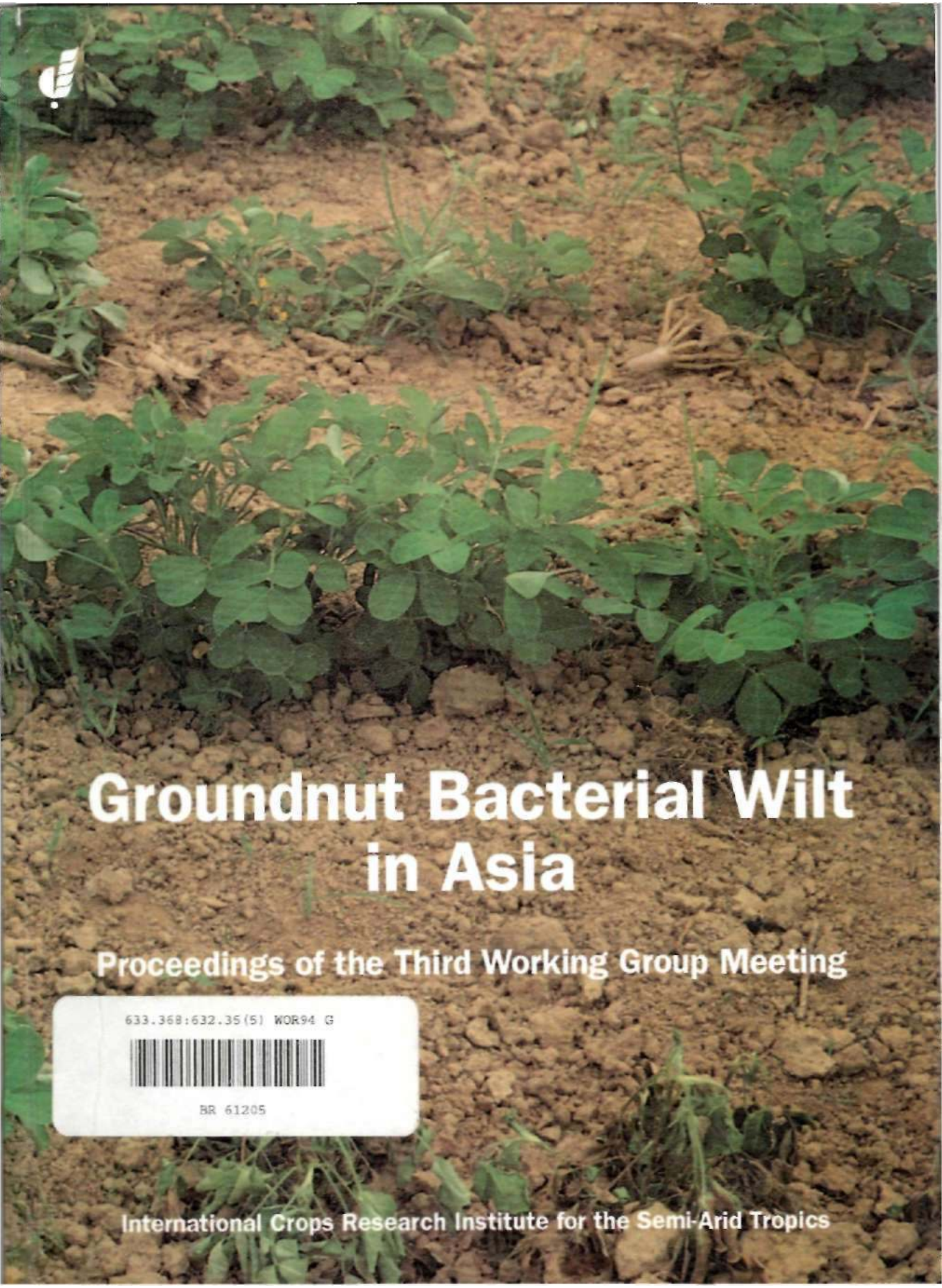


Groundnut Bacterial Wilt in Asia

Proceedings of the Third Working Group Meeting

633.368:632.35(S) WOR94 G

BR 61205

International Crops Research Institute for the Semi-Arid Tropics

Abstract

Citation:Mehan,V.K.,andMcDonald, D. (eds). 1994. Groundnut bacterial wilt in Asia: proceedings of the Third Working Group meeting; 4-5Jul 1994, Oil Crops Research Institute, Wuhan, China. (In En. Summaries in En, Ch, Fr, Es.) Patancheru 502 324, Andhra Pradesh, India: International Crops Research Institute for the Semi-Arid Tropics. 160 pp. ISBN 92-9066-3084. Order code: CPE 095.

The current status of research on bacterial wilt of groundnut in Asia is reviewed. Particular emphasis is given to recent advances in serological and molecular techniques for identification and differentiation of races, biovars, and strains of the wilt pathogen, *Pseudomonas solanacearum*.. Recommendations are made for further collaborative research, and the need to interest potential donors in promoting the Group's activities is emphasized. The publication includes country papers summarizing the status of the disease in China, Indonesia, Malaysia, the Philippines, Thailand, and Vietnam.

本文集对亚洲地区的花生青枯病研究现状作了评述，重点论述了关于青枯菌(*Pseudomonas solanacearum*)的血清学和分子学技术，病原菌的小种，生物型和菌系分化研究的最新进展。提出了进一步合作研究的建 议，强调了争取潜在的资 助以加强花生青枯病工作组活动的必要性。本文集还收集了介绍中国、印度尼西亚、马来西亚、菲律宾、泰国、越南花生青枯病现状的国家报告。

Resume

Le fletrissement bactirien de l'arachide en Asie: comptes rendus de la reunion du Troisieme Groupe de travail, 4-5 juillet 1994, Oil Crops Research Institute, Wuhan, Chine. Cet ouvrage presente une revue du statut actuel des travaux de recherche sur le fletrissement bacterien de l'arachide en Asie, portant surtout sur les progres recents en matiere de techniques serologiques et moleculaires pour l'identification et la differenciation de races, biovars et souches de l'agent pathogene du fletrissement, *Pseudomonas solanacearum.* Les recommandations sont faites pour le soutien de la recherche collaborative future mettant en relief le besoin d'interesser les bailleurs de fonds potentiels dans la promotion des activites du Groupe. La publication comprend egalement les communications faisant le point du statut de la maladie en Chine, en Indonesie, en Malaisie, aux Philippines, en Thailande et au Viet Nam.

Resumen

Marchitamiento bacterial del maní en Asia: Actas de la Tercera Reunión del Grupo de Trabajo, 4–5 de julio de 1994, Oil Crops Research Institute, Wuhan, China. Examina el estado actual de la investigación sobre el marchitamiento bacterial del maní en Asia. Se hace hincapie en los avances recientes en las técnicas serológicas y moleculares para la identificación y diferenciación de estirpes, biovares y clases de patógeno del marchitamiento *Pseudomonas solanacearum*. Se hacen recomendaciones para futura investigacíon colaborativa y se destaca la necesidad de generar interes en posibles donantes hacia la promoción de las actividades del Grupo. La publicación incluye ponencias de países resumiendo el estado de la enfermedad en China, Indonesia, Malasia, Filipinas, Tailandia y Vietnam.

Groundnut Bacterial Wilt in Asia

Proceedings of the Third Working Group Meeting

4-5 Jul 1994
Oil Crops Research Institute,
Wuhan, China

Edited by
V K Mehan and D McDonald

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

1994

Co- sponsors

Chinese Academy of Agricultural Sciences (CAAS), China

Peanut Collaborative Research Support Program (Peanut CRSP), USA

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

Organizing Committee

D McDonald, V K Mehan, and C L L Gowda (ICRISAT Asia Center, India)

Guo Qingyuan, B S Liao, and Zhan Xianhe (Oil Crops Research Institute, China)

A C Hayward (University of Queensland, Australia)

The opinions in this publication are those of the authors and not necessarily those of ICRISAT, The designations employed and the presentation of the material in this publication do not imply the expression of any opinion whatsoever on the part of ICRISAT concerning the legal status of any country, territory, city, or area, or of its authorities, or concerning the delimitation of its frontiers or boundaries. Where trade names are used this does not constitute endorsement of or discrimination against any product by the Institute.

Contents

Groundnut bacterial wilt: past, present, and future

Host-plant resistance and cultural control

Country reports on the status of groundnut bacterial wilt research

Preface

The third meeting of the Groundnut Bacterial Wilt Working Group was held in Wuhan, China, from 4 to 5 Jul 1994. The meeting, and an associated short training course (*Techniques for the diagnosis and identification of the bacterial wilt pathogen, Pseudomonas solanacearum, and for resistance screening against groundnut bacterial wilt*), were co-sponsored by the Chinese Academy of Agricultural Sciences (CAAS), Cereals and Legumes Asia Network (CLAN)/ ICRISAT, and the Peanut Collaborative Research Support Program (Peanut CRSP).

Scientists from eight countries and three international research organizations participated in the meeting. Recently developed procedures for identification of specific biovars and strains of the pathogen were discussed, and accounts of current research on the bacterial wilt disease in cooperating institutions were presented. Good progress has been made, and prospects of continued collaborative research into the management of this important disease are excellent.

We hope this volume will provide a useful guide to the present status of groundnut bacterial wilt in Asia and stimulate more coordinated work on this serious disease.

V K Mehan

D McDonald

Inaugural Session

Welcome address

Liang Keyong[1]

Respected Chairman [Dr Guo Qingyuan], Dr Ryan, Dr Nene, Mr Zhe, distinguished administrators, and participants,

First of all, please allow me, on behalf of President Wang Lianzheng and the Chinese Academy of Agricultural Sciences (CAAS), to express our warm welcome to Dr Ryan; Dr Nene; the experts from Australia, Indonesia, Malaysia, the Philippines, Thailand, UK, and Vietnam, and from ICRISAT; officials from FAO and the International Plant Genetic Resources Institute (IPGRI); and scientists from China for your attendance at this meeting. I would like to take this opportunity to highlight the cooperation between ICRISAT and the Chinese institutions.

The cooperation between ICRISAT and CAAS began in the 1980s, when, in 1988, we signed the first cooperative agreement. Since then we have executed two work plans. On 2 Jul 1994, we discussed and signed the work plan for 1995-1997. In the past years, through training courses, collaborative research, mutual visits, workshops, and germplasm exchange, our collaboration in research on sorghum, pearl millet, chickpea, pigeonpea, and groundnut has been very successful. The number of institutions as well as scientists that cooperate with ICRISAT is increasing. We have new cooperators from several institutions in Shandong, Guangdong, Qinghai, and Henan for collaborative research. As a result of cooperation, young scientists were trained, research work improved, and some results from this research were used in crop production. Also, the cooperation has strengthened the friendship between ICRISAT and CAAS. Of course, there are still some aspects, e.g., exchange of crop germplasm, that need to be improved. We shall do our best to improve this aspect. We hope to enhance our cooperation to a new stage.

Groundnut bacterial wilt has been reported to be a serious problem in several countries in Asia, and it is also a potential threat to groundnut production in other humid tropical regions. Therefore, it has drawn much attention in many countries and international institutions. We greatly appreciate the efforts made by ICRISAT in organizing the international collaborative research activities on groundnut bacterial wilt.

This is the first international scientific meeting held in China for groundnut. I have been much impressed by the success of your Working Group and by the excellent spirit of cooperation that has been manifest. It will strengthen academic exchange between scientists in China and other countries. We hope

1. Vice-President, Chinese Academy of Agricultural Sciences, Beijing 100081, China,

that our scientists will make full use of this opportunity to learn from the work done in other countries. We also hope this international meeting will play an important role in the control of groundnut bacterial wilt in all the countries where the disease is prevalent.

I should like to welcome you again and wish the meeting a great success.

Welcome from FAO

C Y Shen[1]

Respected Vice-President Liang Keyong, Director General Dr Ryan, Chairman Dr Guo Qingyuan, distinguished experts, Ladies and Gentlemen,

It is my great pleasure to have the opportunity to attend this Working Group meeting. First of all, please allow me, on behalf of the Director General of the Food and Agriculture Organization of the United Nations, and on my own behalf, to warmly welcome all of you to attend this important meeting and to sincerely wish the meeting a great success.

During this 2-day Working Group meeting, the experts from different countries will review the progress of research in recent years in diagnosis, biology, and ecology of the wilt pathogen; discuss integrated control of groundnut bacterial wilt; and establish priorities for future research and collaboration. I sincerely hope that this meeting will be a milestone in the progress of the Groundnut Bacterial Wilt Working Group (GBWWG). FAO Regional Office for Asia and the Pacific (RAPA) considers the activities of the GBWWG to be very important in Asia, and has joined the Asia Pacific Association of Agricultural Research Institutions (APAARI) as an Associate Member from 1 Jan 1994. I am pleased that China is one of the member countries of APAARI, with which we have a very close relationship.

Finally, I should like to extend my deep appreciation to our most generous hosts for their excellent arrangements for the meeting, their warm hospitality, and the tremendous work that they have put in both before and during the meeting.

I wish the meeting all success.

1. FAO Regional Office for Asia and the Pacific, Bangkok 10200, Thailand.

Objectives of the Meeting

Y L Nene[1]

Professor Guo Qingyuan, Professor Liang Keyong, Dr J G Ryan, distinguished participants from the Chinese Academy of Agricultural Sciences (CAAS) and from various countries, and my colleagues from ICRISAT,

Let me add my own welcome to you to this 2-day Groundnut Bacterial Wilt Working Group Meeting. I am particularly happy to participate in this meeting because I have spent more than 25 years of my professional life working on diseases of grain legumes, particularly the grain legumes known as pulses, such as mungbean, pigeonpea, and chickpea.

The Cereals and Legumes Asia Network (CLAN), which started off in 1986 as the Asian Grain Legume Network (AGLN), with ICRISAT providing a Coordinating Unit, has successfully supported many collaborative activities in research on ICRISAT's mandate crops, namely sorghum, pearl millet, chickpea, pigeonpea, and groundnut. The mechanism of constituting "Working Groups', to conduct collaborative research on the challenging regional problems, has been effective in achieving quick progress. There is a large number of such international working groups focusing attention on narrow topics—one such working group is meeting here today.

The Groundnut Bacterial Wilt Working Group was formed in 1990 after a planning meeting held in Malaysia. This third meeting of the Group is being held jointly under the auspices of CAAS and ICRISAT. It is particularly gratifying that this meeting is being held at one of the leading institutions, the Oil Crops Research Institute at Wuhan.

The objectives of the meeting are as follows.

- To bring together active researchers to exchange the latest information on the bacterial wilt of groundnut.
- To evaluate the status of research on the disease in different countries and regions.
- To recommend priorities for collaborative research at national and international levels.
- To assign, wherever possible, research modules to individuals or groups.
- To identify specific training needs as well as organizations that can offer training.
- To determine the resources needed and to prepare research proposals for funding.

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

This meeting is being attended by over 40 scientists and administrators from 8 countries. Participants also include representatives of several international organizations. The abstracts of papers to be presented clearly indicate the progress being made towards solving the problem through basic as well as applied research.

We should all feel happy that this 2-day meeting is being followed by a 4-day training course to provide opportunities to young scientists to gain practical experience in the diagnosis of the wilt pathogen, *Pseudomonas solanacearum*, using advanced techniques, as well as to obtain information on the disease management. The Faculty for this course has been drawn from Australia, China, Malaysia, and the United Kingdom, and from ICRISAT.

I am confident that this meeting and the training program will prove useful in finding practical solutions to this very serious disease problem of groundnut.

Inaugural address

J G Ryan[1]

Dr Liang Keyong, Dr Guo Qingyuan, distinguished participants from China and other countries, and my colleagues from ICRISAT,

Let me add my own welcome to you to this 2-day Working Group Meeting on Groundnut Bacterial Wilt. I am very happy to participate in this meeting being held at one of the leading institutions, the Oil Crops Research Institute at Wuhan. The choice of China and Wuhan in particular for this meeting was influenced by the interest of Working Group members in being able to interact with many Chinese scientists who have made such notable contributions to the collaborative research on groundnut bacterial wilt.

I am pleased to take this opportunity to spell out certain aspects of cooperation between ICRISAT and Chinese institutions. The first ICRISAT mission to China, headed by the then Director General, Dr L D Swindale, was in 1979. After this, ICRISAT scientists have made more than 10 visits to China. The formal Memorandum of Understanding (MOU) with the Chinese Academy of Agricultural Sciences (CAAS) was signed in May 1988. We have had three collaborative workplans with China, the most recent being signed on 2 Jul 1994 in Beijing. More than 67 Chinese scientists and technicians have participated in various training programs at ICRISAT. Germplasm exchange has been a major activity ICRISAT has supplied 5420 germplasm accessions and 3280 breeding lines/materials of its five mandate crops. However, only 585 Chinese germplasm accessions (including 217 of groundnut) are currently available in the ICRISAT gene bank. This represents only a minor proportion of the germplasm available in China. We hope that we can work out a way by which the available Chinese germplasm lines can be exchanged with ICRISAT and the outside world.

Collaboration with Chinese institutes, especially on bacterial wilt and groundnut virus diseases, has been very good. We at ICRISAT are not able to conduct research on bacterial wilt of groundnut, and hence depend on the comparative advantage and expertise of Chinese groundnut scientists to assist us and other network member countries in the management of this disease.

Bacterial wilt is an important disease of groundnut, particularly in the countries of East and Southeast Asia and the South Pacific. The disease is recognized as serious in China and Indonesia, and is becoming increasingly important in Malaysia, the Philippines, Thailand, and Vietnam. Bacterial wilt is also a problem in some areas of Africa. Notwithstanding its importance,

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

reliable estimates of yield losses from bacterial wilt are not generally available. In China, over 200 000 ha are affected by the disease. Yield losses of 10-30% are common in the central and southern provinces, and can reach up to 60% in individual fields. In Indonesia, yield losses are estimated at 50 000 to 150 000 t annually.

Basic research on the wilt pathogen, *Pseudomonas solanacearum,* has been carried out in advanced laboratories in several countries, as *P. solanacearum* attacks many other important food crops throughout the world. China and Indonesia have a long history of research on groundnut bacterial wilt, and wilt-resistant groundnut cultivars have been developed in both the countries. The variety Schwarz 21 was released in Indonesia in 1927, the earliest record of a disease-resistant groundnut being developed. Progress has also been made in both the countries in cultural control of bacterial wilt. However, much remains to be done. There is a need for breeding wilt-resistant groundnut varieties with high yield potential and good agronomic characters, and to combine resistance to other diseases and pests. These can be used with appropriate cultural control practices to develop integrated disease management practices for particular situations.

Although good progress has been made in the past, progress could be much more rapid if different groups could cooperate more effectively instead of working in isolation. At the Asian Region Groundnut Scientists' Meeting in Malang, Indonesia in 1988, the participants recommended such cooperation among national, regional, and international groups. The Australian Centre for International Agricultural Research (ACIAR) and ICRISAT took the initiative and organized a planning meeting in March 1990 in Malaysia, as a satellite meeting to the 3rd International Conference on Plant Protection in the Tropics. The International Groundnut Bacterial Wilt Working Group was set up as a result of a recommendation from this meeting. Dr A C Hayward, University of Queensland, Australia, was designated as Technical Coordinator. The Asian Grain Legumes Network (AGLN) was requested to provide administrative and logistic support to the Working Group. The second meeting of the Working Group (sponsored by AGLN/ICRISAT) was organized in November 1992 as a satellite meeting to the International Symposium on Bacterial Wilt held in Taiwan. Unfortunately, not all the Working Group members could attend that meeting. I am happy to see that the Group is in its full strength today.

In 1993, the coordination of the Working Group became the responsibility of Dr V K Mehan of ICRISAT, and he and Dr C L L Gowda, Coordinator of the Cereals and Legumes Asia Network (CLAN), have organized the present meeting in cooperation with the Oil Crops Research Institute, Wuhan, and CAAS, Beijing.

The Groundnut Group based at ICRISAT Asia Center has responsibility for working jointly with Asian NARS scientists to address the constraints to groundnut production in different production systems in Asia. This function is facilitated by CLAN (formerly AGLN).

ICRISAT Asia Center scientists are not directly involved in research on the disease as groundnut bacterial wilt is not prevalent in India. Hence, research has to be through our NARS partners. However, we do assist with exchange of germplasm and information, and provide training. Also, as ICRISAT is an international organization, we can help with coordination of research and networking with members of the Working Group.

Groundnut bacterial wilt is obviously a very serious problem in groundnut production systems in East and Southeast Asia and in similar systems in other parts of the world. The disease could spread and cause substantial losses in new areas where irrigation and multiple cropping are being introduced. Given the necessary support and funding, the efforts of the Working Group should be able to ensure that integrated disease management technology is available to meet such a challenge.

I hope that the recommendations that evolve from this meeting will provide a sound basis for continued international cooperative research to provide solutions to manage this serious disease that constrains production of groundnut in many regions of the world.

Identification and diagnostic methods for Pseudomonas solanacearum

An integrated system for identification and characterization of plant pathogenic bacteria with special reference to *Pseudomonas solanacearum*

R Black[1] and K Y Lum[2]

Abstract

Methods for preliminary identification of bacteria (BACT1D) and metabolic profiling using Biolog are described and presented as an integrated system for the identification and characterization of Pseudomonas solanacearum. The BACTID system helps in quickly eliminating saprophytes and other nontarget organisms. It is recommended as a cost-effective step before metabolic profiling is used to identify P. solanacearum positively and to characterize subspecific isolates. Progress in the development of metabolic profiling of P. solanacearum and related bacteria using user-defined databases, alternative software, and cluster analysis is discussed. Reference is made to isolates of R solanacearum from Malaysia.

该文描述了用细菌初步鉴定(BACTID)和 BIOLOG 代谢谱方法作为青枯菌(*Pseudomonas solanacearum*)的综合鉴定系统。BACTID 系统有助于迅速排除腐生菌和其他非研究对象微生物,它被作为代谢谱方法用于青枯菌鉴定之前一个必要的步骤。讨论了应用代谢谱方法鉴定青枯菌和其他有关细菌,包括设立数据库、代用软件和检索分析方面取得的进展。该文的应用以马来西亚青枯菌株为例。

Introduction

The Biolog system for identifying bacteria by their metabolic profiles has been evaluated and adapted for use in small plant pathology laboratories in developing countries. Special emphasis has been put on *Pseudomonas solanacearum* and related bacteria (Black and Sweetmore 1994). One disadvantage of the system is the high cost of plates for routine diagnostic use. To avoid unproductive use of Biolog plates on saprophytic or other nontarget bacteria, the use of the BACTID system for preliminary identification is suggested in conjunction with Biolog. This paper describes the features of

1, Natural Resources Institute, Central Avenue, Chatham Maritime, Kent ME4 4TB, United Kingdom
2. Malaysian Agricultural Research and Development Institute, PO Box 12301,50774 Kuala Lumpur, Malaysia.

Black, R., and Lum, K.Y. 1994. An integrated system for identification and characterization of plant pathogenic bacteria with special reference to *Pseudomonas solanacearum.* Pages 15-26 *in* Groundnut bacterial wilt in Asia: proceedings of the Third Working Group meeting, 4-5 Jul 1994, Oil Crops Research Institute, Wuhan, China (Mehan, V. K. and McDonald, D., eds.). Patancheru 502 324, Andhra Pradesh, India: International Crops Research Institute for the Semi-Arid Tropics.

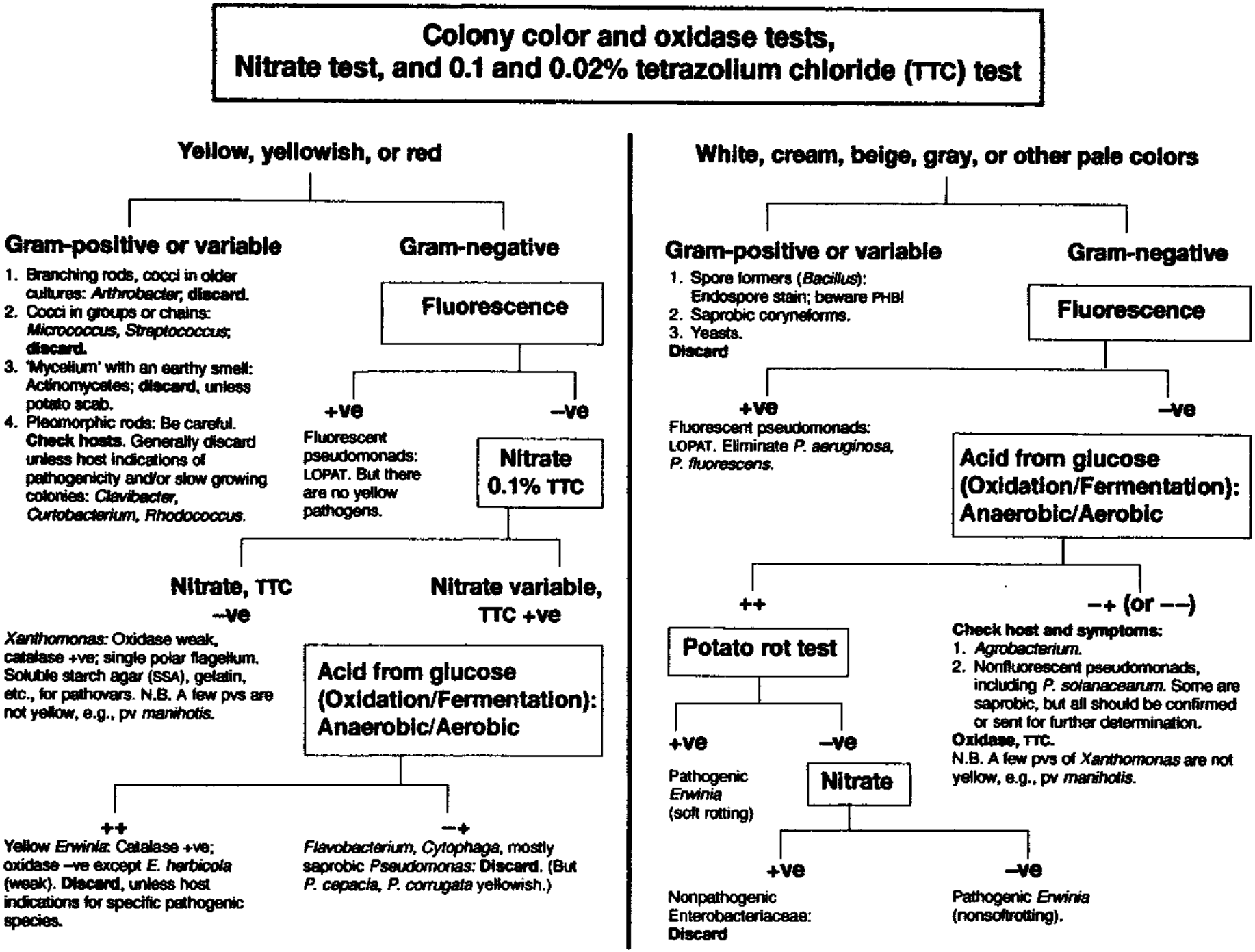


Figure 1. Flow chart of scheme for preliminary identification of plant pathogenic bacteria in the tropics, emphasizing early elimination of nonpathogens. (Originally devised by senior author and J Kolkowsky of International Mycological Institute.)

BACTID and discusses the use of the Biolog method to the best advantage of identification and characterization of *P. solanacearum.*

BACTID system

Scheme for preliminary identification

Bacteriology is generally neglected in small plant pathology laboratories engaged in routine diagnosis. One problem is the wide range of media and reagents required for complete identification of the full range of bacteria that are likely to be encountered (Black and Sweetmore 1994). A scheme was devised to make identification of plant pathogens more accessible to such laboratories. The basic motivation was to develop a scheme that would provide sufficient information for diagnostic purposes without necessarily resorting to complete identification. Originally conceived by the senior author and J Kolkowsky at the International Mycological Institute, and based on Bradbury's key (Bradbury 1970), the flow chart for the scheme is given in Figure 1. An important feature of the scheme is the rapid elimination of saprophytes so that further work can be concentrated on important organisms. This makes the scheme suitable for use as a precursor to the definitive identification of *P. solanacearum* by more expensive methods such as metabolic profiling and fatty acid profiling.

The flow chart in Figure 1 shows the scheme with media in conventional format. The use of such test strips as Oxidase Touch Sticks (Unipath) and nitrate/nitrite strips (Merck) is also recommended.

BACTID plates

Attention was given to developing a scheme in a comprehensive and convenient kit form. Hayward et al. (1990a) described the use of microtiter plates for the phenotypic characterization of plant pathogenic species of *Pseudomonas*, with the wells acting as miniature petri dishes. Some of the required tests involved the use of solid media while others required liquid media. Other tests (such as nitrate reduction and those that require anaerobic conditions) could not be done in this format. In the BACTID kit, eight tests are included in a microtiter plate-based kit in solid form for preliminary identification of bacteria following the BACTID scheme. An innovative feature of the method is the use of freeze-dried plugs of potato for the potato rot test. Gelatin liquefaction and oxidation/fermentation tests are done in 7-mL sterile disposable Bijou bottles. A slope of nutrient agar is also provided for Gram and oxidase tests. Details of these tests are given in Table 1.

In small plant pathology laboratories, one obstacle to more extensive bacteriology work is that media are often not readily available. There will be some delay while media are prepared, or obtained from suppliers. One feature of this system is that kits may be prepared in advance and stored in a refrigerator for several months. With the addition of sterile disposable loops and similar items, the components make a portable kit.

Table 1. Tests for bacterial identification included in the BACTID kit

Medium/Test	Purpose
Microtiter plate[1]	
Nutrient agar	Catalase test: Add a drop of 3% hydrogen peroxide. Bubbling indicates a positive reaction.
Tetrazolium salt (0.1, 0.02%)	Bright red smear indicates tolerance to tetrazolium chloride.
King's medium B	There will be blue-green fluorescence under UV light (or in bright sunlight). With a UV viewer, it is not necessary to remove the lid from plastic microtiter plates.
Sucrose nutrient agar	Thick, creamy, mucilaginous growth indicates levan-positive strain. (Practice is required to recognize a positive result).
Nitrate reduction test	One drop of each of the nitrate test reagents should be added in the usual way. See Lelliott and Stead (1987) for interpretation of results.
Soluble starch agar	Add iodine for starch hydrolysis test. Clear zones in the blue iodine reaction indicate starch hydrolysis.
Potato rot test	Positive strains will break down the plug of freeze-dried potato[2].
Separate tubes	
Nutrient agar (NA) for: Color/Gram reaction	Observe color on the NA slope. Use a loop to remove culture from the slope for the Gram reaction. (The 3% KOH solubility test is recommended. See Lelliott and Stead 1987.)
Oxidase test	Use Oxidase Touch Sticks on NA slope. A deep purple color within 30 seconds indicates a positive result.
Gelatin	Gelatin-positive strains will liquefy the medium. Compare with noninoculated control and/or a known positive.
Oxidation/fermentation test	After stab inoculation, close the anaerobic tube tightly. (No sealant is required.) Yellow color indicates acid production from metabolism of glucose.

1. At least one column should be a noninoculated control. If all 12 columns of the plates are seldom used, multiwell strips can be used instead of the full 96-well microtiter plates.
2. If a freeze drier is not available for the potato plugs, the plugs may be stored separately in the deep freeze, or they may be freshly prepared immediately before use.

BACTID software

A computer expert system has been developed to provide identification of bacteria from the test results in the BACTID scheme. The software was written on the KnowledgePro shell (Knowledge Garden Inc.), but the run-time version is in the public domain. An interim release of BACTID is available for evaluation. BACTID may be used in several ways:

- Following the flow chart for the sequence of tests. This is particularly useful when no particular bacterial group is suspected.

- Performing selected tests in any desired sequence. This is useful when a particular group is suspected (e.g., *Xanthomonas*).
- Performing all necessary tests at one time. BACTID should be used this way with the BACTID plate kit, which provides tests in a convenient, portable format.

The hypertext in the expert system allows the user to access background information on the bacteria and the tests. In addition, full details of media, reagents, test methods, and laboratory safety practices are included, providing a comprehensive laboratory manual. The basic source for most of the methods is Lelliott and Stead (1987). BACTID may also be used in conjunction with the Plant Clinic training system for decision-making and resource management in diagnosis (Black, Sweetmore, and Holt 1994, in press).

Use of the Biolog system for identification and characterization of *Pseudomonas solanacearum*

Biolog methods

Methods that adapt the Biolog method for *P. solanacearum* and related bacteria were described by Black and Sweetmore (1993). A liquid pre-incubation medium (nutrient broth) is used and the cultures are washed by centrifuging. This departure from standard Biolog methods is discussed by Black and Sweetmore (1994).

Software for reading plates and interpreting metabolic profiles

The color reaction in the wells of the Biolog microliter plate must be interpreted as a substrate reaction (positive, negative, or borderline). The complete set of substrate reactions provides the metabolic profile (fingerprint) of the bacterium. This profile has to be matched with a database (library) of profiles of known taxa of bacteria. Several options are available to read Biolog plates, interpret the well-readings, and match them to the database (Figure 2). The Biolog GN database for Gram-negative bacteria may be used with any version of Biolog's MicroLog software to identify at least 90% of isolates of *P. solanacearum*. For identifying a wider range of bacteria, it is necessary to acquire databases compiled from profiles including a wider range of isolates for each taxa, particularly those from the user's own locality. If such a database is used, configuring the Bacterial Identifier program (Blackwell Scientific Software) for the Biolog system is recommended in addition to, or as an alternative to, MicroLog (Black and Sweetmore 1994).

High background readings may cause problems if absorbance readings in the plate reader are converted to substrate reactions by internal or external software with the standard Biolog thresholds. The recommended solution is to use fixed thresholds (Black and Sweetmore 1993). However, recent versions of MicroLog that accept absorbance data files, or control and read the plate reader directly, can interpret poor plates satisfactorily in most cases.

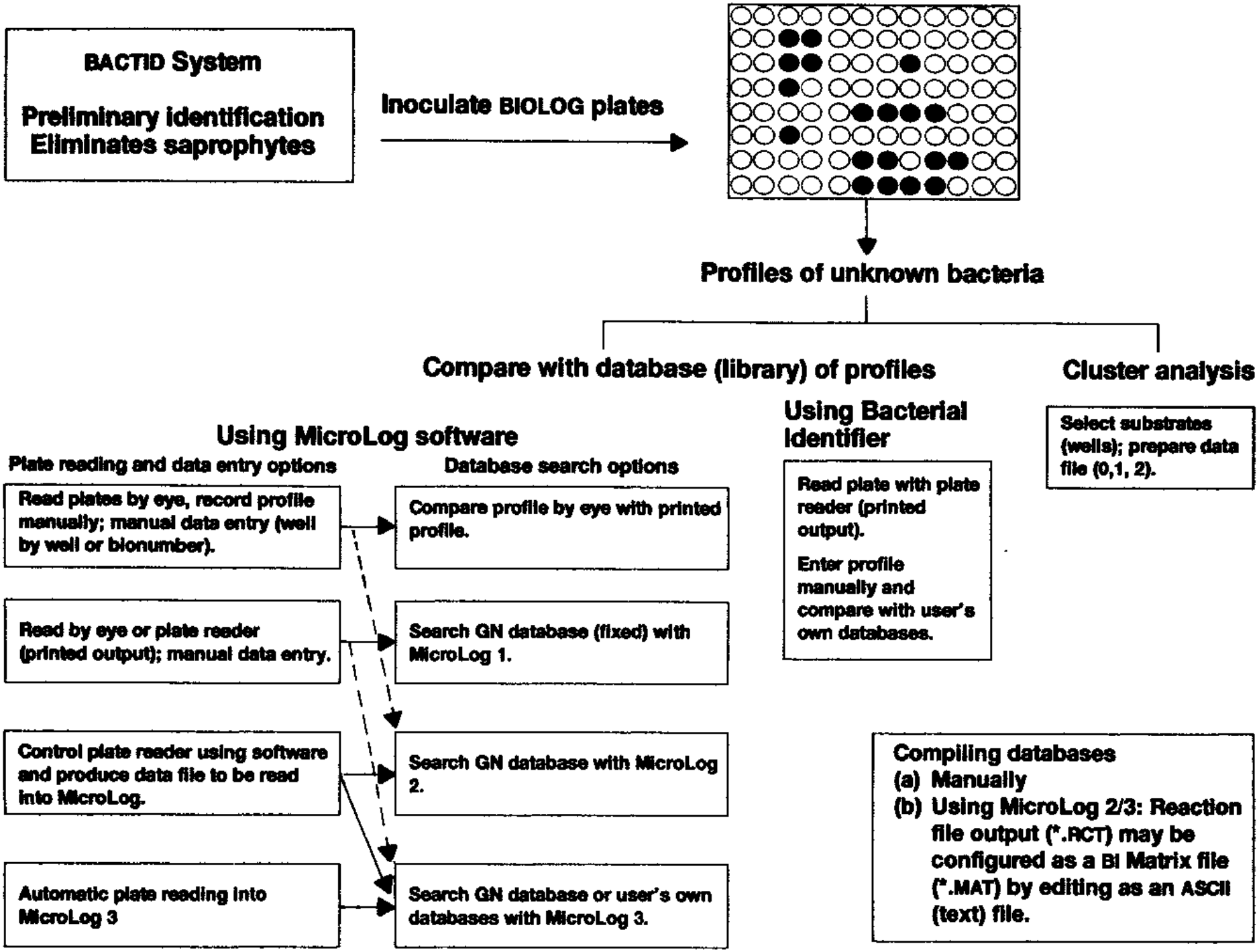


Figure 2. Integration of BACTID system and metabolic profiling for identification of bacteria: software options.

Sub-specific characterization

Biovars

In adapting Biolog for small plant pathology laboratories for routine diagnosis, two databases were compiled for use with both MicroLog software and Bacterial Identifier. The general database includes representative tropical bacterial plant pathogens, some species not included in the GN database, a range of saprophytes and nonpathogens, and many type specimens. There is also a database comprising all the biovars of *P. solanacearum* (Hayward et al. 1990b) together with *P. syzygii,* and the blood disease bacterium (Eden-Green 1993). As reported previously, metabolism of disaccharides used in biovar tests cannot be tested reliably with the GN plates (Black and Sweetmore 1993). However, using the current biovar database with Bacterial Identifier, biovars 1 and 2 can be distinguished from biovars 3 and 4. This partial discrimination is in accordance with the groupings based on nucleic acid characteristics (Seal 1994, these proceedings), suggesting some genetic basis for the groupings rather than the Biolog system's limitations.

However, misleading results may be obtained with weakly metabolizing isolates of biovar 3 as has been found in Malaysia. If there is any doubt, the response of the biovar to sugar alcohols (mannitol, sorbitol) in the Biolog plates should be checked. Arabitol was found to be metabolized by biovars 3 and 4 only (Black and Sweetmore 1993). Results from the analysis of metabolism of Malaysian isolates will be used to improve the biovar database.

Cluster analysis

The use of metabolic profiling in cluster analysis and other types of multivariate analysis has also been described (Black and Sweetmore 1993, Li and Hayward 1993). In Malaysia, profiles were obtained from isolates from several hosts and localities. All the isolates examined were confirmed as *P. solanacearum* by metabolic profiling, pathogenicity, or other means. The data were analyzed, using substrates that were differential (i.e. eliminating those substrates where all or nearly all the isolates are positive or negative). The results from one cluster analysis are shown in Figure 3 (using the substrates in Table 2). Biovar substrates were not included in this analysis as all isolates were found to belong to biovar 3 using standard tests (Hayward 1964), although some other isolates from highland-grown potatoes were found to be of biovar 2. As shown in Table 2, major groupings are separated at a high level of similarity according to the overall versatility of metabolism: three groups with broad versatility (including BR1, which consistently clustered on its own) and two groups with more restricted versatility. The reactions to leucine and lactic acid further differentiate these groups. The ability to metabolize some substrates requires induction by pre-incubation, but these results were obtained under standard conditions. Hence, these results represent real differences between isolates. The origin of the isolates had no apparent connection with the groups, but most of the isolates from a single host fell into

Table 2. Metabolism of Biolog substrates by isolates of Pseudomonas *solanacearum* from Malaysia grouped according to cluster analysis (see Figure 3).

		Metabolism of substrates[1] (Biolog well position indicated)								
Isolate code[2]	Origin	D-galactose (B4)	α-hydroxy butyric acid (D10)	α-keto butyric acid (E3)	D,L-lactic acid (E6)	L-leucine (G3)	L-pyro glutamic acid (G7)	D-serine (G8)	Inosine (H2)	Glycerol (H9)
BR1	Seberang Perak, Perak	2	0	0	2	2	2	2	2	2
BR2	Pulau Gading, Melaka	0	1	0	.2	0	2	0	2	1
BR3	Bachok, Kelantan	0	0	0	2	0	2	0	2	2
R1	Rhu Tapai, Terengganu	0	0	0	2	0	2	0	2	2
BR4	Bachok, Kelantan	0	2	0	2	0	2	0	2	2
P23	Kelang, Selangor	0	1	0	2	0	1	0	2	2
CW3	Serdang, Serdang	0	0	2	2	0	0	0	2	2
P28	Kelang, Selangor	0	1	1	2	0	1	0	2	2
MG4	Shah Alam, Selangor	1	0	0	2	0	0	0	2	2
MG5	Shah Alam, Selangor	1	0	0	2	0	0	1	1	2
GN4	Serdang, Serdang	0	2	2	2	0	0	0	2	2
P18	Kelang, Selangor	0	2	1	2	0	1	1	2	1
CW1	Kelang, Selangor	0	0	0	0	0	0	0	2	0
P1	Kelang, Selangor	0	0	0	0	0	0	0	0	0
P20	Kelang, Selangor	0	0	0	0	0	0	0	0	0
T10	Jelang, Selangor	0	0	0	0	0	0	0	0	0
GN5	Bukit Tangga, Kedah	0	0	0	0	0	2	0	0	0
CW2	Seberang Perak, Perak	0	0	0	2	0	0	0	0	0
P25	Kelang, Selangor	0	0	0	2	0	0	0	0	0
T2	Broga, Selangor	0	0	0	2	0	0	0	0	0
T3	Kelang, Selangor	0	0	0	2	0	0	0	0	0
T4	Kelang, Selangor	0	0	0	2	0	0	0	0	0
T5	Kelang, Selangor	0	0	0	2	0	0	0	0	0
POT3	Cameron Highlands, Pahang	0	0	2	2	0	0	0	0	0
P22	Kelang, Selangor	0	0	0	2	0	1	0	0	2
T1	Serdang, Selangor	2	0	0	2	0	0	0	0	1
MG1	Cameron Highlands, Pahang	0	2	2	2	2	2	2	1	0
P31	Kelang, Selangor	0	2	1	2	2	2	1	1	0
MG2	Shah Alam, Selangor	0	2	2	2	2	2	0	0	2
P26	Kelang, Selangor	0	2	1	2	2	1	1	1	2
P27	Kelang, Selangor	0	2	0	2	2	1	0	1	2
P29	Kelang, Selangor	0	2	1	2	2	0	0	2	2
P30	Kelang, Selangor	0	2	2	2	2	1	1	2	1
P16	Kelang, Selangor	0	0	0	2	2	0	0	1	1
POT1	Cameron Highlands, Selangor	0	1	1	2	2	1	1	0	1
POT8	Cameron Highlands, Selangor	0	0	0	2	2	1	2	0	1
T8	Kelang, Selangor	0	0	0	2	2	2	0	0	0

1. Substrate metabolism. 0: negative; 1: borderline; 2: positive.
2. Host codes. BR: eggplant, CW: chili, GN: groundnut MG: Tagetes, P: sweet pepper, POT potato, *R: Hibiscus sabdariffa,* T: tomato.

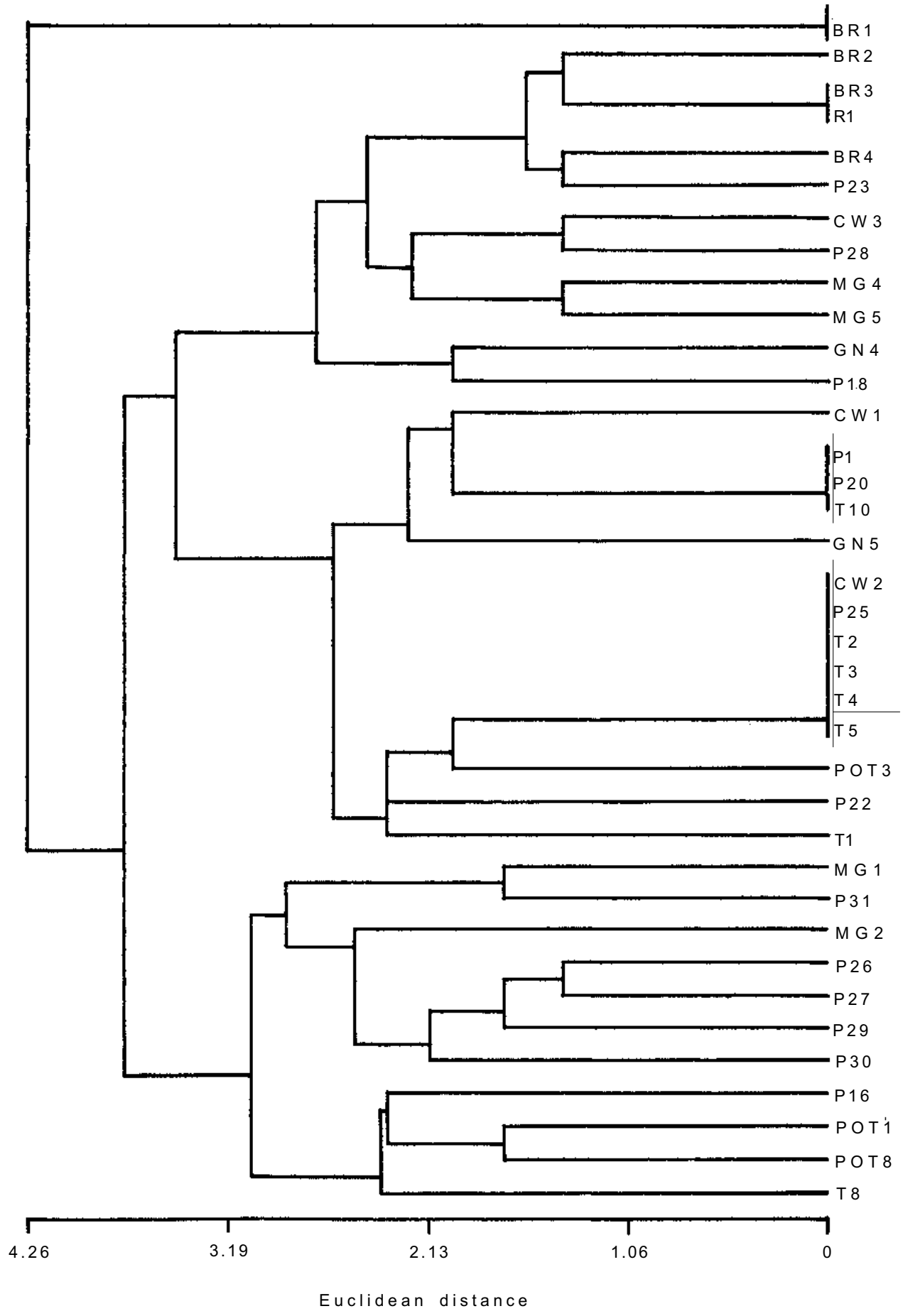


Figure 3. Cluster analysis of substrate use by isolates of *Pseudomonas solanacearum* from Malaysia. Dendrogram produced by UGPMA from Euclidean coefficients. (Details of isolates and substrates are given in Table 2.).

a particular group. This suggests some host specificity for metabolic type, which has implications for breeding for resistance, and for biological control by using avirulent isolates.

Substrate analysis

The Biolog system may be further exploited by analyzing metabolic profiles so as to select substrates for differential media in agar format, either for species or for sub-specific taxa. This is being explored at present. For a given target bacterial species, this could be a cost-effective means of differentiation, particularly if the media were incorporated into a kit. It will be interesting to compare results for *P. solanacearum* with those of Harris (1972), who found that biochemical tests could differentiate strains from different hosts within biovar 1.

Conclusions

The Biolog system is a robust and compact system, which can be used with confidence for the identification and characterization of *P. solanacearum.* Depending on the laboratory and computing facilities available, the user can choose the level of sophistication of data collection, entry, and processing (Figure 2). Metabolic profile data are amenable to analysis by multivariate statistical methods. By combining Biolog and BACTID systems for preliminary identification, the cost-effectiveness of metabolic profiling can be improved.

Acknowledgments

This work was funded by the UK Overseas Development Administration under the Adaptive Research Programme managed by the Natural Resources Institute (Project F0020). Laboratory work in UK was carried out under the Plant Health Licence 1172A/619/63 from the Ministry of Agriculture, Fisheries and Food.

References

Black, R., and Sweetmore, A. 1993. Identification and characterization of *Pseudomonas solanacearum* using metabolic profiles. Pages 32-44 *in* Bacterial wilt: proceedings of an International Conference, 28-31 Oct 1992, Kaohsiung, Taiwan (Hartman, G.L. and Hayward, A.C, eds.). ACIAR Proceedings no. 45. Canberra, Australia: Australian Centre for International Agricultural Research.

Black, R., and Sweetmore, A. 1994. Appropriate bacterial identification systems for small plant-pathology laboratories overseas incorporating the Biolog method. Plant Pathology 43:438-441.

Black, R., Sweetmore, A.,and Holt, J. 1994 (In Press). Plant Clinic: A training system for decision making and resource management in plant disease diagnosis. Chatham Maritime, Kent, UK: Natural Resources Institute.

Bradbury, J. E 1970. Isolation and preliminary study of bacteria from plants. Review of Plant Pathology 49:213-218.

Eden-Green, S. J. 1993. Diversity of *Pseudomonas solanacearum* and related bacteria in Southeast Asia. Pages 28-31 in Bacterial wilt: proceedings of an International Conference, 28-31 Oct 1992, Kaohsiung, Taiwan (Hartman, G.L. and Hayward, A.C, eds.). ACIAR Proceedings no. 45. Canberra, Australia: Australian Centre for International Agricultural Research.

Harris, D. C. 1972. Intra-specific variation in *Pseudomonas solanacearum.* Pages 289-292 *in* Proceedings of the Third International Conference on Plant Pathogenic Bacteria (Maas Geesteranus, H.P., ed.). Wageningen, Netherlands, 14-21 Apr 1971. Wageningen, Netherlands: PUDOC (Centre for Agricultural Publishing and Documentation).

Hayward, A. C. 1964. Characteristics of *Pseudomonas solanacearum.* Journal of Applied Bacteriology 27: 265-277.

Hayward, A. C., El-Nashaar, H. M, De Lindo, L., and Nydegger, U. 1990a. The use of microtiter plates in the phenotypic characterization of phytopathogenic Pseudomonads. Pages 593-598 in Plant pathogenic bacteria: proceedings of the Seventh International Conference on Plant Pathogenic Bacteria, 11-16 Jun 1989, Budapest, Hungary (Klement, Z., ed.). Budapest, Hungary: Akademiai Kiado.

Hayward, A. C., El-Nashaar, H. M., Nydegger, U., and De Lindo, L. 1990b. Variation in nitrate metabolism in biovars of *Pseudomonas solanacearum.* Journal of Applied Bacteriology 69: 269-280.

Lelliott, R. A., and Stead, D. E. 1987. Methods for the diagnosis of bacterial diseases of plants. Oxford: Blackwell Scientific. 216 pp.

Li, X., and Hayward, A. C.1993. The use of the Biolog identification system for the rapid identification of plant pathogenic Pseudomonads. Pages 45-48 in Bacterial wilt: proceedings of an International Conference, 28-31 Oct 1992, Kaohsiung, Taiwan (Hartman, G. L. and Hayward, A. C, eds.). ACIAR Proceedings no. 45. Canberra, Australia: Australian Centre for International Agricultural Research.

Seal, S. 1994. DNA-based diagnostic techniques for *Pseudomonas solanacearum* with emphasis on biovars 3 and 4. Pages 27-34 in Groundnut bacterial wilt in Asia: proceedings of the Third Working Group meeting, 4-5 Jul 1994, Oil Crops Research Institute, Wuhan, China (Mehan, V. K. and McDonald, D., eds.). Patancheru 502 324, Andhra Pradesh, India: International Crops Research Institute for the Semi-Arid Tropics.

Discussion

V K Mehan The BACTID system can eliminate saprophytes in the first step. Is it also possible to eliminate avirulent colonies of *Pseudomonas solanacearum?*

R Black Yes, if it is possible to find some substrates or tests that distinguish between virulent and avirulent isolates. This is certainly worth looking at.

S N Nigam What parameters do you use for multivariate analysis for differentiation of strains?

R Black The strains are differentiated based on substrate utilization (positive or negative).

M P Natural How much do the Biolog kit and the BACTID kit cost?

R Black Biolog plates cost US $3.5-5.0 a plate, depending on the quantity bought. The advantage of the system is that it saves time, labor, and expense of making many different media. The BACTID kit is a do-it-yourself kit. A sterile ELISA plate costs about US $3 but this will do up to 12 isolates. Breaking the kit down to individual tubes (e.g., Eppendorf) that are reusable would reduce the cost further.

DNA-based diagnostic techniques for *Pseudomonas solanacearum* with emphasis on biovars 3 and 4

S Seal[1]

Abstract

Sensitive and specific detection tests are required to identify latent bacterial wilt infections in planting material, to test soil prior to planting, and for epidemiological studies. Pseudomonas solanacearum-specific DNA sequences were identified by 16S rRNA sequencing and genomic subtraction experiments. Oligonucleotide primers were constructed for those regions that helped in detecting single cells by polymerase chain reaction (PCR) amplification. These PCR tests have been adapted for use in less sophisticated laboratories in Asia and Africa. Results from trials in Burundi, Malaysia, and Mauritius are presented.

Pseudomonas solanacearum subgroup identification tests are often needed for quarantine and epidemiological investigations. The use of a rapid identification test for biovars 3, 4, and 5 based on PCR amplification with primers to the 16S rRNA gene is described. Strains are differentiated within biovars 3 and 4, using RFLP probes, and the relevance of these subgroups discussed. The DNA-based tests and traditional diagnostic techniques are compared for their suitability for laboratories in tropical countries.

需要用灵敏的和特异性方法来检测植株中的潜伏侵染，播种前检测土壤和用于病害流行学研究。通过青枯菌 16SrRNA 序列分析和基因组减除试验获得了对青枯菌特异性的 DNA 序列片段，针对这个片断构件的寡核苷酸引物有助于通过聚合酶链式反应(PCR) 扩增检测青枯菌单个细菌。这种 PCR 检测技术已在亚洲和非洲设备不太好的实验室中应用。布隆迪、马来西亚和毛里求斯的试验结果作了阐述。

青枯菌亚组鉴定常常用于检疫和流行学研究。本文介绍了用 16SrRNA 基因引物的 PCR 扩增迅速鉴定生物型 3、4、5 的试验方法，用 RFLP 探针划分生物型 3 和 4 内的菌系及这些亚组的相互关系。比较了 DNA 检测技术和传统的诊断技术在热带国家实验室内应用的情况。

1. Natural Resources Institute, Central Avenue, Chatham Maritime, Kent ME4 4TB, United Kingdom.

Seal, S. 1994. DNA-based diagnostic techniques for *Pseudomonas solanacearum* with emphasis on biovars 3 and 4. Pages 27-34 in Groundnut bacterial wilt in Asia: proceedings of the Third Working Group meeting, 4-5 Jul 1994, Oil Crops Research Institute, Wuhan, China (Mehan, V. K. and McDonald, D., eds.). Patancheru 502 324, Andhra Pradesh, India: International Crops Research Institute for the Semi-Arid Tropics.

Introduction

In the last decade, use of nucleic-acid-based techniques for the detection of microorganisms has increased dramatically, mainly because of their ability to achieve greater specificity and sensitivity more rapidly than other diagnostic techniques. This paper describes the development and application of DNA probe and polymerase chain reaction (PCR) tests for detection and subspecies division of *Pseudomonas solanacearum* (Smith) Smith. The background to these technologies has been covered in detail by Seal and Elphinstone (1994).

DNA-based tests for detection of *Pseudomonas solanacearum:* use of methods in less developed countries

The development of a *P. solanacearum-speciiic* DNA probe and more sensitive PCR detection tests have been described by Seal et al. (1992a, 1993a, 1993b). These tests were used to screen potato tubers in Burundi for latent bacterial wilt infection (Seal et al. 1993a, Skoglund et al 1993). Following the success of these trials, funding was obtained by the Natural Resources Institute (NRI) to adapt the PCR tests for use in less sophisticated laboratories in tropical countries. Equipment has been set up in Malaysia, Mauritius, and Zimbabwe where training has been given in PCR technology and methodology. Although PCR technology is yet to become common in diagnostic laboratories of less developed countries, our experience in such laboratories has shown that it is a robust technology that could, in time, become as widely used as ELISA technology. One of the advantages of PCR technology for tropical countries is the thermostability of all the reagents involved, reducing the degradation of the components during power failures and delays in customs clearance.

The cost of installing the equipment required for PCR is approximately US $6000. This sum would cover an electrophoresis tank and power pack, micropipetters, a small freezer, a UV transilluminator, a polaroid camera, and a basic PCR machine. It is possible to carry out PCR without a PCR machine by manually transferring the PCR reaction mixtures from one water bath to another, all of which are maintained at pre-set temperatures. This would reduce the set-up cost by half and could be worthwhile for countries where labor is relatively inexpensive. The costs of the PCR, gel electrophoresis, and other accessories will generally lie between US $0.5 and 1.5 for each PCR, depending on the number of samples processed at a time. Allowing for a minimum of two replicates for each sample, this is still competitive with such techniques of comparable sensitivity as selective plating, followed by other identification methods.

Development of DNA-based tests for detection and identification of strains of biovars 3 and 4

Restriction fragment length polymorphism (RFLP) analysis is useful in distinguishing among biovars of *P. solanacearum* (Cook et al, 1991). Fingerprints

generated from restricted total genomic DNA are also useful for the identification of biovars (Gillings and Fahy 1993). These methods, although highly discriminatory, are time-consuming as genomic DNA has to be extracted and purified from a pure culture of the bacterium. Therefore, they are not suitable for rapid screening of isolates. A simpler and quicker method has been developed based on PCR amplification with tRNA consensus primers (Seal et al. 1992b, 1993a). This method groups *P. solanacearum* into three divisions, one of which represents all the strains within biovars 3,4, and 5. Using this test, it is possible to determine if a purified strain belongs to Division II (biovars 3, 4, and 5) in less than 5 h.

Recently, a PCR test has been designed at NRI that can determine whether a *P. solanacearum* isolate belongs to RFLP Division I (biovars 1 and 2) or Division II (biovars 3, 4, and 5) directly from crude extracts of infected plants. This PCR test will also determine, at the same time, if the material is infected with *P. solanacearum.* The test is based on PCR amplification using three primers to 16S rDNA sequences. Two of the three primers are 'OLI1' and 'Y2' that amplify all *P. solanacearum* strains (Seal et al. 1993a). The third primer, 'BV4', was designed to discriminate between Division I and II strains based on the base pair differences around positions 458-470 reported by Li et al. (1993). Use of all three primers in PCR reactions resulted in one band for Division J strains, and two bands for Division II strains, after optimization of the ratio of primer concentrations (Figure 1).

DNA-based tests for examining the variability among strains of biovars 3 and 4

To date, the fingerprinting techniques reported for examining the variability among strains of biovars 3 and 4 require purification and enzymic restriction of DNA. These methods have been described by Cook et al. (1991) and by Gillings and Fahy (1993).

A single probe that divides *P. solanacearum* into over 40 RFLP groups was generated by subtraction hybridization experiments. This probe, 5a67, has been found useful for differentiating strains with similar biochemical properties (Figure 2). Strains isolated from a range of hosts in Mauritius were all found to belong to biovar 3 and had similar phenotypic properties. However, RFLP analysis with probe 5a67 revealed four fingerprint types. Biovar 3 and 4 strains isolated from Malaysia could also be divided into four fingerprint groups. The significance of these groups is not known, but it is clear that the patterns are not related to the host from which the strains were isolated.

Infected pepper plants from one field in Malaysia have been shown by researchers in Dr K Y Lum's laboratory at the Malaysian Agricultural Research and Development Institute (MARDI) to contain *P. solanacearum* isolates belonging to at least two 5a67 RFLP groups. Experiments are in progress to determine how many 5a67 RFLP groups are present in the soil from that field, and whether all the types are equally aggressive in attacking pepper. Although the nature of the multicopy sequence targeted by probe 5a67 remains

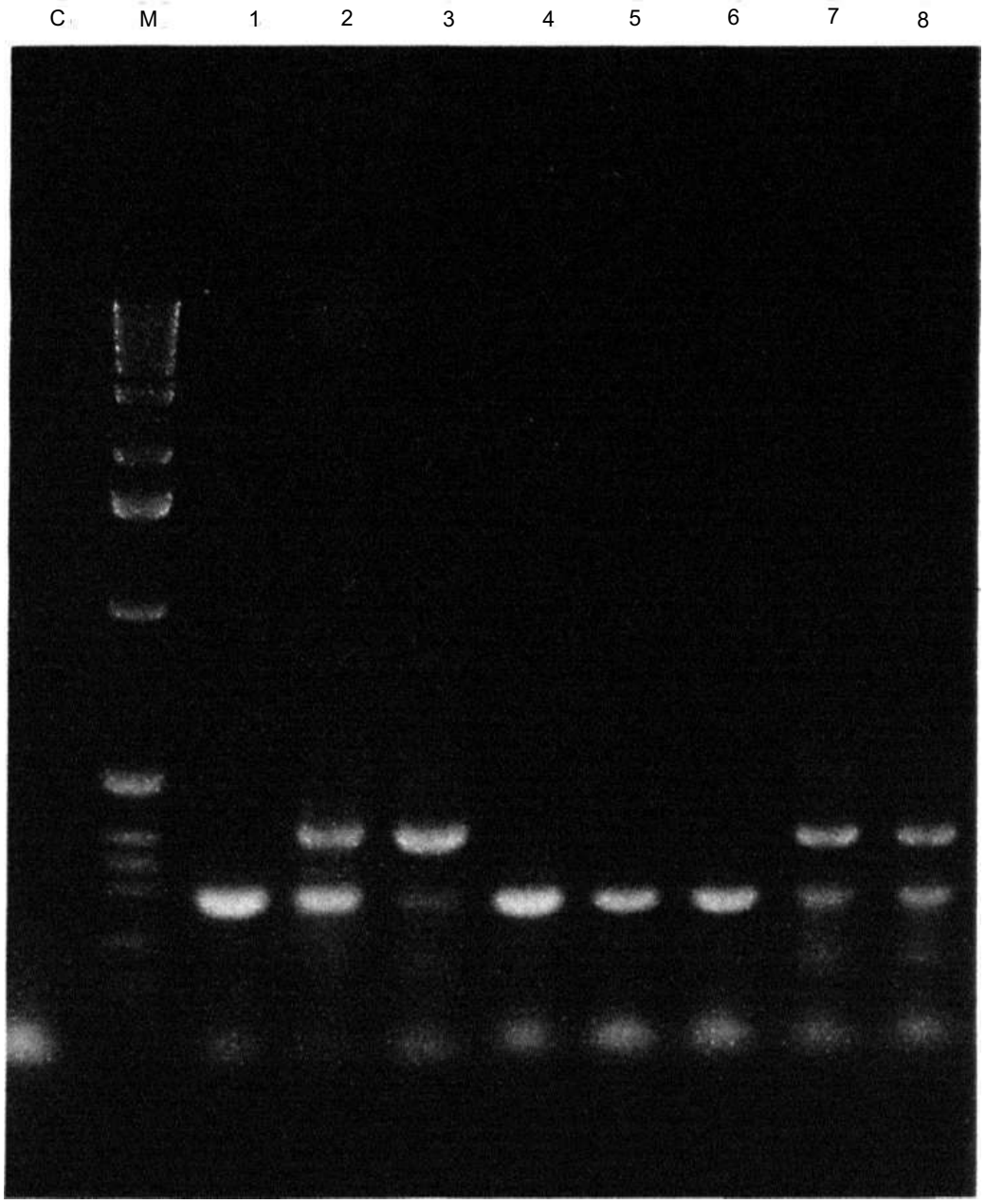


Figure 1. Fingerprints of *Pseudomonas solanacearum* strains produced by PCR amplification with primers OLI1, Y2, and BV4. Biovar 1 and 2 strains produce two main bands whereas biovar 3,4, and 5 strains produce three main bands. Lanes C, M, and 1 to 8 (race/biovar of strain is indicated in parentheses): C, no DNA (control); M, 1 kb molecular weight marker (Gibco BRL); 1, UW26 (1/1); 2, UW369 (1/4); 3, UW141 (1/4); 4, UW19 (3/2); 5, UW160 (2/1); 6, UW30 (1/1); 7, UW360 (1/4); and 8, UW378 (1/4).

unknown, the probe provides a method for differentiating strains that appear biochemically similar. This should be useful for epidemiological studies, and to ensure that breeding programs test host lines against strains that are genetically different and representative of those present in the geographical region of interest.

Comparison of DNA-based techniques with serological tests

Whilst DNA-based methods offer highly specific and sensitive means to detect *P. solanacearum,* they are, at present, more expensive than serological

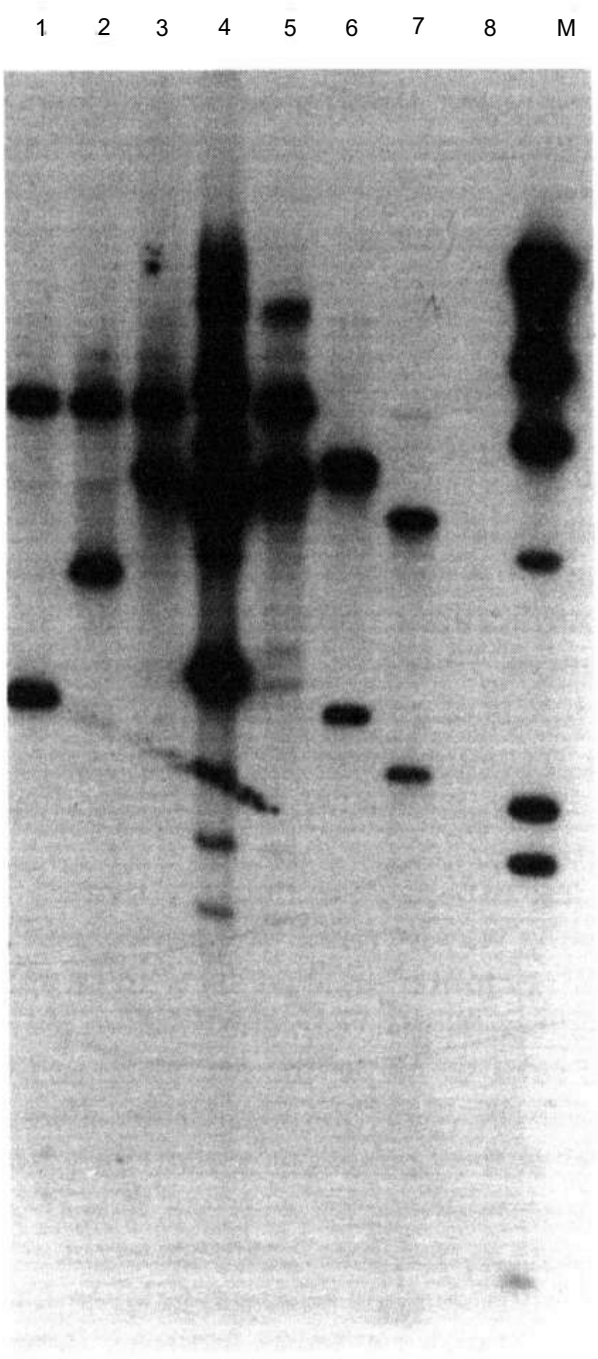


Figure 2. Southern transfer of 5 µg of EcoRI-restricted DNA of *Pseudomonas solanacearum* strains probed with '5a67'. Probe 5a67 was labeled with digoxigenin-11-UTP and detected by chemiluminescence according to the manufacturer's (Boehringer Mannheim) instructions. Lanes 1 to 8 (race/biovar of strain is indicated in parentheses): 1, S9 (1/3); 2, T456 (1/3); 3, T494 (1/3); 4, R39 (3/2); 5, R132 (1/3); 6, R161 (2/1); 7, R589 (2/1); and 8, R604 (blood disease bacterium). Digoxigenin-11-dUTP labeled lambda HindIII (M track) was run as a molecular weight size marker.

techniques. For a rapid and reasonably sensitive assay of total bacterial numbers, immunoassays would therefore generally remain the method of choice. The increased cost of DNA tests is justified only when the required sensitivity must be higher than that possible with ELISA techniques (10^4 cfu mL^{-1}) or if closely related bacteria (e.g., *P. syzygii, P. pickettii,* and *P. cepacia*) that cross-react with the antisera are likely to be present in the sample of interest.

For detection of small genetic differences between closely related strains or subdivisions of *P. solanacearum,* DNA probes and fingerprinting methods are at present the most reliable discriminatory methods. Small differences between isolates of the same species are generally not reflected in surface properties, and hence immunoassays are not suitable.

Future prospects

A limitation of the PGR technology is the inhibition of thermostable DNA polymerase enzymes by compounds present in many plant extracts, leading to false negatives or low detection sensitivities. Although the identities of some of the inhibitory compounds are known (e.g., phenolics and oxidases), many remain uncharacterized. A series of resins and columns is available commercially for purification of nucleic acids from many of these contaminating substances. However, such samples with high PCR-inhibitory activity as soil and extracts of ginger or banana have to be treated with these resins more than once to ensure adequate removal of the inhibitors. This makes the methods prohibitively expensive for routine diagnostic use in many laboratories.

An alternative and less expensive technology, termed 'immunocapture PCR', appears to be a promising way forward. The principle of Immunocapture PCR' is to coat PCR tubes (or microtiter plate wells) with an antibody that is specific, or selective, for the plant pathogen of interest. Soil or plant extracts can then be incubated within the tube to allow the antibody to bind to the pathogen. Compounds that inhibit DNA-polymerase activity are subsequently removed or diluted by washing; PCR reagents are added; and nucleic acid released by such suitable methods as boiling.

An immunocapture PCR test for *P. solanacearum* is being developed in collaboration with Dr J Elphinstone (Central Science Laboratory, Harpenden, UK) and Dr A Dookun (Mauritius Sugar Industry Research Institute). Initial results have shown that PCR-inhibitory compounds found in soil are removed by a few phosphate buffer washes but detection sensitivities in soil are low. The technique has been adapted recently to a magnetic bead format to improve access of the antibody to the bacteria. Magnetic force is also thought to provide a gentler method for removing the bacteria-antibody complexes from the sample, which consequently is less disruptive to the weak bond between antibody and bacterium. Preliminary results with the magnetic bead format have given high detection sensitivities for bacterial suspensions in water (100 cfu mL^{-1}) and encouraging results with soil suspensions GO^{4} cfu g^{-1} soil).

Conclusions

Sensitive PCR and DNA-probe diagnostic tests for detection and differentiation of the strains of biovars 3 and 4 of *P. solanacearum* have been developed. To ensure the widespread use of the PCR tests, simple and inexpensive methods of preparing samples free of PCR-inhibitory substances are being devised.

Immunomagnetic separation of the bacterium from the sample appears the most promising way to meet these criteria- Moreover, such an extraction procedure would benefit from allowing greater sample volumes to be tested, and should overcome false positive results arising from the free DNA released from dead bacteria into the sample. It is hoped that a better understanding of the epidemiology of bacterial wilt disease will result from use of these detection and typing techniques for research, which in the past has been restricted by the lack of suitable methods.

Acknowledgments

This work was commissioned by the Overseas Development Administration and the Natural Resources Institute, Chatham Maritime, UK.

References

Cook, D., Barlow, E., and Sequeira, L. 1991. DNA probes as tools for the study of host-pathogen evolution: the example of *Pseudomonas solanacearum.* Pages 103-108 in Advances in molecular genetics of plant-microbe interactions, vol. 1 (Hennecke, H. and Verma, D. P., eds.). Dordrecht, The Netherlands: Kluwer.

Gillings, M., and Fahy, P. 1993. Genomic fingerprinting and PCR analysis: rapid, sensitive and inexpensive means of differentiating strains of *Pseudomonas solanacearum.* Pages 85-93 in Bacterial wilt: proceedings of an International Conference, 28-31 Oct 1992, Kaohsiung, Taiwan (Hartman, G. L. and Hayward, A. C, eds.). ACIAR Proceedings no. 45. Canberra, Australia: Australian Centre for International Agricultural Research,

Li, X., Dorsch, M., Del Dot, X, Sly, L. I., Stackebrandt, E., and Hayward, A. C. 1993. Phylogenetic studies of the rRNA group II pseudomonads based on 16S rRNA gene sequences. Journal of Applied Bacteriology 74: 324-329.

Seal, S. E., and Elphinstone, J. G. 1994. Advances in identification and detection of *Pseudomonas solanacearum.* Pages 35-57 in Bacterial wilt: the disease and its causative agent, *Pseudomonas solanacearum* (Hayward, A.C and Hartman, G.L., eds.). Wallingford, UK: CAB International. 259 pp.

Seal, S. E., Jackson, L. A., and Daniels, M. J. 1992a. Isolation of a *Pseudomonas solanacearum*-specific DNA probe by subtraction hybridization and construction of species-specific oligonucleotide primers for sensitive detection by the polymerase chain reaction. Applied and Environmental Microbiology 58: 3751-3758.

Seal, S. E., Jackson, L. A., and Daniels, M. J. 1992b. Use of tRNA consensus primers to indicate subgroups of *Pseudomonas solanacearum* by polymerase chain reaction amplification. Applied and Environmental Microbiology 58: 3759-3761.

Seal, S. E., Jackson, L. A., and Daniels, M. J. 1993a. Development of molecular diagnostic techniques for detection of *Pseudomonas solanacearum* and

identification of subgroups within this species. Pages 97-105 in Bacterial wilt: proceedings of an International Conference, 28-31 Oct 1992, Kaohsiung, Taiwan (Hartman, G.L. and Hayward, A.C., eds.). ACIAR Proceedings no. 45. Canberra, Australia: Australian Centre for International Agricultural Research.

Seal, S. E., Jackson, L. A., Young, J. P. W., and Daniels, M. J. 1993b. Differentiation of *Pseudomonas solanacearum, Pseudomonas syzygii, Pseudomonas pickettii* and the blood disease bacterium by partial 16S rRNA sequencing: construction of oligonucleotide primers for sensitive detection by polymerase chain reaction. Journal of General Microbiology 139:1587-1594.

Skoglund, L. G., Seal, S., Elphinstone, J. G., and Berrios, D. E. 1993. Study of latent infection of potato tubers by *Pseudomonas solanacearum* in Burundi. Pages 106-110 in Bacterial wilt: proceedings of an International Conference, 28-31 Oct 1992, Kaohsiung, Taiwan (Hartman, G.L. and Hayward, A.C., eds.). ACIAR Proceedings no. 45. Canberra, Australia: Australian Centre for International Agricultural Research.

Discussion

Y W Kang How large was the primer that you used in the PCR to detect *Pseudomonas solanacearum?*

S Seal To detect 16S rDNA gene, the primers are 21-mer and 24-mer. Other set is of 26-mer and 28-mer primers. The primers are described in two papers: Journal of General Microbiology (1993), 139: 1587-1594 and Applied and Environmental Microbiology (1992), 58:3751-3758.

Z Y Xu Have you used the PCR technique to distinguish between pathotypes or strains of *Pseudomonas solanacearum?*

S Seal The tRNA consensus primer PCR technique can distinguish three broad fingerprint types (with many faint bands giving rise to many more fingerprint types). An RFLP probe can differentiate over 40 groups within *Pseudomonas solanacearum*. To date, I have not designed a finer PCR fingerprinting method for differentiating *P. solanacearum* strains.

Serological and molecular diagnostic techniques

Polyclonal and monoclonal antibody-based enzyme-linked immunosorbent assays for *Pseudomonas solanacearum*

A Robinson-Smith[1]

Abstract

Both monoclonal and polyclonal antibodies have been produced against the glutaraldehyde-fixed whole cells of the bacterial wilt pathogen, Pseudomonas solanacearum. *When used in an indirect enzyme-linked immunosorbent assay (ELISA), the polyclonals detected as few as 1 x 10^2 bacterial cells mL^{-1} in pure culture, and 1 x 10^4 cells mL^{-1} in infected tissues. They could not, however, distinguish between* P. solanacearum, P. celebensis, P. syzygii, *and* P. pickettii. *These antibodies will be useful for the rapid detection of* P. solanacearum *directly from infected tissue, provided the cross-reacting bacteria are not present. The monoclonals developed were used in an identical assay, but they too cross-reacted with* P. celebensis *and* P. syzygii, *and could detect only 1* x 10^6 *cells* mL^{-1}. *However, monoclonals that were produced using more selective immunization schedules did not show any cross-reaction. Although they were less sensitive (1 x 10^6 cells mL^{-1}) than the polyclonals (1* x 10^4 *cells* mL^{-1}), *they should be useful in assays that require differentiation of* R solanacearum *from the cross-reacting bacteria.*

通过对青枯菌细胞戊二醛固定制备了青枯菌的多克隆和单克隆抗体。用间接 ELISA 方法检测，多克隆抗体可检测培养菌液的 100 个细菌/毫升和受感染组织中的 10000 个细菌/毫升，但不能区分 *P. solanacearum*，*P. celebensis*，*P. syzygii* 和 *P. picketii*。如果不存在其它交叉反应的细菌，多克隆抗体可用于迅速检测感病组织中的青枯菌。制备的单克隆抗体运用于相同的检测方法，但非常容易同 *P. celebensis* 和 *P. syzygii* 产生交叉反应，而且只能检测每毫升 1 百万个细菌。但是通过更严格选择性的免疫程序制备的单克隆抗体，未产生任何交叉反应，尽管它的灵敏度比多克隆抗体低（只能检测到 1,000,000 个细菌/毫升），但能用于区分青枯菌和其它可产生交叉反应的细菌。

1. Institute of Arable Crops Research, Rothamsted Experimental Station, Harpenden, Herts, AL5 2JQ, United Kingdom.

Robinson-Smith, A. 1994. Polyclonal and monoclonal antibody-based enzyme-linked immunosorbent assays for *Pseudomonas solanacearum.* Pages 37-47 *in* Groundnut bacterial wilt in Asia: proceedings of the Third Working Group meeting, 4-5 Jul 1994, Oil Crops Research Institute, Wuhan, China (Mehan, V. K. and McDonald, D., eds.). Patancheru 502 324, Andhra Pradesh, India: International Crops Research Institute for the Semi-Arid Tropics.

Introduction

Bacterial diseases of plants are generally diagnosed on the basis of symptoms and subsequent isolation and characterization of the pathogen by the classical morphological and nutritional tests. These tests are tedious and time-consuming. In recent years, there has been much interest in finding quicker methods of diagnosis.

Several new tests for detecting the bacterium *Pseudomonas solanacearum* are currently being developed, and have been reviewed by Seal and Elphinstone (1994). Of these, serological techniques are a good compromise between the sensitivity and specificity of detection and the ease and expense of application.

Serological techniques have been used for the detection of plant viruses for many years, but it was only in 1978 that the first serological assays were used for plant pathogenic bacteria (Cambra and L6pez 1978, Vruggink 1978). antibodies (PAbs), for detecting *P. solanacearum,* including latex agglutination (Nakashima and Nydegger 1986), enzyme-linked immunosorbent assays (ELISAs) (Bellstedt and van der Merwe 1989), and immunofluorescence antibody staining (Janse 1988). Because of non-specific reactions of PAbs, the immunofluorescence technique has been the most widely used though it is laborious and relatively costly.

Very few methods have been described so far that use monoclonal antibodies (MAbs) to detect P. solanacearum, but their use should eliminate many of the problems associated with PAbs.

This paper describes the production of MAbs and PAbs against *P. solanacearum*, compares their sensitivity and specificity, and discusses their usefulness for the rapid detection of *P. solanacearum.*

Materials and methods

Bacterial isolates and antigen preparation

All bacterial isolates were cultured in 9-cm plastic petri dishes on tetrazolium chloride (TZC) medium (Kelman 1954), but without tetrazolium, at 28 °C for 24-48 h, with the exception of *P. syzygii* and *P. celebensis,* which were cultured on PW basal medium (Davis et al. 1981). The cells were harvested in sterile distilled water (SDW), centrifuged for 10 min at 10 000 g and washed three times in SDW. Finally, they were re-suspended in 5 mL SDW, and their number estimated by measuring the OD_{650} (an OD_{650} of 0.1 is equivalent to 1 x 10^8 cells mL^{-1}).

For ELISA, the cells were adjusted to 2 x 10^9 cells mL^{-1} in SDW, fixed with 0.1% formaldehyde (final concentration), and stored at 4 °C. For immunizations (with the exception of schedule C in MAbs production), the cells were adjusted to 1 x 10^9 cells mL^{-1} in 0.85% sterile saline, and fixed with glutaraldehyde, following the method of Allan and Kelman (1977); 0.5 mL aliquots were then prepared and stored at -20 °C until required.

For MAbs production by schedule C, surface washings instead of whole bacterial cells were used as an immunogen. Cell-free washings from a known number of cells were obtained as described earlier, and passed through an Amicon® ultrafiltration cell containing a Diaflo membrane, YM30. The low molecular weight fraction was collected and freeze-dried in aliquots (each containing washings from approximately 2 x 10^9 cells), which were re-dissolved in 1 mL sterile saline as required.

Polyclonal antibody production

Female Dutch x Lop rabbits were injected intramuscularly at two sites, with a total of 5 x 10^8 glutaraldehyde-fixed whole bacterial cells in 0.5 mL sterile saline, emulsified in an equal volume of Freund's complete adjuvant (Difco). Four weeks later, the rabbits were injected with the immunogen, but in Freund's incomplete adjuvant. Blood was collected from the lateral ear vein at 2- to 3-week intervals up to a maximum of ten times. The blood was allowed to clot at room temperature, separated by centrifuging (1500 *g* for 15 min), and the serum fraction collected. Antibody levels in the serum were determined by ELISA.

Monoclonal antibody production

Immunization schedule A

Female Balb/c mice were injected intraperitoneally with 1 x 10^8 glutaraldehyde-fixed whole bacterial cells in 0.4 mL sterile saline, twice, at 4-week intervals. Two to four weeks later, the mice were bled from the tail vein, and antibody titers measured by ELISA. If titers were greater than 1:2000, a final boost was given, as described earlier. Otherwise, immunizations were continued at 4-week intervals until this titer was achieved.

Immunization schedule B

Two female Balb/c mice were injected intraperitoneally with a mixture of 1 x 10^8 glutaraldehyde-fixed *P. syzygii* and *P. celebensis* cells (the cross-reacting bacteria) in 0.4 mL sterile saline. Two days later, one of the two mice was injected with 20 mg kg^{-1} cyclophosphamide (Sigma Chemical Co.) in sterile saline (0.4 mL). The second mouse served as a control. This entire procedure was repeated three times, at weekly intervals. Test bleeds were taken during the intervening weeks and antibody titers compared. After a 3-week rest, the cyclophosphamide-treated mouse was injected with a mixture of 5 x 10^8 *P. solanacearum* strains (glutaraldehyde-fixed whole cells) in 0.4 mL sterile saline, followed by a final identical boost a week later.

Immunization schedule C

Female Balb/c mice were immunized intraperitoneally with low-molecular-weight washings from 5 x 10^8 to 1 x 10^9 cells in 0.5 mL sterile saline, twice, at

4-week intervals. Two to four weeks later, the mice were bled from the tail vein, and antibody titers measured by ELBA. If titers were greater than 1:2000, a final boost was given. Otherwise, immunizations were continued at 4-week intervals until this titer was achieved.

Cell fusion

Three days after the final boosts, the mice were killed and their spleens removed. The spleen cells were fused with the myeloma cell line NSO (European Collection of Animal Cell cultures, no. 85110503), by spinning both of them together in the presence of 50% polyethylene glycol (mol. wt 1500, Boehringer Mannheim) and 10% dimethyl sulfoxide (filter-sterilized, 99.5% pure, Sigma), using the method of Kennet et al. (1978). Growth of the fused cells (hybridomas) was aided by supplementing the routine cell culture media [Dulbecco's Modification of Eagle's Medium (Flow Labs) containing 20% fetal calf serum (Imperial Labs)] with peritoneal macrophages from young mice (Campbell 1986).

Hybridoma production

Fused cells were placed in 96-well plates at 37 °C with 8% CO_2. After 7 to 10 days, when growth of the hybridomas was almost confluent, culture supernatants were screened by ELISA. Clones that produced antibodies reacting with *P. solanacearum* were bulked to 24-well plates and subsequently cloned, twice, by limiting dilution (Harlow and Lane 1988). Subclones were then bulked to 25-cm^3 tissue culture flasks and supernatants drawn for screening when growth was confluent and the media were beginning to show signs of acid production (i.e. turning yellow). All subclones of interest were frozen in a mixture of 90% fetal calf serum and 10% DMSO at -70 °C in a cryo freezing box (Nalge) for 24-48 h, and then transferred to liquid nitrogen.

Antibody screening by ELISA

Microliter plates (Nunc, polysorp) were coated with a suspension of whole bacterial cells (100 uL per well), diluted from 1 x 10^2 to 1 x 10^8 cells mL^{-1} in 0.05 M sodium carbonate coating buffer, pH 9.6. After coating for 1 h at 37°C, the bacterial suspensions were decanted from the wells, and the plates washed three times in phosphate buffered saline plus 0.05% v/v Tween 20 (PBS-T). This washing was repeated after each of the subsequent steps. Culture supematants diluted 1:1, or polyclonal antisera diluted 1:1000 to 1:100 000 in blocking buffer [0.05% v/v Tween 20, 2% w/v polyvinyl pyrrolidine (PVP, mol. wt 44 000), and 0.5% w/v NIDO full-cream milk powder (Nestle) in PBS], were then added to the wells (100 uL), and incubated again for 1 h at 37°C. After washing, a horseradish peroxidase conjugated rabbit anti-mouse antibody diluted 1:2000 (for culture supernatants) or goat anti-rabbit antibody diluted 1:5000 (for polyclonal antisera) (Sigma), in blocking buffer, was added and incubated at 37°C for 1 h. Finally, 100 uL of 3,3', 5,5' tetramethylbenzidine (TMB) substrate

[1 mg mL^{-1} TMB (Sigma), 0.1% v/v hydrogen peroxide, and 10% v/v sodium acetate, pH 5.8, in distilled water] was added to each well, and incubated at room temperature until sufficient color developed. The reaction was stopped by adding 3 M sulfuric acid (25 uL per well). The absorbance at 450 nm was measured on a *Titertek* Multiscan microtiter plate reader (ICN Flow). A positive reading was taken as being three times the mean of the negative controls.

Results

Polyclonal antibodies

Four PAbs against *P. solanacearum* (Rothamsted Culture Collection accessions R 283, R 303, R 608, and R 710), and one against P. celebensis [blood disease bacterium (BDB), R 230] were produced (Eden-Green and Robinson-Smith, unpublished). All *P. solanacearum* PAbs had high titers, and reacted with all the isolates of *P. solanacearum* at 1 x 10^8 cells mL^{-1}, but they also cross-reacted with *P. syzygii, P. celebensis, P. pickettii,* and *P. cepacia.* There was no detectable difference between the PAbs against *P. celebensis* and those produced against *P.*

Table 1. Detection of *Pseudomonas solanacearum* and related bacteria by polyclonal antibodies.

Bacterial isolate	Biovar	Race	Polyclonal[1] (IACR-PS-) 277	278	291	290	322
R232	1	1	+	+	+	+	+
R638	1	2, moko	+	+	+	+	+
R651	1	2, bugtok	+	+	+	+	+
R710	2	3	+	+	+	+	+
R361	N2	?	+	+	+	+	+
R799	3	1	+	+	+	+	+
R812	3	4?	+	+	+	+	+
R277	4	4?	+	+	+	+	+
R471	4	1	+	+	+	+	+
R292	5	5	+	+	+	+	+
R044	*P. cepacia*		+/-	+/-	+/-	+	+/-
R001	*P. syzygii*		+	+	+	+	+
R002	*P. syzygii*		+	+	+	+	+
R011	*B. subtilis*		-	•	-	-	-
R036	*X. campestris*		-	-	-	*	-
R111	*E. coli*		-	-	-	-	-
R137	*A. tumefaciens*		-	-	-	•	-
R226	*P. celebensis*		+	+	+	+	+
R228	*P. celebensis*		+	+	+	+	+
R707	*P. pickettii*		+	+	+/-	+/-	+/-
Homologous titer as found by ELISA with 1 x 10^8 cells			1:25 600	1:300 000	1:12 800	1:25 600	1:409 600

1. IACR-PS-277 was produced against the isolate R283; 278, against R303; 291, against R608; 290, against R230; and 322, against R710.

solanacearum (Table 1). the lowest concentration of bacteria routinely detectable by the PAbs was 1 x 10^4 cells mL^{-1}, although several isolates were detectable at 1 x 10^2 cells mL^{-1} in pure culture.

Monoclonal antibodies

Immunization schedule A

In general, serum with a titer in excess of 1:2000 was obtained after two immunizations, so fusions were done after the third boost. A wide range of MAbs were obtained from the fusion, but none was able to detect all isolates of *P. solanacearum.* They all cross-reacted with isolates of *P. syzygii, P. celebensis,* and *P. pickettii* There was no correlation between the reactions of these MAbs and the grouping systems used to classify *P. solanacearum,* i.e. biovars or races (Table 2). The lowest concentration of bacteria detectable by the MAbs was 1 x 10^6 cells mL^{-1} in pure culture.

Immunization schedule B

When the serum titers of the cyclophosphamide-treated mouse and of the control mouse were compared, it was found that the cyclophosphamide had removed all homologous and heterologous reactions from the treated mouse (Table 3). All MAbs produced after cell fusion were almost identical, showing no cross-reactions to related bacteria (*P. syzygii, P. celebensis,* and *P pickettii*), but giving a similar spectrum of reactions to *P. solanacearum* isolates as those obtained by the immunization schedule A (Table 2). The lowest concentration of bacteria detectable by the MAbs was 1 x 10^6 cells mL^{-1} in pure culture.

Immunization schedule C

Initially, the mice immunized with cell surface washings died. It was found that the washings obtained from bacteria that had been grown for longer than 48 h were toxic. Preparations from 48-h-old bacteria were, however, tolerated by the mice. As with the immunization schedule A, a wide range of MAbs were obtained from the fusion, but instead of showing greater specificity, as expected, most of the MAbs showed a greater range of activity than any of the MAbs produced by the other schedule. In fact, several reacted with almost all isolates of *P. solanacearum* (>260). There were, however, some cross-reactions with related bacteria, although one MAb (IACR-PS-144) only cross-reacted with a single isolate of P syzygii (Table 2). These MAbs could also detect only 1 x 10^6 cells mL^{-1} in pure culture.

Detection of *Pseudomonas solanacearum* in plant samples

Using the ELISA, *P. solanacearum* was detectable in both artificially inoculated and naturally infected tomato and potato plants. With the artificially inoculated plants, *P. solanacearum* was detectable even before wilting was apparent.

Table 2. Detection of *Pseudomonas solanacearum* and related bacteria by monoclonal antibodies.

Bacterial isolate	Biovar	Race	Monoclonal[1] (IACR-PS-) 054	089	117	124	144
R232			-	-	-	.	+
R296		1	-	+/-	-	-	+
R702		1	+	+	+	+	+
R281		1	+	+	+	+	+
R638		2, moko	-	-	-	-	+
R651		2,bugtok	+/-	+/-	-	-	+
R128	2	3	-	-	-	-	+
R710	2	3	-	-	-	-	+
R568	N2	?	-	+	+	+	+
R573	N2	?	+	+	+	+	+
R361	N2	?	-	+	+	+	+
R143	3	1	-	-	-	-	+/-
R304	3	1	-	-	-	-	+
R799	3	1	-	+/-	+	+	+
R811	3	4?	+	+	+	+	+
R812	3	4?	+/-	+	+	+	+
R277	4	4?	-	+/-	+/-	-	+
R289	4	1	-	-	-	-	+
R300	4	1	-	-	+	+	+
R471	4	1	+	+	+	+	+
R293	4	1	-	+/-	+	+	+
R288	5	5	-	+	-	-	+
R292	5	5	+	+	-	-	+
R001	*P. syzygii*		+	+	-	-	-
R002	*P. syzygii*		+	+	-	-	+
R036	*X. campestris*		-	-	-	-	-
ROM	*P. cepacia*		-	-	-	-	-
R227	*P. celebensis*		+	+	-	-	-
R229	*P. celebensis*		+	+	-	-	-
R707	*P. pickettii*		-	+	-	-	-

1. IACR-PS-054 and 089 were produced by schedule A, 117 and 124 by schedule B, and 144 by schedule C.

Discussion

The PAbs, when used in the detailed EUSA, detected all isolates of *P. solanacearum* routinely at concentrations as low as 1 x 10^4 cells mL^{-1}, but were unable to discriminate between *P. solanacearum* and *P. syzygii, P. celebensis, P. pickettii,* or *P. cepacia.* While these cross-reactions are probably of little practical significance when testing infected plant tissues (as the cross-reacting bacteria are unlikely to be present), they may cause problems when testing soil samples where *P. pickettii* and *P. cepacia* can be present in large numbers. Also, in such countries as Indonesia, both *P. syzygii* and *P. solanacearum* are found together in clove trees (*Syzygium aromaticum*), and both *P. celebensis* and *P. solanacearum* in bananas.

Table 3. ELISA readings showing the immune responses of a cyclophosphamide-treated mouse and a control mouse.

Bacterium	OD_{450} Negative control[2]	Mouse A[1] sera at 1:300	Mouse B[1] sera at 1:300
P. soknacearum biovar 1	0.018	0.034	0.254
P. soknacearum biovar 2	0.015	0.027	0.595
P. soknacearum biovar 3	0.017	0.018	0.306
P. soknacearum biovar 4	0.034	0.025	0.491
P. soknacearum biovar 5	0.043	0.025	0.451
P. syzygii	0.017	0.025	0.525
P. syzygii	0.022	0.022	0.687
P. celebensis	0.022	0.030	0.568
P. celebensis	0.020	0.028	0.511
P. pickettii	0.016	0.027	0.714
P. cepacia	0.011	0.026	0.257

1. Cyclophosphamide-treated
2. Control

Therefore, MAbs were produced to improve specificity, initially by injecting the mice with glutaraldehyde-fixed whole cells (immunization schedule A). The use of these MAbs not only removed the cross-reactions with P. cepacia but also the reactions to several isolates of *P. soknacearum* (Table 2). Hence, replacement of PAbs with these MAbs in ELISA was not useful. There was no correlation between the spectrum of reaction of these MAbs and biovars or races of the wilt pathogen. Similar results have been obtained in other laboratories (A Alvarez, personal communication).

To improve the specificity of MAbs, an attempt was made to manipulate the response of the immune system of the mice, so that the production of antibodies to the shared epitopes of *P. solanacearum* and the cross-reacting bacteria was suppressed (immunization schedule B). When an immune response is elicited, B and T lymphocytes proliferate. These dividing cells can be killed by the cytotoxic drug, cyclophosphamide, giving rise to 'temporary tolerance', and permitting manipulation of the immune response such that antibodies to a different set of epitopes can be generated. Cyclophosphamide was, therefore, administered two days after immunizing the mouse with the cross-reacting bacteria. The antibody titers of this mouse, and of the mouse that had received only the cross-reacting bacteria (control mouse), were compared. Titers of the mouse that had been given cyclophosphamide were far lower than those of the control mouse, indicating that cyclophosphamide was destroying the proliferating B and T cells. The injections were continued until no antibody production was detected, with a total of four being required. Table 3 shows that cyclophosphamide knocked out the antibodies to not only the cross-reacting bacteria but also to *P. solanacearum.* Once the mouse had been re-immunized with a mixture of strains of *P. solanacearum,* antibodies that reacted

with *P. solanacearum* were produced. MAbs resulting from this immunization schedule did not cross-react, but could not detect all isolates of *P. solanacearum.* Once again, the spectrum of reactions of the MAbs bore no correlation to the biovars or races of *P. solanacearum,* although they did differentiate between biovars 2 and N2, by recognizing only the N2 isolates. These MAbs were too specific (reacting with only selected isolates of *P. solanacearum*), and were of little use as replacements of the PAbs in ELISA.

It was interesting to note that these MAbs did not cross-react with any related bacterial species. It showed that cyclophosphamide could be used, to some extent, to manipulate the immune response and that, by using different immunization schedules, MAbs of desired specificity could be obtained.

A final attempt at producing MAbs that could be used in ELISA was made by immunizing mice with cell-surface washings instead of whole cells (immunization schedule C). The resulting MAbs differed markedly: some reacted with only a few isolates of *P. solanacearum,* whereas others reacted with every isolate against which they were screened (>260). Cross-reactions with related species also differed but, in general, they were found to be less cross-reactive, with one of the MAbs, IACR-PS-144, cross-reacting only with a single isolate of *P. syzygii.* This MAb belonged to the group of MAbs that detected all isolates of *P. solanacearum.* It was found ideal for use in ELISA. Unfortunately, its limit of detection, like all of the MAbs, was not as great as that of the PAb (i.e. only 1 x 10^6 cells $ml/^1$). Despite this, for soil assays, and for use in Indonesia, lack of cross-reactions will be advantageous, enabling *P. solanacearum* to be distinguished from the related species, namely *P syzygii, P celebensis, P. pickettii*, and *P. cepacia.* For routine use in all other situations, however, the increased sensitivity of the PAbs would probably mean that it would be best to use them in ELISA until further improvements on the sensitivity of the MAbs can be made.

Acknowledgments

The author gratefully acknowledges the work of Mr Stephen Forde and Dr John Elphinstone in preparing some of the bacterial stocks used in this work, and also Dr Simon Eden-Green for his help in producing some of the polyclonal antibodies used. This work was commissioned and funded by the Natural Resources Institute, Chatham Maritime, UK (project X0071), and was carried out under the MAFF licenses PHF1307A/74/22 and PHF1307A/02/51.

References

Allan, E., and Kelman, A. 1977. Immunofluorescent stain procedure for detection and identification of *Erwinia carotovora* var atroseptica. Phytopathology 67:1305-1316.

Bellstedt, D. U., and van der Merwe, K. J. 1989. The development of EUSA kits for the detection of *Pseudomonas solanacearum* bacterial wilt in potatoes. Pages

64-69 in Proceedings of the potato research symposium (Aartapplenavorsingsimposium), 1-2 Aug 1989, South Africa.

Cambra, M., and Lopez, M. M. 1978. Titration of *Agrobacterium radiobacter* var *tumefaciens* antibodies by using enzyme labelled anti-rabbit r-globulins (ELISA indirect method). Pages 327-331 *in* Proceedings of the Fourth International Conference on Plant Pathogenic Bacteria. Angers, France: Institut National de la Recherche Agronomique.

Campbell A. M. 1986. Monoclonal antibody technology: laboratory techniques in biochemistry and molecular biology, 13. Amsterdam: Elsevier. 265 pp.

Davis, M.J., French, W. J., and Schaad, N. W. 1981. Axenic culture of the bacteria associated with phony disease of peach and plum leaf scald. Current Microbiology 6: 309-314.

Harlow, E., and Lane, D. 1988. Antibodies: A laboratory manual. New York, USA: Cold Spring Harbor Laboratory. 726 pp.

Janse, J. D. 1988. A detection method for *Pseudomonas solanacearum* in symptomless potato tubers and some data on its sensitivity and specificity. EPPO Bulletin 18: 343-351.

Kelman, A. 1954. The relationship of pathogenicity in *Pseudomonas solanacearum* to colony appearance on a tetrazolium medium. Phytopathology 44:693-695.

Kennet, R. H., Davis, K. A., Tung, A. S., and Kilman, N. R. 1978. Hybrid plasmocyta. Production: Fusion with adult spleen cells, spleen fragments, neonatal spleen cells and human spleen cells. Current Topics in Immunology 81: 77-91.

Nakashima, J., and Nydegger, U. 1986. Detection de *Pseudomonas solanacearum* mediante las tecnicas serologicas de latex y ELISA. [Detection of *Pseudomonas solanacearum* by serological techniques of latex and ELISA] Fitopatologia 21:46-51.

Seal, S. E., and Elphinstone, J. G. 1994. Advances in identification and detection of *Pseudomonas solanacearum.* Pages 35-57 in Bacterial wilt: the disease and its causative agent, *Pseudomonas solanacearum* (Hayward, A.C. and Hartman, G.L., eds.). Wallingford, UK: CAB International. 259 pp.

Vruggink, H. 1978. Enzyme-linked immunosorbent assay (ELISA) in the serodiagnosis of Plant Pathogenic Bacteria. Pages 307-310 in Proceedings of the Fourth International Conference on plant pathogenic bacteria. Angers, France: Institut National de la Recherche Agronomique.

Discussion

V K Mehan Would you care to comment on the sensitivity of monoclonals (against *Pseudomonas solanacearum*) produced in Dr Alvarez's laboratory in the USA.

A Robinson-Smith We have not compared them in our laboratory. They have been reported to have similar sensitivities.

S Seal How many replicates do you usually run for your ELISA tests?

A Robinson-Smith Generally two wells for each sample, but it depends on the number of samples. If we have many samples, we may not bother about duplicates. If we have only a few samples, we include several replicates. It also depends on how important the results are—if we want absolute positive/negative results, we will probably do several tests with different antibodies.

M Machmud Production of monoclonal antibodies is an expensive and skilled operation. Is it possible to get some help from your institution in the production of monoclonals?

A Robinson-Smith We can provide small quantities of the monoclonals and polyclonals already produced in our laboratory free of charge. However, we can consider developing new monoclonals only if it was relevant to our research projects.

M Machmud Apart from using whole cells to produce polyclonal antibodies against *Pseudomonas solanacearum,* have you used other constituents of the bacterium (e.g., flagella and extracellular polysaccharides)?

A Robinson-Smith No. We have only used whole cells. Our previous experience with *Pseudomonas syzygii* suggested that whole cells are likely to give the best response.

Phenotypic and molecular approaches to strain differentiation in *Pseudomonas solanacearum*

M Taghvi, M Fegan, and A C Hayward[1]

Abstract

Several phenotypes (biovars) of Pseudomonas solanacearum are associated with groundnut bacterial wilt. Molecular methods including restriction fragment length polymorphism (RFLP) analysis and near-total sequencing of nucleotides in the 16S rRNA have shown groupings of isolates that are well correlated with these phenotypes. Based on 16S rRNA gene sequencing data on 16 strains of P. solanacearum, two sets of primers were designed for use in a specific polymerase chain reaction (PCR)-based test. One set of primers was designed to detect P. solanacearum strains belonging to RFLP Division I (biovars 3 and 4), and the other to detect strains belonging to Division II (biovars 1,2, and N2). Eighty strains of P. solanacearum representing all biovars isolated from many hosts and locations were tested together with 11 other bacterial species to evaluate the specificity of the reactions. Following optimization of the conditions for PCR, all strains of P. solanacearum were distributed within the two divisions as expected. None of the other bacterial species gave a PCR product with either set of primers. Primers are now being applied directly to exudates from infected plant material for detection.

花生青枯病病原(*Pseudomonas solanacearum*)存在着几种表型(生物型)。应用分子生物学方法包括 RFLP 分析和 16SrRNA 核苷酸序列分析划分的菌株与表型的划分相符。根据青枯菌 16 个菌系的 16SrRNA 基因序列数据,设计了二种引物用于聚合酶链式反应(PCR)检测。其中一种引物用来检测属于 RFLP 第一类青枯菌(生物型 3 和 4),另一种引物则检测第二类(生物型 1、2 和 N2)。从不同地点和许多寄主上采集代表所有生物型的 80 个青枯菌菌系和 11 种其它细菌用来评价这一反应的专化性。在 PCR 最佳反应条件下,所有青枯菌菌系均属于上述两种类型,而其它种类细菌用二种引物均未得到 PCR 产物。引物现在已直接应用于检测发病植株的渗出物。

1. The University of Queensland, St Lucia 4072, Queensland, Australia.

Taghvi, M., Fegan, ML, and Hayward, A X. 1994. Phenotypic and molecular approaches to strain differentiation in *Pseudomonas solanacearum. Pages* 49-52 in Groundnut bacterial wilt in Asia: proceedings of the Third Working Group meeting, 4-5 Jul 1994, Oil Crops Research Institute, Wuhan, China (Mehan, V. K. and McDonald, D., eds.). Patancheru 502 324, Andhra Pradesh, India: International Crops Research Institute for the Semi-Arid Tropics.

Introduction

Bacterial wilt, caused by *Pseudomonas solanacearum* (Smith) Smith, is one of the most serious and widespread diseases in tropical, subtropical, and some warm temperate regions of the world. The disease can affect a wide range of such plants as potato, tomato, tobacco, groundnut, banana, and ginger. *Pseudomonas solanacearum* is a heterogenous species that has been divided into five races based on host range, and into five biovars based on biochemical properties (Hayward 1991). Rapid and highly sensitive methods are needed to identify different strains of *P. solanacearum,* especially for quarantine purposes and for certification of planting material, because this pathogen can infect potato, tomato, ginger, and banana without any visible symptoms (Seal et al. 1993). This paper reports the construction of two sets of specific primers for the diagnosis of Division I and Division II of *P. solanacearum* by the polymerase chain reaction (PCR).

Materials and methods

The strains of *P. solanacearum* used in this study represented biovars 1,2, N2,3, and 4 from different hosts and countries. All strains of *P. solanacearum* were grown on sucrose peptone agar (SPA) and were incubated at 28°C for 48 h (Hayward 1964). The other bacterial species were grown on peptone yeast extract agar containing 1% glucose (PYEA-G) and were incubated at 28°C for 48h.

The bacterial DNA was extracted by the method described by Marmur and Doty (1962) with minor modifications. Whole cells of *P. solanacearum* were used to provide a template for PCR amplification. The cultures were grown on minimal medium (MM) or SPA for adequate lysis. A loopful of bacterial cells from a colony was re-suspended in 100 uL sterile distilled water, and lysed by boiling this suspension. After cooling to room temperature, 2 uL of boiled suspension was used for each PCR.

The PCR was performed using a total volume of 100 uL as described by Weisburg et al. (1991), and PCR products were purified by using the universal method. The Taq DyeDeoxy™ terminator cycle sequencing kit (Applied Biosystem 373A, USA) with different primers was used to sequence the PCR products directly. The extension products were purified by phenol-chloroform extraction, and the electrophoresis of products was performed on an Applied Biosystem DNA sequencer.

The PCR amplifications with specific primers were performed using DNA thermal cyclers (Perkin Elmer Cetus). The reaction volume was 25 uL and consisted of 4 uL 10 x PCR buffer [500 mM KCl, 15 mM $MgCl_2$, and 0.1% (w/v) gelatine in 100 mM Tris, pH 8.0]; 4 uL dNTP (2 mM dATP, dCTP, dGTP, and dTTP); and the template DNA (either 25 ng DNA or 2 uL of boiled suspension). The specific bvI-2 (forward and reverse) and bv3-4 (forward and reverse) primers were used at a concentration of 40 ng for each primer.

A wide range of annealing temperatures (48, 55, 60, 62, 65, and 70°C) and number of cycles were tested to optimize the PCR conditions. After initial denaturation of the reaction mixture at 96°C for 3 min, 0.1 uL of Taq polymerase was added to each reaction. The thermal profile included 25 cycles and each cycle consisted of annealing at 62°C for 1 min, extension at 72°C for 2 min, and denaturation at 93°C for 1 min. The final extension was carried out at 62°C for 1 min and at 72°C for 5 min. The PCR products were examined by loading 10 uL-aliquots of each PCR product and 5 uL of molecular weight standard on 1% agarose gels. Bands were revealed by staining with ethidium bromide.

Results and discussion

Based on 16S rRNA gene sequencing data for 16 strains of *P. solanacearum,* two sets of specific primers were designed to identify strains of this pathogen using the PCR. One set of primers was specifically for the detection of strains of *P. solanacearum* belonging to Division I (biovars 3 and 4) and the other for those belonging to Division II (biovars 1, 2, and N2). Following optimization of the conditions of PCR at an annealing temperature of 62°C and 25 cycles, 80 strains of P. solanacearum representing biovars 1, 2, 3, 4, and N2 were tested together with 11 strains of other bacterial species used to test the specificity of the reactions. Thirty-seven strains belonging to biovars 1, 2, and N2 reacted with Division II primers, and 42 strains belonging to biovars 3 and 4 reacted with Division I primers. One atypical strain (ACH 0732) of biovar 2 isolated from tomato in Australia reacted with a Division I primer. None of the other representatives of the genus *Pseudomonas,* including *P. cepacia, P. andropogonis, P. gladioli, P. pickettii, P. caryophylli, and P. syringae* pv *syringae*, and such unrelated bacteria as *Erwinia carotovora, Escherichia coli,* and *Xanthomonas campestris* pv *campestris*, reacted with either set of the primers. These two sets of specific primers, now being used directly on exudates from infected plant materials to detect the pathogen, will be useful tools to apply in quarantine laboratories, especially in countries where *P. solanacearum* is not present.

References

Hayward, A. C. 1964. Characteristics of *Pseudomonas solanacearum.* Journal of Applied Bacteriology 27: 265-277.

Hayward, A. C. 1991. Biology and epidemiology of bacterial wilt caused by *Pseudomonas solanacearum.* Annual Review of Phytopathology 29:65-87.

Marmur, J., and Doty, P. 1962. Determination of the base of deoxyribonucleic acid from its thermal denaturation temperature. Journal of Molecular Biology 5:109-118.

Seal, S. E., Jackson, L. A., Young, J. P. W., and Daniels, M. J. 1993. Differentiation of *Pseudomonas solanacearum, Pseudomonas syzygii, Pseudomonas pickettii* and the

blood disease bacterium by partial 16S rRNA sequencing: construction of oligonucleotide primers for sensitive detection by polymerase chain reaction. Journal of General Microbiology 139:1587-1594.

Weisburg, W. G., Barns, S. M., Pelletier, D. A., and Lane, D. J. 1991. 16S ribosomal DNA amplification for phylogenetic study. Journal of Bacteriology 173:697-703.

Discussion

V K Mehan Could you please comment on the virulence of biovar 1 as compared to biovars 3 and 4 on groundnut.

A C Hayward Biovar 1 appears to be much less virulent in the USA than are biovars 3 and 4 under Asian conditions. But we do not know whether this is a question of virulence of different strains (biovar 1 in USA versus biovars 3 and 4 in Indonesia and China etc.) or one of environment. In China and Indonesia, the prevailing temperature and humidity may be much more conducive to disease expression. I suspect that some biovar 3/4 strains are also more virulent. The strain/environment interaction (including soil type) is not well understood.

M P Natural The banana isolates in the Philippines are mostly of biovar 1. They are highly virulent, judging from the incidence of moko disease in bananas.

Factors involved in virulence and pathogenicity of *Pseudomonas solanacearum* and their role in pathogenesis

Y W Kang, G Z Mao, and L Y He[1]

Abstract

Pseudomonas solanacearum is an important wilt-inducing pathogen that infects a wide variety of crops throughout the world. Studies using artificial inoculation methods suggest that some of its extracellular proteins play a significant, but auxiliary, role in causing wilt. Mutants of race 1 and race 3 strains of P. solanacearum with Tn5 insertions at a single locus (eep) were isolated, the culture supernatants of which lack all of its known extracellular enzymes and most other detectable extracellular proteins (EXPs). Analysis of subcellular fractions of eep Tn5 mutants showed that they still synthesized many of these EXPs, but accumulated them inside the cell, implying that eep is involved in protein export. These analyses and others with phoA fusion proteins showed that protein export across the inner membrane was not affected by the eep mutation, suggesting that the eep locus functions only in protein export across the outer membrane. However, neither production nor export of extracellular polysaccharide was obviously affected by the eep mutation. Analysis of the in-plasma behavior of eep mutants after stem inoculation into tomato plants showed that they had lost the ability to cause wilt symptoms or kill the plant, possibly because they colonized plant stems much more slowly than wild-type strains. Plants grown in soil inoculated with the mutants did not develop any visible disease symptoms over a 20-day period and their stems showed no infection by P. solanacearum cells. Under the same conditions, the wild-types killed the plants in 14 days, and more than 10^9 cells were found in their stems. These results indicate that an individual EXP or a group of EXPs of P. solanacearum are required for infection via the roots, as well as for wilting and killing of host plants.

青枯菌是一种重要的引起萎蔫的病原菌，在世界范围内侵染多种作物。人工接种研究表明，某些胞外蛋白对引起萎蔫起着重要的辅助作用。我们分离了青枯菌 1 号和 3 号小种在一位点(eep)有 Tn5 插入片段的突变体，其培养上清液中缺少已知的胞外酶和大部分其它可测的胞外蛋白(EXPs)。eep Tn5 突变体亚细胞成分分析表明，它们仍可以合成 EXPs 中的大部分物质，但这些物质仅积聚在细胞内，说明 eep 位点与蛋白质输出有关。这些分析和其它 Pho A 融合蛋白质表明，蛋白质通过内膜输出不受 eep 突变的影响，eep 位点的功能仅在于蛋白质通过外膜的输出。但胞外多糖的产生和输出显然不受 eep 突变的影响。通过茎刺接种 eep 突

1. Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing 100094, China.

Kang, Y.W., Mao, C.Z., and He, L.Y. 1994. Factors involved in virulence and pathogenicity of *Pseudomonas solanacearum* and their role in pathogenesis. Pages 53-62 *in* Groundnut bacterial wilt in Asia: proceedings of the Third Working Group meeting, 4-5 Jul 1994, Oil Crops Research Institute, Wuhan, China (Mehan, V. K. and McDonald, D., eds.). Patancheru 502 324, Andhra Pradesh, India: International Crops Research Institute for the Semi-Arid Tropics.

变体进入番茄植株后的原生质行为分析表明，它们已失去了引起植株萎蔫和死亡的能力，其原因可能是它们在茎部的繁殖速度比野生型菌株慢得多。用突变体土壤接种，20天后植株不产生任何病症，茎部分离不到青枯菌。同样条件下，野生型菌株14天引起植株死亡，茎部可查到10^9细菌。这些结果说明一种或几种胞外蛋白是青枯菌由根部侵染植株并产生枯死的必要成分。

Introduction

Pseudomonas solanacearum (Smith) Smith may enter the plant through wounds on roots or stem, and then reach the vascular system, causing the plant to wilt. Under field conditions, the pathogen can also infect plants by penetrating the gaps between secondary root caps and surfaces of main roots, and then reach the intercellular spaces of epidermal tissue. The bacteria damage the middle lamellae of cells and degrade the cell walls. This results in destruction of xylem tissues and production of tyloses, which block the vascular system and cause the plants to wilt (Buddenhagen and Kelman 1964, Schmit 1978, Wallis and Truter 1978). Physio-pathological studies show that *P. solanacearum* produces large quantities of extracellular polysaccharides (EPS), which hinder the movement of water in plants, especially between the petioles and leaf laminae (Wallis and Truter 1978, Hussain and Kelman 1958, Kelman and Cowling 1965). In addition, the bacterium can secrete several extracellular enzymes, such as pectinase and cellulase, which may play an important role in destroying vascular tissue. *Pseudomonas solanacearum* can also produce growth regulators such as indole acetic acid, cytokinin (zeatin), and ethylene. But these are produced only in small quantities, and their role in pathogenesis is less important. Therefore, studies on pathogenesis of *P. solanacearum* have concentrated on EPS, pectinase, and cellulase. Since the middle of the 1980s, research on the functioning of these factors in pathogenesis has been extended to the molecular level. The genes responsible for EPS and lipo-polysaccharides (epsA-D and ops A-D) were cloned in Sequeira's laboratory at the University of Wisconsin in the USA (Kao and Sequeira 1991). A *phcA* gene related to EPS phenotype conversion was also cloned, and was identified as a global regulatory gene in Denny's laboratory at the University of Georgia in the USA (Brumbley and Denny 1990, Denny et al 1990). In recent years, many studies have proved that complex regulatory networks control EPS production, reactions to environmental factors or signals, and expressions of some genes responsible for such virulent factors as cellulase, pectinase, and 28KDa export protein in *P. solanacearum.* From these networks, *phcB, xpsR, vsrA,* and *vsrB* genes were cloned and studied . Moreover, the eps gene cluster was controlled by *phcA* (Denny et al 1990). Several studies show that EPS play a very important role in pathogenesis of *P. solanacearum,* but are not absolutely necessary Extracellular polysaccharides are a main factor in wilting of plants. Schellet al (1988) and Allen et al. (1991) made detailed studies of the role of pectinases and cellulases in pathogenesis of *P. solanacearum* at the molecular

level They successfully cloned structure genes of polygalacturonases PehA and PehB genes, or pg1A and pg1B, and the egl gene responsible for endoglucanase production. They concluded that all these extracellular enzymes are involved in pathogenesis, but they are not essential. Pme gene, the PME structure gene, has also been successfully cloned (Spoke et al 1991), but its role in pathogenesis is unknown. However, race 3 produces little PME. In addition, Boucher's group cloned a cluster of hrp genes from race 1 (biovar 4), which was located on a megaplasmid of a tomato strain, GM 100, of P. solanacearum (Boucher et al. 1988). The cluster of hrp genes is required to infect the host plants and to induce a hypersensitive response (HR) in nonhost plants. This gene cluster can hybridize with similar gene clusters cloned from many pathovars of *Xanthomonas campestris* (Gough et al. 1992). A second cluster of *hrp* genes was recently identified by Huang et al. (1990), but it was not homogeneous with Boucher's hrp cluster. According to recent research on Erwinia amylovora, the hrp gene cluster encoded an elicitor designated as harpin, which was a 44KDa protein (Wei et al. 1992). In China, Ma et al. (1988) cloned a host-specific gene from a groundnut strain of *P. solanacearum.* All of the above mentioned studies illustrate that the virulence and pathogenicity of P. solanacearum are determined by multiple genes. We are studying how these extracellular enzymes or other proteins are involved in the pathogenesis of the wilt pathogen. This paper reviews the research on virulence and pathogenesis of P. solanacearum in China.

Construction of mutants of Pseudomonas solanacearum

Obtaining mutants of *P. solanacearum* deficient in multiple traits is a pre-requisite to study pathogenesis. In order to get mutants deficient in producing EG, PG, or EPS, or in pathogenicity or induction of HR, the transposon mutagenesis was used. The sweet potato strain B4, which exists solely in China, and the predominant race 3 potato strain (P041) were selected as recipient strains. *Pseudomonas aeruginosa* PAO 1826::PM075::Tn5 and Escherichia coli S17-I::pSUP2021::Tn5 were selected as donor strains. Mating experiments with four pairs showed that stable Km^r Rif^r resistant transconjugants were obtained only from P041 mixed with PAO 1826 at a high frequency. Screening of 8000 transconjugants led to isolation of (a) 19 mutants that can produce EPS but cannot export EG and PG outside cells, and (b) 16 mutants that cannot produce EPS or produce it only in very small quantities but can synthesize and export EG and PG outside cells. No PGTE or PMTE activities were detected in P041 and its derivative mutants, indicating that either P041 is unable to synthesize these two enzymes, or that their activities are too low to be detected. Stem inoculation of potato plants showed that 16 EPS-deficient mutants had significantly low virulence. Of 19 EG- and PG-deficient mutants, 17 also had decreased virulence, while the other 2 mutants retained the same virulence as the wild-type strains. Tests for HR on tobacco leaves showed that most of the mutants had the same capacity to cause HR as the wild-type did, but 2 mutants were significantly deficient in causing HR, These mutants

deficient in multiple traits are very useful in studying pathogenic factors of *P. solanacearum*. The use of a photobiotin-labeled Tn5 probe to hybridize with chromosome DNA of typical mutants D1, D2, D3, D4, G5, and G7 and the Southern blot assay indicated that all mutants had a positive reaction, which indicated that these mutants resulted from transposition of Tn5 into the chromosome of strain PO41.

Characterization of the defective mutant in export of extracellular enzymes

In order to confirm the activities of PG and EG in the EPS'PG' EG'Vir' mutants, a more accurate analytical method was used (Huang et al. 1992, Roberts et al 1988). The results showed negligible quantities of both the enzymes in culture-supernatants of these mutants. This again indicated that the mutants had lost the ability to produce the two enzymes. However, the question, namely whether the mutants were defective in structure genes or export genes, remained to be answered. So we had to measure the activities of the two enzymes within the cells. We found that the cell-free extracts from eight mutants had high levels of activity of both the enzymes. One of these mutants, D4, was analyzed further.

To ensure that the phenotype of the mutant D4 is due to a single Tn5 insertion, the genomic segment with the Tn5 insertion (Km^r marker) was recombined, using transformation, into the genomes of the wild-type parent strain P041 (race 3) and the more extensively characterized strain AW (race 1). The phenotypes of the resultant Km^r transformants (D4.1 and AD4) were identical to that of the original mutant D4. In vitro analysis showed that levels of EG and PG activity in the transformants and in the wild-type were similar but the transformants failed to export the enzymes outside the cells. The activity could be detected only within the cells and not in the culture-supernatants. Similar analyses of culture supernatants and cell-free extracts of strain AD4 for activity of another extracellular enzyme, pectin methylesterase (PME), showed that it too remained only within the cell. However, its parent strain (wild-type AW) could produce and export PME. These results suggest that a single Tn5 insertion at a homologous locus in both race 1 and race 3 strains of *P. solanacearum* blocks the export of at least three major EXPs.

Effect of mutation on export of other extracellular proteins

To study the effect of eep::Tn5 insertion mutation on other extracellular proteins, the culture supernatants of a wild-type strain AW (race 1), P041 (race 3), and the mutants (D4, D4.1, AD4) were compared, using sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), for enzyme activity. The results showed that the wild-type strains contained more than six major types of EXPs. Three of these EXPs present in culture supernatants of all the wild-type strains appeared identical in size (e.g., 28kDa, 35kDa, and 43kDa). Other EXPs were missing from the supernatants of the mutant cultures. In

contrast, the profiles of membrane-associated and soluble proteins of both eep mutants on SDS-PAGE were very similar to those of their respective wild-type parents. These results suggest that a single Tn5 insertion at a homologous locus in the strains of both race 1 and race 3 of *P. solanacearum* blocks the export of not only PG, EG, and PME, but also of other EXPs. This locus is designated as eep (export of extracellular proteins).

Location of extracellular proteins in eep mutants

The function of export of EXPs is associated with the membrane of the bacteria. Gram-positive bacteria have a single membrane, whereas Gram-negative bacteria have two membranes. *Pseudomonas solanacearum* is Gram-negative and has two membranes. The mechanism of export of extracellular proteins in *P. solanacearum* is much more complicated than that in Gram-positive bacteria. Recent work suggests that most well-characterized extracellular enzymes such as PG and EGL can synthesize precursors with N-terminal signal sequences for processing and exporting them across the inner membrane (Ma et al. 1988, Huang and Schell 1990a and 1990b). However, it is not known how these proteins are exported across the outer membrane. To establish the site at which the eep mutation blocks the export pathway of *P. solanacearum,* the intracellular location of several accumulated extracellular enzymes was determined. Analysis of the soluble (cytoplasmic + periplasmic) and membrane fractions of D4 and AD4 demonstrated that the PG activity was nearly equally divided between the soluble and the membrane fractions. EG or PG activity was detected in both soluble and membrane fractions but was five times higher in the soluble fraction (Table 1). This result was further confirmed by analyzing the ability of *P. solanacearum* strain AD4 to export two previously characterized *phoA* hybrids: one encoded on pJH123 and comprised the signal sequence and

Table 1. Distribution of extracellular enzymes in cultures of eep::Tn5 mutants of *Pseudomonas solanacearum.*

	Specific activity[1]								
	PG			EG			PME		
Strain	Sol[2]	Mem	Ext	Sol	Mem	Ext	Sol	Mem	Ext
AW	und[3]	und	384	und	und	224	und	und	25
AD4	144	192	und	24	124	und	2.6	0.1	und
P041	und	und	48	und	und	21	und	und	und
D4.1	16	24	und	5	23	und	und	und	und

1. Activity of polygalacturonase (PG) and endoglucanase (EG) is given in nmol of reducing sugar released min^{-1} mg^{-1} total cell protein. Pectin methylesterase (PME) activity is given in nmol H^+ released h^{-1} mg^{-1} total cell protein (1 unit).
2. Sol = soluble (cytoplasmic + periplasmic) fraction; Mem = membrane fraction (150 000 x g x h pellet); Ext = extracellular fraction (culture supernatant).
3. und = undetectable (for PG and EG <0.10; for PME <0.01).

17 residues of mature PgLA fused to mature *phoA*; the other encoded on pJH113 and comprised the signal sequence and 65 residues of mature egl fused to mature *phoA*. In wild-type AW of *P. solanacearum,* these hybrids are processed and exported across the inner membrane by the Sec-encoded pathway giving levels of *phoA* activity. AD4 with either p*gIA-phoA or egl-phoA* fusion gene produced the same level of *phoA* activity as in the wild-type with the same genes. The *phoA* requires export across the inner membrane to attain catalytic activity. These results suggest that in the eep mutants the Sec-dependant pathway for export across the inner membrane is intact; eep inactivation only affects the export across the outer membrane.

Virulence of eep mutants

To determine if the loss of the ability to export most major EXPs affects virulence, we compared disease development in tomato plants that were stem-inoculated with eep::Tn5 strains or their wild-type parents. The eep mutants AD4 and D4.1 lost much of their virulence, while the wild-type strains were highly virulent. Plants inoculated with AD4 showed only such mild symptoms as minor chlorosis and stunting.

When tomato plants were inoculated with the eep mutant AD4 via the roots using the soil-inoculation method, no disease symptoms were observed even 20 days after inoculation. Under the same conditions, the wild-type strain AW killed 80% of the plants in less than 14 days. Also, in contrast to its wild-type parent, when tested on its natural host (potato), the race 3 eep mutant D4.1 failed to cause any symptoms.

Results from these experiments suggest that EXPs and/or enzymes are required for infection of host plants by *P. solanacearum.*

Ability of eep mutants to grow, colonize, and spread in plants

To investigate if eep mutants lose the ability to grow and to colonize their hosts, we examined populations of bacteria at certain intervals after inoculations. Examinations of roots of inoculated plants 10 days after inoculation showed that roughly equal numbers (10^5) of bacteria of eep mutants and wild-type strains were associated with the root system. Examination of 1-cm-long stem pieces (taken from a point 2 cm above the soil surface) showed fewer than 10^2 viable cells in the plants inoculated with the epp::Tn5 mutant AD4 and more than 10^9 in those inoculated with wild-type AW. These results indicated that eep mutants could colonize, spread, and multiply in plants, but that they did so much more slowly than the wild-type strains. This suggests that some EXPs are required for infection through the roots.

Cloning of eep gene

Cloning of the eep gene can help in analyzing the role of EXPs in pathogenesis of *P. solanacearum.* Four cosmids were obtained from the gene bank of race 3

strain PO41. They could restore EG and PG activities of the eep mutant to the same level as that in wild-type P041. Tests of virulence of these four merodiploids obtained by conjugating the eep mutant with four cosmids showed that they could cause wilting of plants just as the wild-type did. The four cosmids were designated pOUl, pOU2, pOU3, and pOU4.

Recently, genes for export of EXPs have been cloned from three other species of bacteria, namely xps gene of *Xanthomonas campestris,* out gene of *Erwinia chrysanthemi*, and xep gene of *P. aeruginosa* (Dow et al 1987, Lindberg and Collmer 1992, Bally et al. 1992). To examine the homology within eep gene and other export genes, the out DEE genes provided by Dr M Schell (University of Georgia) were labeled with photobiotin and hybridized with pOU1, pOU2, pOU3, and pOU4. The results showed that out DEF gene of *E. chrysanthemi* could specifically hybridize with genomic fragments cloned in pOUl, pOU2, and pOU3. This suggests some homology between the eep gene and the out gene DEF.

Discussion

Studies on the mechanisms of pathogenesis of *P. solanacearum* have focused on EPS, pectinase, cellulases, and lipopolysaccharides since the 1950s. In addition, research on hypersensitive reaction and host specificity of the pathogen has been intensive. The EXP-deficient mutants of *P. solanacearum* have been well characterized, and the genes responsible for export across the outer membrane also have been cloned. It is thought that EXPs have a greater influence on the virulence of *P. solanacearum* compared to EPS, PG, or EG. Although eep mutants retained the ability to produce EPS in culture as well as in plants, they lost the ability to cause wilting. The fact that eep mutants proliferate and spread in inoculated stems much more slowly than wild-types do indicates that EXPs are probably essential for degradation of plant tissues or for efficient release of nutrients. It also suggests that EXPs are necessary for the bacteria to invade the roots of plants and penetrate the vascular tissue. The eep mutants can colonize roots but cannot spread well within stems.

According to EXPs profiles obtained by SDS-PAGE, *P. solanacearum* can secrete many types of EXPs. Of these, at least six proteins are produced in large amounts. However, the kinds of proteins involved in the pathogenesis remain unknown except for some characterized extracellular enzymes.

The molecular mechanism of *P. solanacearum* to export EXPs across outer membranes is still not clear. N-terminal signal sequences are essential for EG and PG of the pathogen in export across the inner membrane (Huang and Schell 1990a and 1990b). It suggests that a special apparatus exists in the outer membrane and is involved in the transport of these EXPs across the outer membrane (Bally et al. 1992, Huang and Schell 1990a). What is this apparatus? And how does it work? Answers are needed to these questions. Understanding the functioning of this apparatus should provide us with a basis for effective management of bacterial wilt.

References

Allen, C., Huang, Y., and Sequeira, L. 1991. Cloning of genes affecting polygalacturonase production in *Pseudomonas solanacearum.* Molecular Plant-Microbe Interactions 4:147-154.

Bally, M., Filloux, A.M., Ball, G., Lazdunski, A., and Tommassen, J. 1992. Pectin secretion in *Pseudomonas aeruginosa:* Characterization of seven xcp genes and processing of secretory apparatus components by prepolin peptidase. Molecular Microbiology 6:1121-1131.

Boucher, C. A., Barberis, P. A., and Arlat, M. 1988. Acridine orange selects for deletion of *hrp* genes in all races of *Pseudomonas solanacearum.* Molecular Plant-Microbe Interactions 1:282-288.

Brumbley, S, M., and Denny, T. R 1990. Cloning of wild-type *Pseudomonas solanacearum phcA*, a gene that when mutated alters expression of multiple traits that contribute to virulence. Journal of Bacteriology 172: 5677-5685.

Buddenhagen, I. W., and Kelman, A. 1964. Biological and physiological aspects of bacterial wilt caused by *Pseudomonas solanacearum.* Annual Review of Phytopathology 2: 203-230.

Denny, T.P., Carney, B. F., and Schell, M. A. 1990. Inactivation of multiple virulence genes reduces the ability of *Pseudomonas solanacearum* to cause wilt symptoms. Molecular Plant-Microbe Interactions 3: 293-300.

Dow, J. M., Scofield, G., Trafford, K., Turner, P.C., and Daniels, M. J. 1987. A gene cluster in *Xanthomonas campestris* pv *campestris* required for pathogenicity controls the excretion of polygalacturonate lyase and other enzymes. Physiological Molecular Plant Pathology 31: 261-271.

Gough, C. L., Genin, S., Zischek, C., and Boucher, C A. 1992. *Hrp* genes of *Pseudomonas solanacearum* are homologous to pathogenicity determinants of animal pathogenic bacteria and are conserved among plant pathogenic bacteria. Molecular Plant-Microbe Interactions 5: 384-389.

Huang, J., and Schell, M. A. 1990a. Evidence that extracellular export of the endoglucanase encoded by *egl* of *Pseudomonas solanacearum* occurs by a two-step process involving a lipoprotein intermediate. Journal of Biological Chemistry 265:11628-11632.

Huang, J., and Schell, M. A. 1990b. DNA sequence analysis of pglA and mechanism of export of its polygalacturonase product from *Pseudomonas solanacearum*. Journal of Bacteriology 172:3879-3887.

Huang, J., and Schell, M. A. 1992. Role of the two-component leader sequence and mature amino acid sequences in extracellular export of endoglucanase Egl from *Pseudomonas solanacearum.* Journal of Bacteriology 174:1314-1323.

Huang, Y., Xu, R, and Sequeira, L. 1990. A second cluster of genes that specify pathogenicity and host response in *Pseudomonas solanacearum.* Molecular Plant-Microbe Interactions 3:48-53.

Hussain, A., and Kelman, A. 1958. Relation of slime production to mechanisms of wilting and pathogenicity of *Pseudomonas solanacearum.* Phytopathology 48:155-165.

Kao, C, and Sequeira, L. 1991. A gene cluster required for the coordinated biosynthesis of both lipopolysaccharides and exopolysaccharide also affects virulence of *Pseudomonas solanacearum.* Journal of Bacteriology 173: 7841-7847.

Kelman, A., and Cowling, E. B. 1965. Cellulase of *Pseudomonas solanacearum* in relation to pathogenesis. Phytopathology 55:148-155.

Lindberg, M., and Collmer, A. 1992. Analysis of eight out genes in a cluster required for pectic enzyme secretion by *Erwinia chrysanthemi:* Sequence comparison with secretion genes from other Gram-negative bacteria. Journal of Bacteriology 174: 7385-7397.

Ma, Q. S., Chang, M. F., Tang, J. L., Feng, J. X., Fan, M. J., Han, B., and Liu, T. 1988. Identification of DNA sequence involved in host specificity in the pathogenesis of *Pseudomonas solanacearum* strain T 2005. Molecular Plant-Microbe Interactions 4:169-174.

Roberts, D. P., Denny, T. P., and Schell, M. A. 1988. Cloning of the egl gene of *Pseudomonas solanacearum* and analysis of its role in phytopathogenicity. Journal of Bacteriology 170:1445-1451.

Schell, M. A., Roberts, D. P., and Denny, T. P. 1988. Analysis of the *Pseudomonas solanacearum* polygalacturonase encoded by pglA and its involvement in phytopathogenicity. Journal of Bacteriology 170:4501-4508.

Schmit, J. 1978. Microscopic study of early stages of infection by *Pseudomonas solanacearum* on 'in vitro' grown tomato seedlings. Pages 841-856 in Proceedings of the Fourth International Conference on Plant Pathogenic Bacteria.

Spoke, A., Stubenrauch, G., Schorgendorfer, K., and Schwab, H. 1991. Molecular cloning and sequencing of a pectin esterase gene from *Pseudomonas solanacearum*. Journal of General Microbiology 137:131-140.

Wallis, F. M., and Truter, S. J. 1978. Histopathology of tomato plants infected with *Pseudomonas solanacearum,* with emphasis on ultrastructure. Physiological Plant Pathology 13: 307-317.

Wei, Z. M., Laby, R. J., Zumoff, G H., Bauer, D., He, S. Y., Collmer, A., and Beer, S.V. 1992. Harpin elicitor of the hypersensitive response produced by the plant pathogen *Erwinia amylovora.* Science 257: 85-88.

Discussion

V K Mehan Do you think it would be easy to get these mutants established in soil, particularly when there is selection pressure for wild-type strains?

Y W Kang I think it would be easy to get these mutants established in soil because the mutants can colonize the root systems.

S Seal Due to localized growth and production of EPS by the bacterium, will an inoculated plant wilt above the site of stab inoculation on the stem?

Y W Kang Of course not.

Y D Tang Is it difficult to get an out mutant?

Y W Kang I was lucky to get such mutants. From 8000 transconjugants screened, 19 out mutants were isolated. Dr Schell's group in the USA worked for two years, but failed to get an out mutant.

Z Y Xu Out mutants of *Pseudomonas solanacearum* could produce the same amounts of EPS as produced by a virulent isolate. What is the reason for inability of the out mutants to multiply in the plant and to cause wilting?

Y W Kang Extracellular proteins are very important for the bacterium to spread and to move within the plants. So, the out mutants cannot spread very well in the plants and then multiply enough to block water transportation.

A C Hayward In biocontrol of groundnut bacterial wilt, are the plants showing tolerance to out mutants? Is the situation similar to that in Trigalet's mutants?

Y W Kang No, because the out mutants multiply only to a limited extent. They are not similar to Trigalet's mutants.

A C Hayward Are your mutants the same as the Tn5-induced invasive mutants of Trigalet?

Y W Kang No, probably not, but we have not made a direct comparison.

Isolation of Tn5-induced exopolysaccharide (EPS)-deficient and motile mutant E1 of *Pseudomonas solanacearum*

G Z Mao, Y W Kang, and L Y He[1]

Abstract

When Pseudomonas aeruginosa PAO1826 containing plasmid $_p$MO75 (Tn5, Km^r) and Escherichia coli S17-1 containing plasmid pSUP 2021 (Tn5, Km^r) were each mated with six isolates of Pseudomonas solanacearum (Smith) Smith from groundnut, potato, and sweet potato, stable Km^r Rif transconjugants were obtained only from a potato isolate P041 and from a groundnut isolate P8, with frequency of 1 x 10^6 for each recipient cell Although the conjugation cell number ratio was changed, and the kanamycin concentration was reduced, transconjugants were either not obtained at all from the other combinations, or only unstable ones were obtained, which became easily susceptible to kanamycin. Screening of 8000 transconjugants led to the isolation of one mutant E1, which was EPS-deficient, motile, and moderately virulent. Southern blot assay showed that there was only one transposition site in the chromosome of E1. Mutant E1 is deficient in motility and pathogenicity compared to the butyrous variant type of P041. Cosmids were obtained from a P041 genomic library that can totally revert E1 to wild-type status, or revert it to synthesize EPS. The relevant genes are being characterized.

用含质粒 $P^{MO\,75}$(*Tn*5,K_m^r)的 *Pseudomonas aeruginosa* PAO 1826 和含质粒 P^{sup}2021(T。5,K_m^r)的 E. coli S17－1 作为转座子诱变的供体菌，以分离自花生、马铃薯和甘薯的 6 株青枯菌(*P. solanacearum*) 作为受体菌株，进行转座子诱变。在 12 个诱变组合中，只有用 *P. aeruginosa*PAO 1826 诱变马铃薯菌株 P041 和花生青枯菌 P8 可以获得大量稳定的转移接合子，诱变频率为 1×10^{-6}。其他的组合诱变频率太低，检测不出，或接合子不稳定，易丧失卡那霉素抗性。用 *P. aeruginosa*PAO 1826 诱变青枯菌 P041，筛选了 8000 个转移接合子，获得了 1 株胞外多糖缺失、异运动性、致病力中等的菌株 *E*1。*Southern* 杂交表明在突变株 E1 的染色体 DNA 上有一个 Tn5 插入位点。突变株 E1 的性状变异不同于 P041 的自发突变，P041 的自发突变株没有运动性，致病力完全丧失。从 P041 的染色体基因文库里获得了一系列柯斯质粒，它们分别可以完全或部分恢复突变株 E1 的野生型表型，即完全恢复野生型表型或只恢复产生胞外多糖的能力。相关基因正在鉴定之中。

1. Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing 100094, China.

Mao, G.Z., Kang, Y.W., and He, L.Y. 1994. Isolation of Tn5-induced exopolysaccharide (EPS)-deficient and motile mutant E1 of *Pseudomonas solanacearum.* Page 63 *in* Groundnut bacterial wilt in Asia: proceedings of the Third Working Group meeting, 4-5 Jul 1994, Oil Crops Research Institute, Wuhan, China (Mehan, V. K. and McDonald, D., eds,). Patancheru 502 324, Andhra Pradesh, India: International Crops Research Institute for the Semi-Arid Tropics.

Groundnut bacterial wilt: past, present, and future

Groundnut bacterial wilt: past, present, and future

V K Mehan[1] and B S Liao[2]

Abstract

Groundnut bacterial wilt has been a major constraint to crop production in Indonesia and China, and in limited areas of Uganda, for several decades. Many reports of the disease occurrence from several countries of South and Southeast Asia and the South Pacific in the late 1980s and early 1990s highlight the increasing seriousness of groundnut bacterial wilt in the region. The disease is considered a potential threat to groundnut production in several other parts of the world, particularly in warm, humid areas. The destructiveness of the disease is compounded by the wide host range of the wilt pathogen, Pseudomonas solanacearum. Considerable research in Indonesia and China provided an excellent understanding of the effects of cultural practices and environmental factors on groundnut bacterial wilt and the results have been used to formulate recommendations to reduce wilt incidence and severity. Research on genetic resistance has been given the highest priority since the early 1920s, and much progress has been made in both Indonesia and China in the development of various wilt-resistant, high-yielding groundnut cultivars. Several of these cultivars are now grown in parts of Indonesia, and southern and central China. Despite the reported variation in cultivar susceptibility and the interactions of environmental factors with strain variation in the pathogen, breeding for wilt resistance is likely to remain the primary control measure. Future research into stability and durability of wilt resistance, and strategies for incorporating resistance to wilt and other important stress factors into high-yielding cultivars are discussed.

Recent research in China has highlighted the existence of pathotypes in the wilt pathogen, and this necessitates critical genetic evaluation of wilt resistance to delineate host-pathogen interactions. Future research into the distribution of distinct pathotypes in relation to ecology and etiology of groundnut bacterial wilt is needed. Basic and strategic research on the wilt pathogen has been carried out in advanced laboratories in several countries, as P. solanacearum attacks many other crops throughout the world; and the availability of improved diagnostic tools (e.g., monoclonal antisera and polymerase chain reaction techniques) is expected to result in improved disease control. An integrated approach involving the use of resistant cultivars, appropriate cultural practices, and crop sanitation is recommended.

1. ICRISAT Asia Center, Patancheru 502 324, Andhra Pradesh, India.
2. Oil Crops Research Institute, Chinese Academy of Agricultural Sciences, Wuhan 430 062, China.
ICRISAT Conference Paper no. CP 903.

Mehan, V. K., and Liao, B. S. 1994. Groundnut bacterial wilt: past, present, and future. Pages 67-88 in Groundnut bacterial wilt in Asia: proceedings of the Third Working Group meeting, 4-5 Jul 1994, Oil Crops Research Institute, Wuhan, China (Mehan, V. K. and McDonald, D., eds). Patancheru 502 324, Andhra Pradesh, India: International Crops Research Institute for the Semi-Arid Tropics.

In the past, research efforts have been scattered, relatively uncoordinated, and seldom given enough importance. Recently, many scientists, including pathologists, breeders, and geneticists, have accorded high research priority to the disease. The increasing collaboration on bacterial wilt research through the Groundnut Bacterial Wilt Working Group should be able to provide answers to specific problems, and contribute to improved disease control. To increase the effectiveness of research on genetic resistance, it is imperative to assemble (through collection and exchange) various groundnut germplasm/varieties with putative resistance, in countries where the disease is severe.

几十年来，花生青枯病是印尼、中国和乌干达局部地区花生生产的重要限制因素。八十年代末到九十年代初在东南亚、南亚和南太平洋的几个国家关于花生青枯病的报导说明了这一病害在该区域不断增长的重要性。青枯病被认为是世界其它一些地区，尤其是湿热地区花生生产的潜在威胁。青枯病的危害性还表现在该病原细菌(*Pseudomonas solanacearum*)的很广的寄主范围。印尼和中国的研究工作可使人们更好地理解栽培措施对防治花生青枯病的作用和环境因子对病害的影响，而且这些研究结果已被用于减轻病害损失的措施中。花生对青枯病的遗传抗性自二十代年早期以来一直是最重要的研究内容，在培育抗病、高产品种方面，印尼和中国均取得了很大进展，一些抗病品种正在印尼，中国中、南部应用。尽管有报告指出了品种感病的普遍性，环境因子与病原菌系分化交互作用的存在，但培育抗病品种是防治这一病害最基本的方法。文中还讨论了对抗性的稳定性和持久性、以及将青枯病抗性与对其它主要限制因子的抗性结合起来并转移到高产花生品种中去的策略。

中国近来的研究指出了青枯菌致病型的存在，有必要对抗性作细致的遗传评价并弄清寄主与病原菌间的交互作用。需要对青枯菌不同致病型的分布进行生态学等方面的深入研究。由于青枯菌在全球范围内也危害其它许多作物，一些国家条件先进的实验室做了许多基础性的研究，而且已有先进的诊断手段(如单克隆抗血清和 PCR 技术)，有希望改进病害的防治。一种包括利用抗病品种、适宜的栽培措施和田间清除病株体的综合方法值得应用。

过去的研究工作较为分散，相对缺乏协调，未引起足够的重视。近来包括病理、育种、遗传学方面的科学家对这一病害的研究更加重视。通过"花生青枯病工作组"而进行的日益增长的合作研究将有希望解决一些特殊问题，有利于防治工作的改进。为了增强花生抗性研究的有效性，那些病害严重的国家必须通过收集和交换而获得抗病花生种质。

Introduction

Bacterial wilt caused by *Pseudomonas solanacearum* (Smith) Smith has been a serious constraint to groundnut production in Indonesia and China for several decades. Although the disease was reported from several countries of Africa (Ethiopia, Libya, Madagascar, Mauritius, Somalia, South Africa, Uganda, and Zimbabwe) in the 1930s and 1940s, it is not considered economically important in these countries with the exception of Uganda (Mehan et al. 1986, 1994). Recent reports of the occurrence of the disease from several countries of South and Southeast Asia and the South Pacific (Hayward 1990, Mehan et al. 1994) highlight its increasing seriousness in Asia. It is now recognized as a serious

problem in major groundnut-growing areas of Vietnam (Mehan et al 1993, Hong et al 1994).

Bacterial wilt poses a threat to groundnut production in warm, humid areas of the world where groundnut cultivation is expanding, or where cultural practices are changing. It takes no great foresight to predict that the wilt problem will be more serious as a consequence of predicted global warming.

The destructiveness of the disease is compounded by the wide host range of the wilt pathogen. Thus, in areas where intercropping and continuous cropping are practiced, the severity of the problem increases each year. The persistence of the pathogen in different types of soils, and its wide host range, often hamper the effectiveness of cultural control practices. Despite the variation in the susceptibility of cultivars and the variability of the pathogen, breeding for disease resistance is likely to remain the most widespread control measure and the most important component of integrated control strategies. This paper presents a brief review of the history, distribution, and economic importance of bacterial wilt; its present status; and advances in research on various aspects of the disease worldwide. Host-plant resistance and cultural control measures are considered for use in integrated disease management systems. Future research directions are indicated.

Early history, current status, and economic importance

Little is known about groundnut bacterial wilt prior to the very early part of the twentieth century, although bacterial wilt affecting various solanaceous crops was recognized as a problem in the 1880s in many areas of the world.

Bacterial wilt of groundnut was first observed in 1905 in the Cirebon area of West Java, Indonesia (van Breda de Haan 1906). The disease became widespread within Indonesia in the next 15 years, and in the 1920s it was regarded as the most serious disease of groundnut in Indonesia where heavy losses (25%) continued for many years (Bolhuis 1955). Recent surveys indicate that the disease is still widely distributed in Indonesia, causing significant yield losses in West Java, South Sumatra, and South Sulawesi, particularly where susceptible cultivars are grown (Machmud and Hayward 1992).

In the USA, groundnut bacterial wilt was first reported in 1912 from Granville County, North Carolina, an area notorious for its earlier problems with bacterial wilt of tobacco (Fulton and Winston 1914), and was later reported to occur in many counties of Georgia and North Carolina (Miller and Harvey 1932), where it caused heavy yield losses in the 1930s. It is intriguing that the disease is currently of only minor importance in the USA.

In China, the disease was first reported in the late 1930s (Ma and Gao 1956). Prior to the 1960s, it was limited to a few provinces of southern China, but now the disease occurs in 16 groundnut-growing provinces (Mehan et al 1994). It is most severe in the central and southern provinces, where it is estimated that over 200 000 ha of groundnut fields are infested with *P. solanacearum.* Yield losses are commonly in the range of 10 to 30%, but can exceed 60% in heavily

infested fields when susceptible cultivars are grown (Liao et al. 1990). Annual losses in pod yield for the country are estimated at around 45000 t.

In Malaysia, the disease was first observed in 1949 and is now reported to be widespread. It is severe in Kelantan and Serdang (Hamidah and Lum 1993).

In Uganda, bacterial wilt of groundnut was first reported in 1938 at Bukalasa farm near Kampala. Heavy losses have been reported in some localities in the Lake Crescent area. The disease is now economically important in some central and north-western regions (Simbwa-Bunnya 1972, Opio and Busolo-Bulafu 1990).

Groundnut bacterial wilt was reported from several other countries of Africa—Ethiopia, Libya, Madagascar, Mauritius, Somalia, South Africa, and Zimbabwe—in the early 1930s, but information is sparse and the present status of the disease in these countries is unknown. The disease has also been reported from Japan and Taiwan (Hayward 1990).

In the late 1980s, bacterial wilt of groundnut was reported from several countries of South and Southeast Asia (Fiji, India, Papua New Guinea, the Philippines, Sri Lanka, and Thailand) (Hayward 1990, Mehan et al. 1994), which were previously thought to be free from it. In Fiji and Thailand, it is reported that in some areas groundnut is severely attacked but that, in general, the losses are lower than those caused by *P. solanacearum* on other crops. The disease has recently been reported as severe in parts of the major groundnut-growing areas of both northern and southern Vietnam (Hong and Mehan 1993, Hong et al. 1994, these proceedings). It is becoming increasingly serious in Vietnam.

Bacterial wilt caused by *P. solanacearum* is common on many crops throughout the tropics and subtropics, but on groundnut its incidence is relatively isolated. It is not known whether the sporadic occurrence of groundnut wilt reflects the restricted distribution of specific highly virulent strains of the pathogen, or whether environmental factors are involved. The recent spread of the disease may be due to changes in cultural practices and varieties, or due to the movement of infected seed. Recent reports of occurrence of bacterial wilt on groundnut could also reflect greater awareness of the disease.

Survival of the pathogen

Although there are many reports of the survival of *P. solanacearum* in different soils (Graham et al. 1979, Quimio and Chan 1979a, Moffett et al. 1981), critical studies on natural conditions, and physical factors, are largely lacking. The population dynamics of the wilt pathogen in soil also need to be separated from its dynamics in soil as such and in weed hosts. Little is known of the effects of soil pH on survival. What happens to the pathogen population as the soil goes through its normal cycles of wet to dry and dry to wet, at different soil temperatures and moisture conditions? What are the dynamics of the population changes if local hosts or non-wilting hosts are added to the production system? Answers to such questions are crucial to devise rational

disease control measures. A persistent problem in attempts to control bacterial wilt by crop rotation has been the unpredictability of the survival of *P. solanacearum* in the soil. The early research in Indonesia pointed to the need for long periods (up to 8 years) of rotation with non-susceptible crops to allow planting of tobacco in certain soils, and then only for a single season (Kelman 1953). The extensive experiments of Smith (1944) in North Carolina led to the notion that the wilt pathogen could persist for several years in fallow soil, even in the absence of vegetation. However, many investigators believe that *P. solanacearum* does not survive in the soil for prolonged periods since it is not a strong competitor. The bacterium survives on or in plant roots; it appears to survive by continually infecting the roots of susceptible or carrier plants, or by colonizing the rhizosphere of nonhost plants (Quimio and Chan 1979b, Granada and Sequeira 1983). The most logical explanation for the long-term survival of the pathogen is its association with plant roots. Research on population dynamics remained hampered as researchers could not measure populations of the bacterium below a certain number. Until very recently, using even the most selective media could not provide reliable information below a concentration of 10^2 cells g^{-1} soil (Granada and Sequeira 1983). Immunological procedures, e.g., enzyme-linked immunosorbent assays (ELISA) also fail to detect the bacterium below these population levels.

There is also the possibility that *P. solanacearum* exists as a leaf epiphyte under some environments. In view of the report of the wilt pathogen causing a leaf spot disease of capsicum (Hayward and Moffett 1978), it is likely that, under conditions of high relative humidity, epiphytic colonization of the host plant can occur and, in fact, can lead to lesion development on the leaves (Moffett et al. 1981). The epiphytic phase in the life cycle of the bacterium may be an important source of inoculum for the renewal of soil populations. Future research should focus on field studies designed to determine whether such epiphytic growth plays an important role in the epidemiology.

Survival is an issue that is fundamental to our understanding of both the biology of the wilt pathogen and the design of crop rotation for effective disease control.

Seed transmission

Some early evidence from Indonesia indicated that groundnut seed could serve as a means of dispersal of the wilt pathogen (Palm 1922). Although seed transmission is of obvious significance in relation to safe international movement of seed and to integrated disease management, research on this important topic was neglected for several decades. Only in the late 1980s, a few reports from Indonesia (Machmud and Middleton 1990a and 1990b) confirmed seed transmission in groundnut. Recent research in China, Malaysia, and Vietnam has also reported seed transmission of groundnut bacterial wilt (Zhang et al 1993, Hong et al. 1994, Lum and Hamidah 1994). Seed transmission is reported to be at the rate of 4-8%, particularly through freshly harvested seed. However, there is rapid loss of viability of the bacterium as

seeds dry out to moisture contents below 9% (Zhang et al. 1993). The pathogen is not likely to survive in the well-dried seeds that are normally used for sowing. However, at high seed moisture content (10-12%), survival and transmission are possible. In the tropics and subtropics, infected seed is likely to be the primary source of inoculum, particularly in disease-free areas. More research is needed to determine the degree to which *P. solanacearum* can be transmitted through seed of varying moisture contents.

Strains of *Pseudomonas solanacearum* affecting groundnut

Groundnut has been found to be attacked by isolates of biovars 1,3, and 4 of race 1 (Buddenhagen and Kelman 1964). Biovars 2 and 5 have not been reported as pathogenic to groundnut. Only few systematic studies have been made to determine the occurrence of biovars and strains, and their virulence to groundnut. Only biovars 3 and 4 have been reported to cause groundnut wilt in many countries in Asia and Africa (Hayward 1990, Mehan et al 1994), while biovar 1 caused groundnut wilt in the USA. Isolates of biovar 3 appear to be predominant in China, Indonesia, and Malaysia and, in general, more virulent to groundnut than isolates of biovar 4 (Machmud 1993). With the exception of some tomato isolates, in general, isolates from groundnut are reported to be more virulent on groundnut than isolates from other host crops.

Strains of *P. solanacearum* have been found to differ greatly in their virulence on groundnut Limited effort has been made to describe pathotypes of the bacterium pathogenic to groundnut in areas where bacterial wilt is a serious problem. Only one study in China demonstrated the existence of pathotypes, and reported seven pathotypes based on their pathogenicity/virulence on six indicator cultivars with different levels of wilt resistance (Tan et al. 1992). It is highly desirable that more systematic research is carried out on this aspect of the pathogen because of its direct relevance to breeding programs.

Disease management

Cultural control measures

The use of rotations as a means of reducing yield losses from *P. solanacearum*-induced wilt was first suggested by Smith (1896). Effective control of bacterial wilt by crop rotation has been extensively reported since the late 1910s (Garner et al 1917, Schwarz 1926, Smith 1941, Li 1958, Wang and Hou 1982, Wang et al. 1983, Machmud 1993). These reports indicate that several nonhost crops in rotation with groundnut or solanaceous host crops can significantly reduce the incidence and severity of bacterial wilt.

Rotation of groundnut with nonhost crops such as maize, rice, and sugarcane has proved to be an effective means of disease control. It is possible to achieve near-complete control by rotating groundnut with rice for 4-5 years (Wang et al 1983, He 1990). Groundnut-rice rotation systems are now widely adopted in irrigated areas of the southern provinces of China where the

disease is a constraint to production. Crop rotation with rice is feasible in irrigated areas or relatively high rainfall areas of South and Southeast Asia.

In Guangdong province of China, rotation of groundnut with sugarcane for 2-3 years has been found to significantly reduce bacterial wilt incidence (OCRI 1977). Crop rotation with wheat, soybean, and cotton is also effective in this area.

In drylands, rotation of groundnut with maize and sorghum for longer periods (4-5 years) is a useful way to contain the disease. In such areas, intercropping groundnut and maize can also be used to reduce wilt incidence. Rotating or intercropping groundnut with maize is feasible in many regions of Southeast Asia where the disease is serious.

Although experimental evidence indicates the value of crop rotation in greatly reducing the disease, the level of disease control achieved by rotation or fallowing in farmers' fields often varies. These variable results can be attributed to a failure to eliminate weed hosts during periods of rotations with nonhost crops or in fallows, since many weed hosts promote the bacterial survival. This survival can also be dependent on the ability of local strains to maintain a low but infective soil population of the wilt pathogen in the absence of a host. Since many weeds serve as alternative hosts of P. solanacearum, weed control is essential if the beneficial effects of crop rotation are to be fully realized.

Although rotating groundnut for shorter periods with immune crops has been effective in containing the disease, a gap of at least 3-4 years between successive crops of groundnut should prove more effective, especially in fields that are heavily infested with the pathogen.

Very little is known as to how these cropping systems affect the survival and populations of the wilt pathogen. Early researchers attributed the control obtained by rotations to 'starving out' the bacterium. However, it was later realized that factors other than the absence of a susceptible host were involved in the decline in wilt incidence in groundnut crops that followed a nonhost crop in rotation. Some nonhost crops (e.g., maize, rice, and soybean) have been reported to actually reduce the populations of the bacterium in the soil (Quimio and Chan 1979). The maize rhizosphere appears to promote the activity of some bacteria (e.g., P. cepacia) antagonistic to *P. solanacearum* (Elphinstone and Aley 1993). It is obvious that much remains to be known about the effects of different cropping systems and rotations on the survival of *P. solanacearum,* and about the microbiological changes in the soil that may influence its survival.

It is appreciated that long-term crop rotation is obviously not a practical solution for small farmers, since land is scarce and they prefer to grow more profitable crops.

An alternative is to apply complementary crop protection measures to suppress pathogen populations as much as possible/Fertilizer application and weed control are common practices that can be readily adopted in most farming systems.

Soil treatments

In the early phases of work on control of bacterial wilt diseases of many crops, many workers attempted to modify soil pH. Among the first to take this approach to control were Earle and Orr (1898), who observed that heavy applications of lime could sometimes reduce severity of tomato wilt. Much research was later carried out on the effects of sulfur and lime on bacterial wilt, but heavy sulfur and/or lime treatments were reported to give erratic results (Poole 1937, Kelman 1953). With few exceptions, researchers discontinued such studies, convinced that the approach was impractical The costs of changing the pH were prohibitive for small farmers in developing countries.

Some soil amendments containing urea and calcium oxide, together with organic and inorganic components, have proved useful in reducing bacterial wilt incidence (Chang and Hsu 1988, Hsu and Chang 1989). These substances probably stimulate soil microbial activity against the wilt pathogen. Such components as urea and calcium are likely to enhance host resistance to bacterial wilt (Prior and Beramis 1990), but more research is required to elucidate the mechanisms of bacterial wilt control following soil amendments. The role of nitrogen in bacterial wilt should also receive more attention.

In areas where groundnut is grown under irrigation in the dry season, it should be possible to reduce the level of the disease considerably by dry-season fallowing, since the bacterium is highly susceptible to desiccation. The effects of such fallows can be enhanced by cultivation to hasten soil drying and to reduce weed growth.

More research is needed on the effects of organic amendments in Oxisols, and of water management in Vertisols, on bacterial wilt.

Flooding

Flooding groundnut fields for 15-30 days before sowing has been reported to effectively reduce bacterial wilt incidence (Li et al. 1981, He 1990). In the Philippines, bacterial wilt does not appear to be a problem in flooded rice fields and river floodplains. There are conflicting reports about the influence of flooding on the survival of *P. solanacearum* (Senevirante 1976). In northern Vietnam, groundnut bacterial wilt is reported to be serious in many river floodplains. The type and fertility status of soil can influence the rate of decline of the bacterial population. The mechanism by which flooding reduces the disease, or controls it completely, in some environments merits critical investigation.

Biological control

Biological control of *P. solanacearum* has been extensively studied over the last three decades (Averre and Kelman 1964, Akiew 1985, McLaughlin and Sequeira 1988, He 1990, Trigalet and Trigalet-Demery 1990). The biological agents used to control bacterial wilt include several antagonistic rhizobacteria (*P. cepacia, P. fluorescens, P. glumae,* and *Bacillus sp*) and avirulent strains of

P. solanacearum (Trigalet et al. 1994). Although found promising under laboratory and greenhouse conditions, most of these reported biological control agents have not proved highly effective against *P. solanacearum* in the field. Biological control is based on the ability of the controlling agent to compete in the rhizosphere, to produce bacteriocins, or to induce a response in the host that favors growth of the biological control agent and/or inhibits the growth *P. solanacearum.*

Of considerable interest is the more recent work involving some genetically engineered *hrp* avirulent mutants of *P. solanacearum* (Trigalet and Trigalet-Demery 1990). Fluorescent pseudomonads have also been reported to suppress bacterial wilt; they can be useful biocontrol agents as they can improve plant growth by colonizing the rhizosphere and facilitating rapid uptake of nutrients. It would be useful to develop such biocontrol agents into effective commercial products for seed treatment. However, it will be a long time before approaches to biological control reach the stage of commercial application.

Host-plant resistance

The use of resistant cultivars is the most effective and practical way to control bacterial wilt, especially in countries where agriculture is not highly developed. Resistance in groundnut cultivars has been known since 1910 (van der Stock 1910). The first successful attempt to breed or select for resistance among the important crops attacked by P. solanacearum was made with groundnut in Indonesia. In the early 1920s, considerable breeding efforts by Dutch scientists in Java led to the development of a highly resistant cultivar, Schwarz 21, which was released for commercial cultivation in 1927 (Schwarz 1926, Schwarz and Hartley 1950). This cultivar has since been widely grown in Indonesia and was used in the early 1950s as a resistant parent to develop such improved cultivars as Banteng, Gajah, Kidang, and Macan. Since then, several other wilt-resistant cultivars, including Pelanduk, Tapir, and Tupai, have been bred (Anonymous 1983,1984).

In China also, much emphasis has been placed on breeding for wilt resistance since the early 1960s. Two active breeding programs at the Industrial Crops Research Institute (ICRI), Guangzhou, and the Oil Crops Research Institute (OCRI), Wuhan, have led to several wilt-resistant cultivars (Guiyou 28, El Hua 5, Lu Hua 3, and Zhonghua 2). These cultivars play an important role in disease control, and in increasing groundnut production, in heavily wilt-infested areas in the central and southern provinces (Liao et al. 1990, Mehan et al. 1993, Wang et al. 1990).

Resistance to wilt has been a high-priority objective throughout the history of crop improvement both in Indonesia and China. From extensive screening of several thousand germplasm and breeding lines in wilt-sick plots in Indonesia and China, many lines with varying levels of resistance have been identified (Machmud 1993, Sharma and Soekarno 1992, Duan et at 1993, Mehan et al. 1993, 1994). The lines/cultivars with high levels of resistance

include Gajah, Schwarz 21,Taishan Sanlirou, and Xiekangqing and many lines of Chinese 'dragon' type. Many lines/cultivars resistant to *P. solanacearum* have also been reported from Mauritius (Orian 1949), the Philippines (Natural et al 1988), South Africa (Sellschop 1947), Sri Lanka (Jayasena and Rajapaksa 1990), Uganda (Simbwa-Bunnya 1972, Busolo-Bulafu 1993), and the USA (Jenkins et al. 1966). Resistant genotypes have been bred or selected mostly in warm and humid areas of the lowland tropics, where disease pressure is the greatest (Sun et al, 1981, Mehan et al. 1986). This indicates a need for extensive evaluation of landraces from low-latitude areas for sources of resistance to *P. solanacearum*. It appears that the expression of resistance to bacterial wilt is a function of adaptation to high temperature.

Only a few lines have been used extensively as resistance donors in wilt-resistance breeding programs. For instance, only Schwarz 21 and its derivatives have been used as resistant parents in Indonesia, while Xiekangqing and Taishan Sanlirou have been used extensively in China. This points to the narrow genetic base of the available wilt-resistant cultivars. Broadening the genetic base for resistance and adaptation to the environment, particularly heat tolerance, is obviously important in breeding for resistance to bacterial wilt.

Recently, improved wilt-resistant Chinese cultivars have been hybridized with other accessions, with particular emphasis on incorporating resistance to rust and leaf spot diseases (Liao et al. 1990, Mehan et al. 1993). Some Peruvian accessions possess combined resistance to bacterial wilt, rust, and late leaf spot diseases (Mehan et al 1986, Yeh 1990). Some high-yielding breeding lines (e.g., Yue You 202 and Yue You 266) resistant to both rust and bacterial wilt have been developed in China (Liao et al. 1990). Such recently bred wilt- and rust-resistant lines as Zhonghua 112 and Zhonghua 212 have consistently shown slightly higher yield potential than the wilt-resistant cultivar Zhonghua 2. They are now in multilocational trials to assess their wilt resistance and yield, and are expected to be released in the near future. Most wilt-resistant lines are sensitive to drought, while drought-tolerant lines are susceptible to wilt (Liao et aL 1990, Hong et al. 1994). It is noteworthy that the wilt-resistant line Zhonghua 212 is both drought-tolerant and adaptable to low soil fertility.

Since wilt resistance is controlled by additive gene effects, it is desirable to use highly resistant parents to obtain high levels of resistance in hybrid progenies. However, multi-directional crossing should also prove an effective way to use moderately resistant genotypes with desirable traits (Liao et al. 1990).

In recent years, differential disease reactions of some lines/cultivars have been reported from different locations. For instance, the lines PI 414332 and NC Ac 17130, found resistant in China (Yeh 1990), were susceptible in Indonesia (Machmud and Middleton 1990), while some other cultivars (Banteng, Gajah, Kidang, and Macan) reported resistant in Indonesia showed only moderate resistance in China (Yeh 1990). These differential reactions can be attributed to changes in the pathogenicity/virulence of *P. solanacearum*

strains and/or to environmental variations. In the light of some recent reports of the existence of highly virulent strains or pathotypes (Tan et al. 1992, Tan Yujun, personal communication 1994), critical genetic evaluation of wilt resistance is essential to elucidate host-pathogen interactions. It would be useful to study the stability of wilt resistance at various disease hot-spots and in areas where different strains or pathotypes are suspected. It is also important to test selected resistant lines, including differentials, for their reaction to several strains or pathotypes of the wilt pathogen from different regions of the world.

Wilt resistance has been expressed in terms of the survival percentage of plants. Most of the reported sources of resistance have only field resistance to bacterial wilt; they show substantial wilt incidence in greenhouse inoculation tests. Very few lines have shown resistance in both field and greenhouse tests (Machmud and Middleton 1990, Mehan et al. 1993). Field screening under high disease pressure is the best way to identify sources of resistance. In greenhouse tests, it is important to use inoculation techniques that simulate natural field conditions, especially in terms of the route of infection.

Genetics and mechanisms of resistance

The understanding of the inheritance of wilt resistance in groundnut has progressed slowly and views have only recently converged. Limited inheritance studies (Liao et al. 1986) with Spanish groundnuts indicate partial dominance of resistance and the involvement of three pairs of major genes and some minor genes. In another report, Wang et al. (1985) indicated that resistance is a recessive trait. There are also conflicting reports on inheritance of resistance to bacterial wilt in tomato, where resistance has been found to be dominant in some parents and recessive in others (Scott et al. 1988, Messiaen et al. 1991). Recent preliminary results indicate that the resistance mechanisms involved and their inheritance are different in different botanical types (Liao et al. 1994); cytoplasmic factors for resistance have been observed in some of the Chinese dragon types (e.g., Feilongxiang), while only nuclear resistance factors have been found in Spanish types. The resistance levels in F_1 hybrids in four crosses with Feilongxiang as the female parent were markedly higher than those in their corresponding reciprocal crosses. This phenomenon has been observed under both artificial inoculation and natural disease conditions.

Since the 1960s, research emphasis has largely been on mechanisms involved in compatible host-pathogen interactions that result in wilt (Buddenhagen and Kelman 1964, Petrolini et al. 1986). The resistance mechanisms that result in absence of or reduction in bacterial wilt have not been fully investigated.

Recent research at the Rothamsted Experimental Station, UK, has indicated differential colonization of host tissues in wilt-resistant and wilt-susceptible cultivars/lines (John Elphinstone, personal communication 1993). Some accessions of Schwarz 21 and Kidang have shown limited or no colonization of the stem by the wilt pathogen. Selections for resistance to both expression of

symptoms and colonization by the pathogen in the stem should prove useful in developing stable and durable resistance. Some other genotypes (e.g., ICG 1703 and ICG 1704) resistant to rust and late leaf spot also show high levels of wilt resistance and pathogen colonization. Similar results of differential colonization of the stem in resistant and susceptible cultivars have been reported for tomato (Prior et al. 1994). However, some resistant non-wilted cultivars show significant bacterial colonization of the stem. Research so far has been focused on detection of the pathogen at specific sites but the rates of plant response to infection in susceptible and resistant cultivars have not been compared, and the time-space relationship in the invasion of stems remains to be investigated.

Resistance appears to manifest itself mainly through physiological defense mechanism of the host to disease development.

Conclusions and future research needs

Although bacterial wilt has been extensively studied worldwide for several decades, efficient and complete control measures are not available. Appreciable progress has been made in the development and release of resistant cultivars, but varietal resistance has not solved the problem completely. It may be quite some time before highly resistant and high-yielding cultivars, endowed with other desirable characteristics, are available to farmers in regions where the disease is a major constraint to crop production. Extension, and supplying the seed of these newly developed resistant cultivars to farmers, should be given high priority. To increase the effectiveness of research into genetic resistance, it is imperative to assemble (through collection and exchange) various germplasm accessions/varieties with putative resistance in countries where the disease is severe. It is important to broaden the genetic base of wilt resistance by using different parents with high levels of stable resistance. As different genetic backgrounds are discernible in some Spanish and Chinese dragon-type wilt-resistant genotypes, it would also be useful to integrate both nuclear and cytoplasmic resistance genes into high-yielding cultivars for stable and durable wilt-resistance. Sources of resistance from different botanical accessions will be useful in increasing the diversity and stability of resistance. Research efforts are also needed to characterize resistance genes in groundnut, using molecular genetic methods.

High priority should be given to establishing an international groundnut bacterial wilt nursery to determine the stability of wilt resistance through multilocational testing. Such a nursery was earlier proposed at the Second Meeting of the Groundnut Bacterial Wilt Working Group in Taiwan (Mehan and Hayward 1993), but has not been established so far due to nonavailability of various entries, especially those from China. Sincere efforts are necessary to foster the exchange of wilt-resistant germplasm and breeding lines. Priority should also be given to testing selected resistant lines, including possible differentials, for their reaction to various groundnut-adapted strains or

pathotypes of the wilt pathogen from different regions of the world. This work should be done under controlled laboratory conditions, preferably in a country where groundnut is not grown. Concerted efforts are also needed to elucidate the mechanisms and components of wilt resistance. Such strategic research is obviously imperative to utilize the available resistant germplasm most effectively in breeding programs. To increase the effectiveness of research into genetic resistance, it is imperative to assemble various groundnut germplasm/varieties with putative resistance in countries where the disease is severe. Considerable progress has been made in elucidating the cropping systems/cultural practices that significantly reduce wilt incidence and severity, but more information is needed on how they affect the survival and perpetuation of the wilt pathogen. Crop rotations with nonhost crops are often not acceptable to many farmers, especially if such rotations are prolonged, and this highlights the need for complementary control measures. Although some cultural practices, namely crop sanitation, the use of soil amendments, and water- and crop-residues-management, have been shown to be helpful in reducing disease levels, on-farm research is needed to demonstrate their full impact. Emphasis should be placed on an integrated approach to manage the disease, involving wilt-resistant cultivars, rotation with nonhost crops, and crop sanitation. More research is needed to devise appropriate packages of these strategies for local disease-endemic areas. It is likely that strategies involving biological control will, in future, need local adaptations. This prospect will offer a new challenge.

If effective means of control are to be found, more ecological studies are necessary, in different agroecosystems where bacterial wilt is serious, to establish the ecology of the disease caused by a particular strain in a specific environment. Little is known of the strain(s) affecting groundnuts, or the ability of these strains to survive in soil or in association with the roots of host and nonhost crops. For such studies, it would be useful to employ highly specific monoclonal antisera to permit the identification of individual strains/pathotypes of the bacterium in soil, in the rhizosphere, and in root and seed tissues. This field should be an exciting one, since it calls for innovative research on the ecology of this important disease in the tropics and subtropics. Information from research on these lines should provide a sound ecological basis on which to devise disease control measures, irrespective of whether they are based on the development of resistant cultivars and/or agronomic management practices.

In the past, research efforts have been scattered, relatively uncoordinated, and seldom given top priority. Recently, many scientists, including pathologists, breeders, and geneticists, have accorded a high research priority to this disease. The increasing collaboration in research on bacterial wilt through the Groundnut Bacterial Wilt Working Group should be able to provide solutions to specific problems, and to contribute to improved disease control.

References

Alvarez, A. M., Berestecky, J., Stiles, J. I., Ferreira, S.A., and Benedict, A. A. 1993. Serological and molecular approaches to identification of *Pseudomonas solanacearum*: strains from Heliconia. Pages 62-69 *in* Bacterial wilt: proceedings of an International Conference, 28-31 Oct 1992, Kaohsiung, Taiwan (Hartman, G.L. and Hayward, A.C., eds), ACIAR Proceedings no, 45. Canberra, Australia: Australian Centre for International Agricultural Research.

Anonymous. 1983. Varietas kacang tanah Rusa dan Anoa. Warta Penelitian dan Pengembangan Pertanian. Departemen Pertanian Republik Indonesia 5: 11.

Anonymous. 1984. Varietas unggul baru kacang tanah dan kacang hijau. Warta Penelitian dan Pengembangan Pertanian. Departemen Pertanian Republik Indonesia 6: 8.

Averre, G W., and Kelman, A. 1964. Severity of bacterial wilt as influenced by ratio of virulent to avirulent cells of Pseudomonas solanacearum in inoculum. Phytopathology 54: 779-783.

Bolhuis, G. G. 1955. La culture de l'arachide en Indonesia. Oleagineux 10:157-160.

Buddenhagen, I. W., and Kelman, A. 1964. Biological and physiological aspects of bacterial wilt caused by *Pseudomonas solanacearum.* Annual Review of Phytopathology 2: 203-230.

Busolo-Bulafu, C. M. 1993. Breeding for resistance to bacterial wilt of groundnuts in Uganda. Pages 228-229 *in* Bacterial wilt: proceedings of an International Conference, Kaohsiung, Taiwan, 28-31 Oct 1992. (Hartman, G.L. and Hayward, A.C., eds.). ACIAR Proceedings no. 45. Canberra, Australia: Australian Centre for International Agricultural Research.

Chang, M. L., and Hsu, S. T. 1988. Suppression of bacterial wilt of tomato by soil amendments. Plant Protection Bulletin, Taiwan 30: 349-359.

Duan, N, X., Tan, Y. J., Jiang, H., and Hu, D. B. 1993. Screening groundnut germplasm for resistance to bacterial wilt. Oil Crops of China 1:22-25.

Elphinstone, J. G., and Aley, P. 1993. Integrated control of bacterial wilt of potato in the warm tropics of Peru. Pages 276-283 *in* Bacterial wilt: proceedings of an International Conference, 28-31 Oct 1992, Kaohsiung, Taiwan (Hartman, G.L. and Hayward, A.C., eds.). ACIAR Proceedings no. 45. Canberra, Australia: Australian Centre for International Agricultural Research.

Fulton, H. R., and Winston, J. R. 1914. A disease of peanut plants caused by *Bacterium solanacearum.* Phytopathology 3; 72-73.

Gamer, W. W., Wolf, E A., and Moss, E.G. 1917. The control of tobacco wilt in the flue-cured district. U.S. Department of Agriculture. Bureau of Plant Industry Bulletin 562. 20 pp.

Graham, J., Jones, D. A., and Lloyd, A. B. 1979. Survival of *Pseudomonas solanacearum* race 3 in plant debris and in latently infected potato tubers. Phytopathology 69:1100-1103.

Granada, G. A., and Sequeira, L. 1983. Survival of *Pseudomonas solanacearum* in soil, rhizosphere and plant roots. Canadian Journal of Microbiology 29: 433-440.

Hamidah, S., and Lum, K. Y. 1993. Bacterial wilt of groundnut in Malaysia. Pages 6-10 in Groundnut bacterial wilt: proceedings of the Second Working Group Meeting, 2 Nov 1992, Asian Vegetable Research and Development Center, Tainan, Taiwan (Mehan, V. K. and Hayward, A. C., eds.). Patancheru 502 324, Andhra Pradesh, India: International Crops Research Institute for the Semi-Arid Tropics.

Hayward, A. C. 1990. Diagnosis, distribution and status of groundnut bacterial wilt. Pages 12-17 in Bacterial wilt of groundnut: proceedings of an ACIAR/ICRISAT collaborative research planning meeting, 18-19 Mar 1990, Genting Highlands, Malaysia (Middleton, K. J. and Hayward, A. C, eds.). ACIAR Proceedings no. 31. Canberra, Australia: Australian Centre for International Agricultural Research.

Hayward, A. G, and Moffett, M. L. 1978. Leaf spot on capsicum and tomato caused by *Pseudomonas solanacearum.* Plant Disease Reporter 62: 75-78.

He, L. Y. 1990. Control of bacterial wilt of groundnut in China with emphasis on cultural and biological methods, Pages 22-25 in Bacterial wilt of groundnut: proceedings of an ACIAR/ICRISAT collaborative research planning meeting, 18-19 Mar 1990, Genting Highlands, Malaysia (Middleton, K. J. and Hayward, A. G, eds.). ACIAR Proceedings no. 31. Canberra, Australia: Australian Centre for International Agricultural Research.

Hong, N. X., and Mehan, V. K. 1993. Research on bacterial wilt of groundnut in Vietnam. Pages 219-220 in Bacterial wilt: proceedings of an International Conference, 28-31 Oct 1992, Kaohsiung, Taiwan (Hartman, G. L. and Hayward, A. G, eds.). ACIAR Proceedings no. 45. Canberra, Australia: Australian Centre for International Agricultural Research.

Hong, N. X., Mehan, V. K-, Ly, N. X, and Vinh, M. T.1994. Status of groundnut bacterial wilt research in Vietnam. Pages 135-141 in Bacterial wilt of groundnut in Asia: proceedings of the Third Working Group meeting, 4-5 Jul 1994, Wuhan, China (Mehan, V. K. and McDonald, D., eds.). Patancheru, 502 324, Andhra Pradesh, India: International Crops Research Institute for the Semi-Arid Tropics,

Hsu, S. X, and Chang, J. R. 1989. Effect of soil amendments on survival of *Pseudomonas solanacearum.* Plant Protection Bulletin, Taiwan 31:21-33.

Jayasena, K. W., and Rajapaksa, R. H. S. 1990. Present status of groundnut bacterial wilt in Sri Lanka. Pages 52-53 *in* Bacterial wilt of groundnut:

proceedings of an ACIAR/ICRISAT collaborative research planning meeting, 18-19 Mar 1990, Genting Highlands, Malaysia (Middleton, K. J. and Hayward, A G , eds.). ACIAR Proceedings no. 31. Canberra, Australia: Australian Centre for International Agricultural Research.

Jenkins, S. F., Jr., Hammons, R. O., and Dukes, P. D. 1966. Disease reaction and symptom expression of seventeen peanut cultivars to bacterial wilt. Plant Disease Reporter 50:520-523.

Kelman, A. 1953. The bacterial wilt caused by *Pseudomonas solanacearum:* a literature review and bibliography. North Carolina Agricultural Experiment Station, Technical Bulletin No. 99.194 pp.

Li, T. J. 1958. Occurrence of bacterial wilt. Plant Disease Knowledge 2:174-176.

Li, W. R., Chen, C. R., and Xu, Z. Y. 1981. Studies on bacterial wilt of peanut. II. Investigation of disease epidemiology in east Hubei. Oil Crops of China 1:43-47.

Li, W. R., and Tan, Y, J. 1984. Inoculation techniques for groundnut bacterial wilt. Oil Crops of China 2:77-81.

Liao, B. S., Duan, N. X., Wang, Y. Y., Sun, D. R., and Mehan, V. K. 1994. Host-plant resistance to groundnut bacterial wilt: genetic diversity and enhancement. Pages 91-96 *in* Bacterial wilt of groundnut in Asia: proceedings of the Third Working Group meeting, 4-5 Jul 1994, Wuhan, China (Mehan, V. K. and McDonald, D., eds.). Patancheru 502 324, Andhra Pradesh, India: International Crops Research Institute for the Semi-Arid Tropics.

Liao, B. S., Li, W. R., and Sun, D. R. 1986. A study on inheritance of resistance to *Pseudomonas solanacearum* E.F. Smith in *Arachis hypogaea* L. Oil Crops of China 3:1-8.

Liao, B. S., Wang, Y. Y., Xia, X. M., Tang, G. Y., Tan, Y. J., and Sun, D. R. 1990. Genetic and breeding aspects of resistance to bacterial wilt in groundnut. Pages 39-43 *in* Bacterial wilt of groundnut: proceedings of an ACIAR/ICRISAT collaborative research planning meeting, 18-19 Mar 1990, Genting Highlands, Malaysia (Middleton, K. J. and Hayward, A. C, eds.). ACIAR Proceedings no. 31. Canberra, Australia: Australian Centre for International Agricultural Research.

Lum, K. Y., and Hamidah, S. 1994. Status of groundnut bacterial wilt research in Malaysia. Pages 121-124 in Bacterial wilt of groundnut in Asia: proceedings of the Third Working Group meeting, 4-5 Jul 1994, Wuhan, China (Mehan, V. K. and McDonald, D., eds.) Patancheru 502 324, Andhra Pradesh, India: International Crops Research Institute for the Semi-Arid Tropics.

Ma, Q. C., and Gao, Y. C. 1956. Wilt disease of peanut. Bulletin of Fujian Academy of Agricultural Science 2:89-98.

Machmud, M. 1986. Bacterial wilt in Indonesia. Pages 30-34 in Bacterial wilt disease in Asia and the South Pacific, 8-10 Oct 1985, Los Banos, Philippines

(Persley, G.J., ed.). ACIAR Proceedings no. 13. Canberra, Australia: Australian Centre for International Agricultural Research.

Machmud, M. 1993. Present status of groundnut bacterial wilt research in Indonesia. Pages 15-24 *in* Bacterial wilt of groundnut: proceedings of the Second Working Group Meeting, 2 Nov 1992, Tainan, Taiwan (Mehan, V.K. and Hayward, A.C., eds.). Patancheru 502 324, Andhra Pradesh, India: International Crops Research Institute for the Semi-Arid Tropics.

Machmud, M., and Hayward, A. G 1992. Genetic and cultural control of peanut bacterial wilt. Pages 19-25 *in* Peanut improvement—a case study in Indonesia: proceedings of an ACIAR/AARD/QDPI Collaborative Review Meeting, 19-23 Aug 1991, Malang, Indonesia (Wright, G. C. and Middleton, K. J., eds.). ACIAR Proceedings no. 40. Canberra, Australia: Australian Centre for International Agricultural Research.

Machmud, M., and Middleton, K. J. 1990a. Seed infection and transmission of *Pseudomonas solanacearum* on groundnut. Page 57 *in* Bacterial wilt of groundnut: proceedings of an ACIAR/ICRISAT collaborative research planning meeting, 18-19 Mar 1990, Genting Highlands, Malaysia (Middleton, K. J. and Hayward, A. C, eds.). ACIAR Proceedings no. 31. Canberra, Australia: Australian Centre for International Agricultural Research.

Machmud, M., and Middleton, K. J. 1990b. Progress of research on bacterial wilt of peanut in Indonesia: 1989-1990. Presented at the Annual Review of the Collaborative Research between ACIAR and CRIFC on Peanut Improvement in Indonesia, 26-28 Nov 1990, Malang, Indonesia.

McLaughlin, R. J., and Sequeira, L. 1988. Evaluation of an avirulent strain of *Pseudomonas solanacearum* for biological control of bacterial wilt of potato. American Potato Journal 65: 255-268.

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 502 324, Andhra Pradesh, India: International Crops Research Institute for the Semi-Arid Tropics. 32 pp.

Mehan, V. K., Liao, B. S., Tan, Y. J., Robinson-Smith, A., McDonald, D., and Hayward, A. C. 1994. Bacterial wilt of groundnut. Information Bulletin no. 35. Patancheru 502 324, Andhra Pradesh, India: International Crops Research Institute for the Semi-Arid Tropics. 28 pp.

Mehan, V. K., McDonald, D., and Subrahmanyam, P. 1986. Bacterial wilt of groundnut: control with emphasis on host plant resistance. Pages 112-119 in Bacterial wilt disease in Asia and the South Pacific, 8-10 Oct 1985, Los Banos, Philippines (Persley, G. J., ed.). ACIAR Proceedings no. 13. Canberra, Australia: Australian Centre for International Agricultural Research.

Mehan, V. K., Nigam, S. N., and McDonald, D. 1993. Management of bacterial wilt of groundnut with genetic resistance and cultural practices. Pages 211-218

in Bacterial wilt: proceedings of an International Conference, 28-31 Oct 1992, Kaohsiung, Taiwan (Hartman, G. L. and Hayward, A. C, eds.). ACIAR Proceedings no. 45. Canberra, Australia: Australian Centre for International Agricultural Research.

Messiaen, C. M., Blancard, D., Rouxel, F., and Lafon, R. 1991. Les maladies des Plantes Maraicheres, 3rd edn., Paris, France: Institut National de la Recherche Agronomique.

Miller, J. H., and Harvey, H. W. 1932. Peanut wilt in Georgia. Phytopathology 22:371-383.

Moffett, M. L., Wood, B. A., and Hayward, A. C.1981. Seed and soil: sources of inoculum for the colonization of the foliage of solanaceous hosts by *Pseudomonas solanacearum.* Annals of Applied Biology 98: 403-411.

Natural, M. P., Valencia, L. D., and Pua, A. R. 1988. Peanut—a natural host of *Pseudomonas solanacearum* in the Philippines. Bacterial Wilt Newsletter 3: 3.

OCRI (Oil Crops Research Institute, Chinese Academy of Agricultural Sciences). 1976. An evaluation of peanut sources of resistance to bacterial wilt. Science and Technology of Oil Crops 1: 29-35.

Opio, A. E., and Busolo-Bulafu, C. M. 1990. Status of bacterial wilt on groundnut in Uganda. Pages 54-55 *in* Bacterial wilt of groundnut: proceedings of an ACIAR/ICRISAT collaborative research planning meeting, 18-19 Mar 1990, Genting Highlands, Malaysia (Middleton, K. J. and Hayward, A. C, eds.). ACIAR Proceedings No. 31. Canberra, Australia: Australian Centre for International Agricultural Research.

Orian, G. 1949. Division of Plant Pathology, Department of Agriculture, Mauritius, Annual Report 1948, 69-74.

Palm, B. T. 1922. Aanteekeningen over slijmziekte in *Arachis hypogaea* (Katjang tanah). (In Nl. Summary in En.) Instituut voor Plantenziekten (Dutch East Indies), Mededeeling 52.

Petrolini, B., Quaroni, S., and Saracchi, M. 1986. Scanning electron microscopy investigations on the relationship between bacteria and plant tissues. II. Investigations on the initial process of *Pseudomonas solanacearum* pathogenesis. Rivista di Patologia Vegetale SIV 22:100-115.

Poole, R. F. 1937. Further studies on the effects of soil treatment with chemicals for the control of *Bacterium solanacearum.* North Carolina Agricultural Experiment Station Annual Report 59 (1936); 34-35.

Prior, P., and Beramis, M. 1990. Induction de la resistance au fletrissement bacterien du a *Pseudomonas solanacearum* E.F.S. chez un cultivar de tomate repute sensible. Agronomie 10:391-401.

Prior, P., Grimault, V., and Schmit, J. 1994. Resistance to bacterial wilt (*Pseudomonas solanacearum*) in tomato: present status and prospects. Pages

209-223 in Bacterial wilt: The disease and its causative agent, *Pseudomonas solanacearum* (Hayward, A. C and Hartman, G L., eds.). Wallingford, UK: CAB International.

Quimio, A. J., and Chan, H. H. 1979a. Survival of *Pseudomonas solanacearum* under diverse agroclimates in India. Indian Journal of Agricultural Sciences 49: 735-738.

Quimio, A. J., and Chan, H. H. 1979b. Survival of *Pseudomonas solanacearum* E.F. Smith in the rhizosphere of some weeds and economic plant species. Philippine Phytopathology 15:108-121.

Robinson, A. 1993. Serological detection of *Pseudomonas solanacearum* by ELISA. Pages 54-61 *in* Bacterial wilt: proceedings of an International Conference, 28-31 Oct 1992, Kaohsiung, Taiwan (Hartman, G. L. and Hayward, A. C, eds.). ACIAR Proceedings no. 45 Canberra, Australia: Australian Centre for International Agricultural Research.

Schwarz, M. B. 1926. De invloed van de voorvrucht op het optreden van slijmziekte (*Bacterium solanacearum*) in *Arachis hypogaea* en eenige anderegewassen. Instituut voor Plantenziekten (Dutch East Indies), Mededeeling 71: 37 pp. (English summary).

Schwarz, M. B., and Hartley, C. 1950. De selectie van Schwarz 21, een tegen slijmziekte resistente lijn van *Arachis hypogaea.* Landbouw (Bogor) 22: 223-244.

Sellschop, J. 1947. Groundnuts. Farming in South Africa 22: 705-712.

Sharma, D., and Soekarno, B. 1992. Identification of additional groundnut sources resistant to bacterial wilt under field conditions in East Java, Indonesia. International Arachis Newsletter 11: 23-25.

Simbwa-Bunnya, M. 1972. Resistance of groundnut varieties to bacterial wilt (*Pseudomonas solanacearum*) in Uganda. East African Agricultural and Forestry Journal 37: 341-343.

Smith, E. F. 1896. A bacterial disease of the tomato, eggplant, and Irish potato (*Bacillus solanacearum* nov. sp.). US Department of Agriculture, Division of Vegetable Physiology and Pathology, Bulletin 12:1-28.

Smith, T. E. 1941. Critical tests with crop rotations for control of Granville wilt of tobacco. Phytopathology 31: 20-21.

Smith, T. E. 1944. Control of bacterial wilt of tobacco as influenced by crop rotation and chemical treatment of the soil. US Department of Agriculture Circular 692.16 pp.

Sun, D. R., Chen, C. R., and Wang, Y. R. 1981. Resistance evaluation of bacterial wilt (*Pseudomonas solanacearum* E.F. Smith) of peanut (*Arachis hypogaea L.*) in the People's Republic of China. Proceedings of the American Peanut Research and Education Society 13:21-28.

Tan, Y. J., Duan, N. X., Liao, B. S., Li, W. R., and Xu, Z. Y. 1992. Research on groundnut bacterial wilt. Page 486 in Groundnut - A global perspective: proceedings of an International Workshop, 25-29 Nov 1991, ICRISAT Center, India/ (Nigam, S. N., ed.). Patancheru 502 324, Andhra Pradesh, India: International Crops Research Institute for the Semi-Arid Tropics.

Tan, Y. J., and Liao, B. S. 1990. General aspects of groundnut bacterial wilt in China. Pages 44-47 in Bacterial wilt of groundnut: proceedings of an ACIAR/ICRISAT collaborative research planning meeting, 18-19 Mar 1990, Genting Highlands, Malaysia (Middleton, K. J. and Hayward, A. C, eds.). ACIAR Proceedings No. 31. Canberra, Australia: Australian Centre for International Agricultural Research.

Trigalet, A., Frey, P., and Trigalet-Demery, D. 1994. Biological control of bacterial wilt caused by *Pseudomonas solanacearum:* State of the art and understanding. Pages 225-233 in Bacterial wilt: The disease and its causative agent, *Pseudomonas solanacearum* (Hayward, A. C and Hartman, G. L., eds.). Wallingford, UK: CAB International.

Trigalet, A., and Trigalet-Demery, D. 1990. Use of avirulent mutants of *Pseudomonas solanacearum* for the biological control of bacterial wilt of tomato plants. Physiological and Molecular Plant Pathology 36: 27-38.

van Breda de Haan, J. 1906. Rapport over ziekte in den aanplant van *Arachis hypogaea* (Katjang holle) in de Afdeelingen Koeningen en Cheribon der Residentie Cheribon. Teysmannia 17: 52-63.

van der Stok, J. E. 1910. Onderzoeking omtrent rijst en tweede gewasen. Departement van Landbouw, Nijverheid en Handel (Dutch East Indies), Mededeeling 12:176-221.

Wang, J. S., and Hou, X. Y. 1982. A study on control of bacterial wilt of peanut. Agricultural Science and Technology in Lingyi 1:1-11.

Wang, J. S., Hou, X. Y., and Hu, B. J. 1983. Studies on the control of bacterial wilt of peanut. Acta Phytophylactica Sinica 10: 79-84.

Wang, Y. Y., Wang, G. H., and Xia, X. M. 1985. A preliminary study on inheritance of resistance to bacterial wilt in peanut. Oil Crops of China 4:15-17.

Yeh, W. L. 1990. A review of bacterial wilt on groundnut in Guangdong Province, People's Republic of China. Pages 48-51 *in* Bacterial wilt of groundnut: proceedings of an ACIAR/ICRISAT collaborative research planning meeting, 18-19 Mar 1990, Genting Highlands, Malaysia (Middleton, K. J. and Hayward, A. C., eds.). ACIAR Proceedings no. 31. Canberra, Australia: Australian Centre for International Agricultural Research.

Zhang, Y. X., Hua, J, Y., and He, L. Y. 1993. Effect of infected groundnut seeds on transmission of *Pseudomonas solanacearum*. ACIAR Bacterial Wilt Newsletter 9: 9-10.

Discussion

J G Ryan You noted that rice, sorghum, etc. are not infected with *Pseudomonas solanacearum*. Is it feasible to explore the genetic sources of this resistance in these plant species and transfer the genes to Arachis spp?

V K Mehan It may be possible to transfer resistance genes from nonhost crops to the cultivated groundnut through transformation techniques but such genes need to be located and characterized first, before they can be exploited. However, a high degree of resistance is already available in Arachis spp and this resistance is known to be simply inherited. Our research emphasis at this stage should be on transfer of such resistance from Arachis spp through conventional breeding methods.

Y L Nene You mentioned five biovars of the pathogen and then showed us a slide of seven pathotypes. Could you explain the relationship between the biovars and the pathotypes?

V K Mehan Of the five biovars of *Pseudomonas solanacearum,* only biovars 1,3, and 4 are reported to infect groundnut. The seven pathotypes reported in China belong to biovars 3 and 4. The biovars are differentiated based on the utilization/oxidation of sugars, while the pathotypes are differentiated on the basis of their pathogenicity/virulence on certain groundnut genotypes.

C L L Gowda If seed transmission is not prevalent in well-dried seed (<9% moisture), what is the purpose of continuing research on seed transmission? Why worry about the quarantine implications when usually only well-dried seed is transported?

V K Mehan This is important in humid areas where final seed moisture can often be around 10%. It can also have quarantine implications within a country. It will be worthwhile to investigate if some strains of the wilt pathogen could be transmitted through seed with moisture contents lower than 9%.

Y L Nene As a pathologist who has not worked on this disease, I find it confusing to note that pathologists working on the bacterial wilt of groundnut are using the terms 'race', 'biovar', and 'pathotypes'. Is there some way of removing this confusion?

A C Hayward Races of the wilt pathogen are generally defined based on different host species. Races differ in their host ranges and geographic distribution. Using a classical bacteriological approach, *P. solanacearum* has been divided into five biovars based on their ability to oxidize different sugars and on other bacteriological reactions. Though the race classification scheme may be imperfect and the scope of biovar determinations limited, these two systems are useful, complement each other, and have gained acceptance. There may be some confusion regarding the usage of such terms, but researchers engaged in bacterial wilt research do recognize these terms in the right

perspective. Race 1 has a wide host range, and is distributed throughout the lowlands of the tropics and subtropics. It is associated with groundnut bacterial wilt, and it includes biovars 1, 3, and 4; the latter two biovars are prevalent in Asia. The concept of race for the bacterial pathogen is different from that used for fungal pathogens.

S Seal Researchers are familiar with such terms, and are not confused with them. At present, there are no other criteria to replace these terms. It is difficult to suggest alternatives.

Host-plant resistance and cultural control

Host-plant resistance to groundnut bacterial wilt: genetic diversity and enhancement

B S Liao[1], N X Duan[1], Y Y Wang[1], D R Sun[1], and V K Mehan[2]

Abstract

Worldwide, about 90 groundnut genotypes have been reported resistant to bacterial wilt caused by Pseudomonas solanacearum. Most of these resistant genotypes originated in China and Indonesia. While resistance has been found in materials of different botanical types, most of the resistant genotypes belong to the 'Chinese dragon' type. These were identified from heavily wilt-infested locations in southern China. Research indicates that resistance mechanisms and inheritance may be different in different botanical types. Cytoplasmic factors for resistance have been observed in some Chinese dragon types while only nuclear resistance factors have been found in other types. Different components of resistance have been recognized. Possible resistance mechanisms include morphological/anatomical and biochemical features. Evaluation of genetic resistance against different strains of the pathogen is required to incorporate different resistance mechanisms. Resistance levels and stability may be enhanced by integrating different genes or loci through conventional and mutation breeding.

In many regions where groundnut bacterial wilt reaches epidemic proportions, rust disease caused by Puccinia arachidis commonly occurs and causes significant losses in groundnut yields. It is important to combine resistances into groundnut varieties with desirable agronomic traits.

迄今世界上发现了90份抗青枯病(*Pseudomonas solanacearum*)花生资源,它们主要来自中国和印度尼西亚.不同植物学类型中均已发现抗性材料,但中国龙生型花生占多数.这些龙生型抗源主要来自南方重病区。研究表明,不同植物学类型在抗性遗传和抗性机制方面可能存在差异.龙生型花生抗性中存在细胞质作用,而在其它类型中仅发现了细胞核基因的作用。抗性存在不同的成分,抗性机制可能与形态、组织和生化特性有关。为综合不同的抗性机制,需要针对不同的病原菌系进行抗性的遗传评价。可以通过常规杂交及诱变的方法,结合利用不同的抗性基因或等位点来改良抗性的水平和稳定性。由于在许多青枯病流行的地方,花生锈病(*Puccinia arachidis*)也严重发生,因此需要培育兼抗高产的花生品种。

1. Oil Crops Research Institute, Chinese Academy of Agricultural Sciences, Wuhan 430062, China.
2. ICRISAT Asia Center, Patancheru 502 324, Andhra Pradesh, India.
ICRISAT Conference Paper no. CP 907.

Liao, B. S., Duan, N.X., Wang, Y. Y., Sun, D. R., and Mehan, V. K. 1994. Host-plant resistance to groundnut bacterial wilt: genetic diversity and enhancement. Pages 91-96 in Groundnut bacterial wilt in Asia: proceedings of the Third Working Group meeting, 4-5 Jul 1994, Oil Crops Research Institute, Wuhan, China (Mehan, V. K. and McDonald, D., eds.). Patancheru 502 324, Andhra Pradesh, India: International Crops Research Institute for the Semi-Arid Tropics.

Introduction

Bacterial wilt, caused by *Pseudomonas solanacearum* (Smith) Smith, is a serious constraint to groundnut production in several Asian countries. As chemical control of this disease is not feasible, and rotation with nonhost crops might also be limited by some factors, growing wilt-resistant groundnut varieties has been the approach of choice in countries where bacterial wilt is serious. Cultural control measures also continue to be important, since no genotype has been found to be completely resistant. Over the past several decades, extensive research efforts have been made to develop resistant varieties in Indonesia and China, and several resistant cultivars have been bred and widely used. However, many aspects of the available resistant cultivars including the degree and stability of resistance, yield, quality, and resistance to other biotic constraints need to be further improved. This paper reviews the research on genetic diversity of groundnut as regards the resistance to bacterial wilt and its enhancement.

Status of resistant sources

Worldwide, some 90 germplasm accessions and improved cultivars of groundnut have been reported resistant to bacterial wilt. Most of these resistant genotypes are from China and Indonesia (Mehan et al. 1994). Resistant lines have also originated in or been collected from Argentina, Brazil, India, Israel, Peru, Uganda, and USA (Machmud 1993). Most of the screening efforts have been made in China and Indonesia, where more than 6000 germplasm accessions have been tested.

Among the available resistant genotypes, 31 belong to the Spanish type (var *vulgaris*) including more than 20 improved cultivars bred in China and Indonesia. A few Valencia (var *fastigiata*) lines resistant to foliar diseases have also been found resistant to bacterial wilt in China and Indonesia (Yeh 1990, Mehan et al. 1993). Resistance to bacterial wilt in these genotypes (e.g., NC Ac 17127 and PI 393641) might be less stable than that in the resistant Spanish lines. Recently, these Valencia lines showed varying levels of resistance at different locations in China. Only three resistant genotypes belong to the Virginia type (var *hypogaea*) and they are important in breeding programs (Duan et al. 1993).

In recent years, 50 'Chinese dragon' type genotypes have been identified as resistant to bacterial wilt (Duan et al. 1993). All the resistant dragon lines were collected from heavily infested locations in southern China. No resistant material of this type has been obtained from the northern provinces. The dragon lines are landraces that had long been cultivated in China (Sun et al. 1963); archaeological evidence indicates that they had been cultivated for at least five hundred years before the Virginia type groundnuts were introduced towards the end of 19th century. As resistant genotypes are far more common in the dragon type than in the other three botanical types, there might be a close relationship between the evolution of resistance and the disease because

bacterial wilt has long been serious in southern China. The characterization of some two hundred dragon genotypes indicates considerable variation in many characters—the genetic diversity for resistance among the dragon lines merits further investigation. Screening on a limited scale has led to the identification of some resistant accessions of wild *Arachis* species. Resistance has also been found in some interspecific hybrid derivatives that also have resistance to foliar fungal diseases (Mehan et al. 1994).

More than half of the global groundnut germplasm collection has still to be screened for resistance to bacterial wilt, and new sources of resistance could still be identified. Special emphasis should be given to combining wilt resistance with high yield potential and resistance to foliar fungal diseases.

Genetics of resistance

There have been few reports of studies on the genetics of wilt resistance and these have given conflicting information. One report indicated that the resistance is a recessive trait (Wang et al. 1985) whereas another reported it to be partially dominant (Liao et al. 1986). Only a few resistant parents of the Spanish type (e.g., Xiekangqing and Taishan Zhengzhu) were used in these studies. Recent studies at the Oil Crops Research Institute (OCRI) in Wuhan have supported the view that resistance is partially dominant. It is interesting to note that cytoplasmic effect for resistance was found in some dragon lines. In a cross involving two resistant parents, the dragon type genotype Feilongxiang and the Valencia genotype Taishan Sanlirou, the hybrids in F_1 and F_2 generations were resistant. However, a few F_3 progenies (each developed from an F_2 plant) were susceptible. As both parents are highly resistant, this segregation of susceptible plants points to possible differences in the genetic backgrounds of the two subspecies for wilt resistance. Further investigations are in progress.

Genetic diversity of wilt resistance is also thought to exist in the two botanical types of subsp *fastigiata*. The Spanish genotype Xiekangqing and the Valencia genotype Taishan Sanlirou have been identified as highly resistant under natural disease pressure in many environments in China, but their resistance appeared to be different in artificial inoculation tests. These results need further confirmation.

Components and mechanisms of resistance

Resistance to bacterial wilt in groundnut might be expressed through several components, including latent period, degree of vascular browning, hypersensitive reaction showing partial symptoms, and rate of wilting. Resistant lines, generally, have long latent periods. Among different botanical types, the late-maturing Virginia runner and dragon genotypes have longer latent periods than the early-maturing Spanish and Valencia types. Partial symptoms of wilting in a branch or a leaflet appear to be a defense reaction of the host plant, and partial wilting is more common in artificially inoculated plants.

Discoloration of vascular tissues is a diagnostic feature of bacterial wilt, but the degree of vascular browning in different genotypes needs to be assessed.

In some Spanish genotypes, wilt resistance is believed to be related to some morphological features of roots, and to *Rhizobium* spp associated with groundnuts (Liao et al. 1992). The susceptible cultivars generally have long and strong main roots, while the resistant genotypes have long lateral roots. Resistant genotypes have fewer nodules on roots and a lower nitrogen content than the susceptible lines. These observations suggest that resistance to bacterial invasion is an important component of resistance. The lower nitrogen content in the resistant genotypes might be a reason for their low yield potential.

Biochemical investigations reveal that infection by virulent isolates of *P. solanacearum* can cause increased levels of polyphenoloxidase in resistant lines. Gallic acid, catechin, and p-coumaric acid may be related to resistance to bacterial wilt in groundnut (Peng et al. 1994, personal communication). Peroxidase in resistant genotypes appears to be activated after bacterial infection.

Enhancement of resistance

To date, only a few resistant germplasm lines of subsp *fastigiata* have been used in breeding. It is very important to broaden the genetic background of the host resistance. Extensive screening of groundnut germplasm for resistance is necessary to identify more desirable resistant genotypes. Most of the landraces evolved at the hot-spot locations of China and Indonesia have been screened, but most of the groundnut germplasm collection in ICRISAT remains to be tested. New resistant genotypes may be found in the landraces collected from South America with its vast genetic diversity, and also in those from several countries of Southeast Asia. Though highly resistant lines have been obtained only from areas where the disease is prevalent, moderately resistant or tolerant genotypes have been identified in germplasm collected from other regions.

Intensive genetic evaluation of the available resistant germplasm is important to identify resistant genotypes with different genes and mechanisms of resistance to improve the level and stability of wilt resistance. To be successful, such genetic evaluation requires further characterization of the pathogen strains including pathotypes.

The level and stability of wilt resistance can be enhanced by crossing amongst the genotypes that possess different genes/resistance mechanisms. As wilt resistance in groundnut is simply inherited, it is feasible to improve or modify the resistance through mutation breeding.

Several resistant cultivars have been developed by crossing resistant parents with high-yielding but susceptible cultivars. Though it is easy to transfer the resistance by such one-way hybridization, the selected resistant materials might inherit such undesirable traits as low yield and poor tolerance to other constraints from the resistant parents. However, multiple crossing can overcome these problems. In Guangdong province, several wilt-resistant

groundnut cultivars such as Yue You 92 and Yue You 256 have been bred by multiple crossing; these have more desirable agronomic traits. Besides the use of better resistance donors, modified selection methods should also be used. Morphological features of roots, latent infection, and degree of vascular discoloration should be taken as complementary criteria in selection. Such inoculation procedures as stem injection and seed inoculation using high levels of inoculum can be used to detect greater levels of resistance, and inoculation with different strains of the pathogen can be used to test the stability of resistance.

In many regions where groundnut bacterial wilt reaches epidemic proportions, rust disease caused by *Puccinia arachidis* Speg. is common and causes significant losses. It is important to combine the resistances to both the diseases in varieties with desirable agronomic traits. Multiple crossing using germplasm with enhanced resistance and using some bridging materials is highly recommended.

References

Duan, N. X., Tan, Y. J., Jiang, H., and Hu, D. H. 1993. Screening groundnut germplasm for resistance to bacterial wilt. Oil Crops of China 1: 22-25.

Liao, B. S., Duan, N. X., Tan, Y. J., Li, D., and Tang, G. Y. 1994. Cytoplasmic effect for resistance to bacterial wilt in Chinese dragon groundnut (Abstract). ACIAR Bacterial Wilt Newsletter 10: 2.

Liao, B. S., Jiang, R. W., Tan, Y. J., Rao, R. H., and Tang, G. Y. 1992. A study on characters of nitrogen fixation in some groundnut lines resistant to bacterial wilt. Oil Crops of China 4: 34-37.

Liao, B. S., Li, W. R., and Sun, D. R. 1986. A study on inheritance of resistance to *Pseudomonas solanacearum* E.F. Smith in *Arachis hypogaea* L. Oil Crops of China 3:1-8.

Machmud, M. 1993. Present status of groundnut bacterial wilt research in Indonesia. Pages 15-24 in Bacterial wilt of groundnut: proceedings of the Second Working Group Meeting, 2 Nov 1992, Tainan, Taiwan (Mehan, V.K. and Hayward, A.C., eds.). Patancheru 502 324, Andhra Pradesh, India: International Crops Research Institute for the Semi-Arid Tropics.

Mehan, V. K., Liao, B. S., Tan, Y. J., Robinson-Smith, A., McDonald, P., and Hayward, A. C. 1994. Bacterial wilt of groundnut. Information Bulletin no. 35. Patancheru 502 324, Andhra Pradesh, India: International Crops Research Institute for the Semi-Arid Tropics. 28 pp.

Mehan, V. K., Nigam, S. N., and McDonald, D. 1993. Management of bacterial wilt of groundnut with genetic resistance and cultural practices. Pages 211-218 in Bacterial wilt: proceedings of an International Conference, 28-31 Oct 1992, Kaohsiung, Taiwan (Hartman, G.L. and Hayward, A.C., eds.). ACIAR Proceedings no. 45. Canberra, Australia: Australian Centre for International Agricultural Research.

Sun, D. R., Wang, Y. Y., Liu, Y. Q., Wan, W. Z., and Zhang, C. X. 1963. Classification of cultivated groundnut in China. Annual Research Report. Wuhan, China: Oil Crops Research Institute.

Wang, Y. Y., Wang, C. H., and Xia, X. M. 1985. A preliminary study on inheritance of resistance to bacterial wilt in peanut. Oil Crops of China 4:15-17.

Yeh, W.L. 1990. A review of bacterial wilt on groundnut in Guangdong Province, People's Republic of China. Pages 48-51 in Bacterial wilt of groundnut: proceedings of an ACIAR/ICRISAT collaborative research planning meeting, 18-19 Mar 1990, Genting Highlands, Malaysia (Middleton, KJ. and Hayward, A C, eds.). ACIAR Proceedings no. 31. Canberra, Australia: Australian Centre for International Agricultural Research.

Discussion

Y L Nene You mentioned that you need to investigate the components of resistance and use them in breeding disease resistant cultivars. From my experience with chickpea in breeding for wilt resistance, I would say that you need to be concerned only with percent mortality and the time of wilting (i.e., late wilting) when developing a practical disease resistance breeding program. You may use other components only to carry out detailed studies on pathogen variability.

V K Mehan Yes, we agree with your views. While screening large numbers of germplasm and breeding lines, we do emphasize only percent mortality and late wilting. Several components of resistance can be investigated only in a small number of resistant lines, and genotypes with different components of resistance can be used in breeding programs to enhance and stabilize the resistance to the wilt pathogen.

B S Liao I agree with Dr Mehan's views.

A C Hayward Your map showed a record in the southeastern comer of Queensland—actually at Nambour, which is 100 km south of Brisbane. This is not a groundnut production area. Bacterial wilt on groundnut has never been recorded in any production area in Australia. The circumstances are that in 1969/70, Moffett and Pegg grew Red Spanish groundnut in a known wilt-infested soil (infested with biovar 3 endemic to the region) only in order to find out whether the plants would wilt. They did. That is all that that record means.

B S Liao Could Dr Mehan comment on this?

V K Mehan I agree with Dr Hayward's comments. It is true that Moffett and Pegg isolated the wilt pathogen from some Red Spanish groundnut plants in a disease nursery established near Nambour.

Genetic evaluation of resistance to bacterial wilt in Chinese 'dragon' groundnut accessions

H F Jiang, N X Duan, Y J Tan, and B S Liao[1]

Abstract

The Chinese 'dragon' type groundnut has been found to be similar to var hirsuta or Peruvian type. Chinese dragon varieties were traditionally cultivated in many regions of China before germplasm of the other three types of cultivated groundnuts (Virginia, Valencia, and Spanish) was introduced into the country in the last century. Germplasm accessions of the dragon type were systematically evaluated for resistance to bacterial wilt caused by Pseudomonas solanacearum (Smith) Smith. Of the 250 dragon type accessions collected in China, 51 were identified as highly resistant. The frequency of resistant landraces was obviously much higher in the dragon type than in the other three types. All the resistant dragon lines were collected from heavily wilt-infested areas in southern China including Guangxi, Guangdong, and Jiangxi provinces. No resistant landrace of this type was obtained from the northern provinces. The results of characterization showed that the resistant dragon lines generally possessed desirable drought tolerance and strong seed dormancy. There was a positive correlation between resistance and (1) protein content of seed (r = 0.5114) and (2) proline content of the seed protein. Preliminary genetic observations revealed that there was a cytoplasmic effect in bacterial wilt resistance in some dragon genotypes.

中国龙生型花生与国际上通称的秘鲁型花生相似。龙生型品种是最早引入到中国的栽培花生。在中国已经系统地鉴定了龙生型花生品种对青枯病的抗性，在250份龙生型材料中有51份高抗青枯病，高抗品种频率明显高于其它类型，所有这些抗病龙花生基本上都是从南方重病区收集到的，包括广西，广东和江西，没发现来源于北方抗青的龙花生材料。抗病的龙花生材料一般也耐旱，种子休眠性强。龙生型花生对青枯病的抗性与种子蛋白质含量之间呈正相关($r=0.5114$)，与脯氨酸含量之间也呈正相关。初步遗传分析表明龙生型花生对青枯病的抗性存在细胞质效应。

1. Oil Crops Research Institute, Chinese Academy of Agricultural Sciences, Wuhan 430062, China.

Jiang, H.F., Duan, N.X., Tan, Y.J., and Liao, B.S. 1994. Genetic evaluation of resistance to bacterial wilt in Chinese 'dragon' groundnut accessions. Page 97 in Groundnut bacterial wilt in Asia: proceedings of the Third Working Group meeting, 4-5 Jul 1994, Oil Crops Research Institute, Wuhan, China (Mehan, V. K. and McDonald, D., eds.). Patancheru 502 324, Andhra Pradesh, India: International Crops Research Institute for the Semi-Arid Tropics.

A preliminary study of groundnut bacterial wilt resistance mechanisms

Z H Shan and Y J Tan[1]

Abstract

Seeds of several groundnut cultivars with varying levels of resistance to bacterial wilt were inoculated with Pseudomonas solanacearum (Smith) Smith. Plants were grown from these inoculated seeds under hydroponics. Forty days after sowing, the morphological traits and isoenzymes activities of roots were investigated. It was found that the bacterium could infect plants of both resistant and susceptible cultivars. Development of the roots of plants grown from noninoculated seeds, and of susceptible and resistant plants grown from inoculated seeds, was monitored. Infection by P. solanacearum had little influence on root development in resistant cultivars, but significantly reduced root growth in susceptible cultivars. In resistant cultivars, peroxidase activity was increased and esterase was inhibited, while the reverse was true for susceptible cultivars. Roots are the most appropriate plant part for studying biochemical changes that occur after invasion by the wilt pathogen.

用青枯菌 *Pseudomonas solanacearum* 浸种抗性水平不同的花生种子，浸种后水培。播种后 40 天观察根部形态和同工酶活性。结果表明，抗、感品种都能受青枯菌侵染。对不接种(CK)和接种的抗、感品种的根部形态观察发现青枯菌对抗病品种的根系生长影响很小，而严重影响感病品种的根系生长。抗病品种的过氧化物酶活性增强，酯酶活性降低，而感病品种的结果则相反。花生受青枯菌侵染后以根为材料来观察生化变化是可靠的。

1. Oil Crops Research Institute, Chinese Academy of Agricultural Sciences, Wuhan 430062, China.

Shan, Z.H., and Tan, Y.J. 1994. A preliminary study of groundnut bacterial wilt resistance mechanisms. Page 99 in Groundnut bacterial wilt in Asia: proceedings of the Third Working Group meeting, 4-5 Jul 1994, Oil Crops Research Institute, Wuhan, China (Mehan, V. K. and McDonald, D., eds.). Patancheru 502 324, Andhra Pradesh, India: International Crops Research Institute for the Semi-Arid Tropics.

Preliminary studies on the biochemical mechanisms of resistance to *Pseudomonas solanacearum* in groundnut

N X Duan, Z Peng, Y J Tan, and H F Jiang[1]

Abstract

Breeding for resistance is an effective strategy to control groundnut bacterial wilt caused by P s e u d o m o n a s s o l a n a c e a r u m *(Smith) Smith. Knowledge of the mechanisms of host-plant resistance is obviously important for evaluation and selection in breeding, but only limited research has been conducted on this aspect. Eight resistant and two susceptible groundnut genotypes were used in a study to investigate the correlation between resistance and polyphenol oxidase (PPO) activity, and the constitution of some polyphenols. Four-week-old plants with six to eight fully expanded leaves were inoculated with* P. s o l a n a c e a r u m *isolates by the stem-inoculation method.*

No significant difference was observed in PPO activities between the resistant and susceptible genotypes before inoculation, but after inoculation, differences were significant. Peak PPO activity occurred earlier (about six days after infection) in resistant lines than in susceptible ones (about ten days after infection). In resistant cultivars, PPO activity increased by 1.5-1.6 times after infection, whereas in the susceptible cultivars the increase was only 1-1.5 times. This suggests that resistance may be associated with increase in PPO activity after infection.

High pressure liquid chromatography (HPLC) was used to carry out quantitative and qualitative comparisons between cultivars. Gallic acid, catechin, p-coumaric acid, and 2,6-D-tert-butyl-p-crosel were found to be related to resistance to bacterial wilt. The gallic acid contents were very high after inoculation in the eight resistant cultivars tested, but no change was found in the susceptible ones. The contents of catechin and p-coumaric acid also changed after inoculation. The 2,6-D-tert-butyl-p-crosel contents were high in non-inoculated resistant and susceptible cultivars but, after inoculation, the contents were reduced significantly only in the susceptible genotypes. These results indicate that resistance is associated with increased levels of polyphenols after infection.

1. Oil Crops Research Institute, Chinese Academy of Agricultural Sciences, Wuhan 430062, China.

Duan, N. X., Peng, Z., Tan, Y. J., and Jiang, H. F.1994. Preliminary studies on the biochemical mechanisms of resistance to *Pseudomonas solanacearum* in groundnut. Pages 101-102 *in* Groundnut bacterial wilt in Asia: proceedings of the Third Working Group meeting, 4-5 Jul 1994, Oil Crops Research Institute, Wuhan, China (Mehan, V. K. and McDonald, D., eds.). Patancheru 502 324, Andhra Pradesh, India: International Crops Research Institute for the Semi-Arid Tropics.

利用抗病品种是防治花生青枯病的有效途径。在花生抗青枯病育种工作中，了解寄主植物的抗病机制是非常重要的，但是有关这方面的报道很少。本文以 8 个抗病品种和两个感病品种为材料，分析了青枯病抗性与多酚氧化酶(PPO)之间的关系及多酚类物质的组成。利用茎接种方法对 4 周龄植株(大约有 6—8 片全展叶)接种青枯菌 *P. solanacearum*。

接种前，抗感品种间多酚氧化酶(PPO)活性差异不明显，但是，接种后其差异显著。抗病品种的多酚氧化酶活性高峰(接种后约 6 天)早于感病品种的 PPO 活性高峰(接种后约 10 天)。接种后，抗病品种的 PPO 活性以 1.5—1.6 的倍数增加，而感病品种的 PPO 活性只以 1—1.5 的倍数增加，这表明花生品种对青枯病的抗性可能与 PPO 活性有关。

高压液相色谱(HPLC)测定结果表明没食子酸，儿茶酚，对香豆酸和 2.6—二特丁基对甲酚与花生品种对青枯病的抗性有关。接种后，8 个抗病品种的没食子酸含量明显增加，而感病品种中其含量不变。接种后儿茶酚和对香豆酸的含量也有变化。不接种的抗、感品种中，2.6—二特丁基对甲酚含量都很高，但是接种后感病品种中其含量明显下降。这些结果表明花生品种对青枯病的抗性与多酚类化合物有关。

Research on groundnut bacterial wilt in Guangdong province, China

G R Zheng, X Y Li, W L Yeh, X Q Liang, and Y H Li[1]

Abstract

Groundnut bacterial wilt, caused by Pseudomonas solanacearum (Smith) Smith, is economically important in Guangdong province. In this province, the total area under groundnut is 310 000 ha, of which over 18 000 ha are naturally infested with the wilt pathogen. The wilt incidence in Guangdong ranges from 10 to 20%, and the average yield loss ranges from 20 to 30%. Using wilt-resistant groundnut cultivars has been the most important measure to control the disease. Much research effort has gone into breeding wilt-resistant cultivars at the Industrial Crops Research Institute, Guangzhou. Two wilt-resistant cultivars, Yue You 92 and Yue You 256, have been released and extensively used in the province. Yue You 92 is about as resistant as Schwarz 21, but can outyield it by over 10%. It contains 54% oil. Yue You 256 outyielded Yue You 92 by 8.75%, and showed only 3.6% wilt incidence in the Wilt-Resistant Varietal Trial of South China (1988-1990). A new wilt-resistant breeding line, Yue You 200, with a high yield potential, is now at the demonstration stage, and may be released soon. As another economically important disease, namely rust, caused by Puccinia arachidis Speg., always occurs together with bacterial wilt in Guangdong province, breeding efforts have been directed towards integrating wilt resistance with rust resistance, and some lines with multiple disease resistance have been developed and found to be promising.

由 *Pseudomonas solancearum* 引起的花生青枯病是广东省一种重要的花生病害。在该省，花生总面积 31 万公顷，其中有 18000 多公顷受此病危害，发病率为 10－20%，引起产量损失 20－30%。种植抗病品种是防治该病最为重要的途径。广东省农科院经济作物研究所在抗青育种方面取得了很大进展，育成并推广了两个抗病品种"粤油 92"和"粤油 256"。粤油 92 的抗性水平与 Schwarz 21 相当，但是产量比 Schwarz 21 高 10%，含油量为 54%；粤油 256 比粤油 92 增产 8.75%，在南方抗青区域中(1988－1990)发病率为 3.16%。一个新的抗青高产品系"粤油 200"已育成并正在示范，不久就会推广。在广东省，花生锈病与青枯病交叉发生，育种工作者正在向培育兼抗品种努力，已经育成了一些有希望的多抗品系。

1. Industrial Crops Research Institute, Guangdong Academy of Agricultural Sciences, Guangzhou 51040/ China.

Zheng, G. R., Li, X. Y., Yeh, W. L., Liang, X. Q., and Li, Y. H. 1994. Research on groundnut bacterial wilt in Guangdong province, China. Page 103 in Groundnut bacterial wilt in Asia: proceedings of the Third Working Group meeting, 4-5 Jul 1994, Oil Crops Research Institute, Wuhan, China (Mehan, V. K. and McDonald, D., eds). Patancheru 502 324, Andhra Pradesh, India: International Crops Research Institute for the Semi-Arid Tropics.

Country reports on the status of groundnut bacterial wilt research

Status of groundnut bacterial wilt research in China

Y J Tan[1], N X Duan[1], B S Liao[1], Z Y Xu[1], L Y He[2], and G R Zheng[3]

Abstract

Groundnut bacterial wilt has been reported from 16 provinces in China. The area under groundnut cultivation naturally infested with the pathogen is estimated at over 200 000 ha. The annual incidence of the disease is estimated to range from 4 to 8% on resistant cultivars. The annual loss in groundnut pod yield due to the disease is estimated at around 36 000 t. The wilt pathogen affecting groundnut in China belongs to race 1 of Pseudomonas solanacearum, and biovars 3 and 4 have been identified. Its pathogenicity varies greatly with regions, and the disease is generally more serious in southern provinces.

Rotation of groundnut with rice for 1 to 2 years is the most effective cultural control. Rotation with such other nonhost crops as maize, wheat, sorghum, and sugarcane for 2 to 5 years is also effective. However, the use of wilt-resistant cultivars has been the most important measure to control the disease in China. The resistant cultivars used at present are E Hua 5, Gui You 28, Lu Hua 3, Yue You 92, Yue You 256, and Zhonghua 2. Fifty-five highly resistant germplasm accessions have been identified, and some of them have been used in breeding programs. Genetics and mechanisms of resistance have been studied. Research has also been conducted on the molecular basis of virulence and pathogenicity of P. solanacearum, seed transmission, and biological control.

花生青枯病已在中国 16 个省有发生的报道，全国病地面积估计在 20 万公顷以上。目前所种植抗病品种的发病率约为 4-8%，每年全国由青枯病而引起的花生减产约 36000 吨。在中国侵染花生的病原为 *Pseudomonas solanacearum* 小种 1 号，生物型 3 和 4，不同地区病原菌的致病力有很大差异，南方地区病害普遍较重。

花生与水稻轮作 1—2 年对防治病害非常有效，与其它非寄主作物如玉米、小麦、高粱、甘蔗等轮作 2—5 年也有防治效果。但在中国利用抗病品种是首要的防病办法。现在推广应用的抗病品种有鄂花 5 号，中花 2 号，鲁花 3 号，粤油 92，桂油 28 和粤油 256。已鉴定出 55 份高抗青枯病资源材料，并已用于育种中。对抗性遗传和机制也作了研究，而且在病原菌致病性的分子基础、种传问题和生物防治方面也开展了研究。

1. Oil Crops Research Institute, Chinese Academy of Agricultural Sciences, Wuhan 430062, China.
2. Plant Protection Institute, Chinese Academy of Agricultural Sciences, Beijing 100094, China.
3. Industrial Crops Research Institute, Guangzhou 510640, China.

Tan, Y.J., Duan, N.X., Liao, B.S., Xu, Z.Y., He, L.Y., and Zheng, G. R. 1994. Status of groundnut bacterial wilt research in China, Pages 107-113 *in* Groundnut bacterial wilt in Asia: proceedings of the Third Working Group meeting, 4-5 Jul 1994, Oil Crops Research Institute, Wuhan, China (Mehaa V. K. and McDonald, D., eds.). Patancheru 502 324, Andhra Pradesh, India: International Crops Research Institute for the Semi-Arid Tropics.

Introduction

Bacterial wilt, caused by *Pseudomonas solanacearum* (Smith) Smith, has long been recognized as a serious disease of groundnut in China, where it was first reported in 1938. In Hubei province, extensive investigation and research on this disease were initiated in 1955, followed by similar research activities in Fujian and Guangdong provinces. Research efforts in the 1950s and 1960s were concentrated on disease surveys, and on control through cultural measures. Since the 1970s, emphasis has been on the identification of races and biovars, the pathogenicity of the causal bacterium, and disease epidemiology. Considerable research efforts have been directed at germplasm evaluation, the genetics of wilt resistance, and breeding for resistance. Several resistant cultivars have been released and are extensively used. They have played an important role in reducing yield losses due to the disease.

Occurrence and distribution

To date, bacterial wilt of groundnut has been reported in the following provinces of China: Anhui, Fujian, Guangdong, Guangxi, Guizhoun, Hainan, Hebei, Henan, Hubei, Hunan, Jiangsu, Jiangxi, Liaoning, Shandong, Sichuan, and Zhejiang. Generally, the disease is more serious in the central and southern parts of the country. In northern China, the disease causes considerable yield losses in the southeastern part of Shandong province.

The cultivars grown in most of the naturally infested areas in China until the beginning of the 1980s were susceptible. Wilt incidence ranged from 10 to 30% in moderately infested regions, and from 50 to 100% in heavily infested fields, sometime causing total crop failure. Historically, the wilt incidence in Guangdong ranged from 10 to 20%, but in the most heavily infested fields it reached 100%. In Guangxi, 40% of the total groundnut-growing area was infested with the wilt pathogen, the average wilt incidence being 10%. In the eastern part of Hubei province, 26% of groundnut fields were infested and wilt incidence ranged from 10 to 30%. Since the 1980s, several resistant groundnut varieties have been grown extensively in most of the areas where the disease is endemic. This has markedly reduced wilt incidence and associated yield losses. However, the total infested area in China is believed to be over 200 000 ha, with the average wilt incidence being 4-8%, and annual yield losses due to bacterial wilt are estimated at 36 000 t. Losses may be even higher as many farmers are still growing susceptible cultivars because they cannot get seed of resistant cultivars or because of the shortcomings of some of the released resistant cultivars in terms of low yield and susceptibility to other serious diseases.

Races, biovars and pathogenicity of *Pseudomonas solanacearum*

Pseudomonas solanacearum has a wide host range, and isolates of the pathogen have been classified into different races based on their host ranges

(Buddenhagen and Kelman 1964). In China, *P. solanacearum* isolates that infect groundnut belong to race 1, which can also infect eggplant, olive, pepper, sweet potato, and tomato. Isolates that infect mulberry have been identified as race 5 (He et al. 1983).

In China, strains of biovars 3 and 4 have been found to infect groundnut. Among 17 isolates collected from southern and central China, six belonged to biovar 3 and 11 to biovar 4. Among 10 isolates from Guangxi, six belonged to biovar 3 and four to biovar 4. All isolates from Hubei belonged to biovar 4 only. Apparently, isolates of both biovars coexist in the endemic regions, but in varying proportions.

Isolates from different parts of the country vary widely in their pathogenicity. Generally, the isolates from southern and central China are more virulent than those from northern China. Seven pathotypes were observed by Li et al. (1987).

With reference to the molecular basis of virulence and pathogenicity of *P. solanacearum,* the role of extracellular proteins and polysaccharides (EPS) in pathogenesis has been highlighted and the EPS genes have been cloned.

Several experiments on seed transmission have been conducted at the Plant Protection Institute (PPI) and the Oil Crops Research Institute (OCRI). The bacterium was isolated from fresh seeds harvested from naturally infested fields. The seeds were sown in wilt-free plots. Seed transmission ranged from 3.8 to 6%. *Pseudomonas solanacearum* could not be isolated from seed after 2 months of natural drying. The extent of seed infection was positively related to the water content of the seeds—when it dropped below 8.9%, the bacterium lost its viability Drying the seeds after harvest and keeping the water content below 8% prevent seed transmission of the pathogen (Zhang et al. 1993).

Integrated disease management

Rotation

Rotation of groundnut with nonhost crops has proved effective in reducing the inoculum in soil and the wilt incidence. Rotation with rice was the most effective. Rotating groundnut with rice for one to two years, depending upon the degree of infestation, can have a significant effect, but this practice is feasible only in some areas because most wilt-infested fields in China are in upland areas. However, rotating groundnut with such cereal crops as maize, wheat, sorghum, or sugarcane for 2-5 years could be effective in upland areas.

Field Management

Crop sanitation can help in reducing the population of the pathogen in soil, and in avoiding transmission of the pathogen. Eliminating weeds also reduces disease incidence.

As the pathogen is easily transmitted through flowing water, it is advisable to dig deep drainage ditches to ensure that water from infested plots does not flow to other parts of the field.

Biological control

In recent years, scientists at PPI have conducted some experiments on biological control using antagonistic bacteria. Some treatments have given promising results in the laboratory, and further experiments under natural field conditions are in progress.

Use of resistant cultivars

Sowing improved resistant cultivars has proved to be the most effective and feasible way to control bacterial wilt of groundnut, and currently E Hua 5, Gui You 28, Lu Hua 3, Yue You 92, Yue You 256, and Zhonghua 2 are cultivated extensively in different regions of China. Several recently bred lines have proved promising.

Research on genetic improvement for host resistance

Germplasm screening

In recent years, groundnut germplasm accessions have been extensively evaluated for resistance to bacterial wilt. Nearly 4000 accessions, including some 1000 lines from foreign countries, have been screened. Of these, 55 have been identified as resistant and 43 as moderately resistant. Most of the resistant lines are of the Chinese 'dragon' type. Five exotic lines (Bentang, Gajah, Kidang, Macan, and Schwarz 21) have also been identified as highly resistant. All the Chinese resistant genotypes (landraces) were collected from southern China where the disease is most serious.

Genetics and breeding for resistance

Studies have been conducted on the genetics of resistance, using different resistance sources. Liao et al. (1986) studied the inheritance patterns and the genes conferring resistance in Spanish type groundnuts. At present, cytoplasmic effects have been identified in some resistant sources of the dragon type. These are obviously different from the Spanish types (Liao et al. 1994). Preliminary results have indicated genetic diversity in the resistant lines of both Chinese dragon and Valencia types (Liao et al, unpublished data).

To date, many resistant cultivars have been developed in China, using a few resistance donors belonging to subsp *fastigiata*. These Spanish or Valencia resistance sources usually have an adequate level of resistance and early maturity, but their yield potential is generally low. They also exhibit a short seed-dormancy. Liao et al. (1992) found that resistant Spanish lines had poorer nodulation than susceptible lines, which might account for their low yield potential. This highlights the narrow genetic background of the currently available resistant cultivars. Both disease resistance and yield need to be further improved.

Recently, several large-seeded Virginia resistant lines have been used in crossing. Efforts have also been made to cross resistant materials of different

botanical types in order to create more desirable resistant genotypes for further breeding.

Mechanisms of resistance

Resistance to bacterial wilt can be expressed in groundnut in various ways including a latent period, the degree of vascular browning, and the rate of wilting (Mehan et al. 1994). Peroxidase activity in host plant was found to be related to resistance (Kang and He 1994). Genotypes with varying levels of resistance have also been observed to differ in esterase levels.

A polyphenolic substance has recently been observed to play a role in resistance (Peng Zhong, unpublished data). The mechanisms involved might differ among botanical types.

Present staffing, resources available, and collaborative research

Eight scientists involved in research on groundnut bacterial wilt are at the OCRI, Wuhan. These include phytopathologists, botanists, and breeders. In the 1970s, OCRI established a rural base for work on groundnut bacterial wilt in Hong An county in Hubei province. Facilities and methodologies for artificial inoculation have been established. In recent years, a nationwide network has been established through collaboration with provincial staff for multi-locational identification of resistant groundnut germplasm, and greater emphasis has been placed on identifying genetic variations among different sources of resistance and on investigating the mechanisms of resistance.

At PPI, Beijing, a group of scientists led by Dr He, the director of the State Key Laboratory for Biology of Plant Diseases and Insect Pests, is working on bacterial wilt of groundnut and potato with emphasis on identification of pathogen strains, mechanisms of pathogenesis, and biological control. This is a well-equipped laboratory.

At the Industrial Crops Research Institute (ICRI), Guangzhou, several breeders and phytopathologists are working on groundnut bacterial wilt with special emphasis on breeding resistant cultivars.

Besides these institutions, research on disease management is being conducted in Guangxi, Henan, and Sichuan provinces. Molecular work on *P. solanacearum* is in progress at the Guangxi Agricultural College.

Further research plans

Further genetic evaluation of the available sources of resistance is needed to elucidate possible differentiation of genes conferring resistance. The variation in resistance mechanisms among different sources will also be investigated. This is a necessary basic work to utilize available germplasm more effectively and to breed more desirable cultivars. Efforts should also be made to improve the yield and resistance to other major diseases.

The variation in *P. solanacearum* that infects groundnut in different regions should be further investigated. Mechanisms of pathogenesis including the role

of extracellular polysaccharides, polygalacturonase, and other enzymes need to be studied further. Newly developed biotechnological methods should be applied in this regard.

Integrated management of the disease is important. Promoting the newly developed resistant cultivars should be a priority for disease control. The mechanisms whereby cultural practices can reduce wilt incidence should be studied, and more effective complementary measures should be worked out including biological control and crop rotation. Collaboration with international organizations in different aspects of research should be strengthened further.

Acknowledgments

The authors are grateful to Drs Sun Darong and Wang Yuying for their advice and for their comments about this paper, and to Dr Song Xiesong, Mr Li Shuilin, and Mr Tang Ronghua for providing information.

References

Buddenhagen, I. W., and Kelman, A. 1964. Biological and physiological aspects of bacterial wilt caused by *Pseudomonas solanacearum.* Annual Review of Phytopathology 2: 203-230.

Duan, N. X., Tan, Y. J., Jiang, H., and Hu, D. H. 1993. Screening groundnut germplasm for resistance to bacterial wilt. Oil Crops of China 1: 22-25.

He, L. Y., Sequeira, L., and Kelman, A. 1983. Characteristics of strains of *Pseudomonas solanacearum* from China. Plant Disease 67:1357-1361.

Kang, Y. W., and He, L. Y. 1994. Physiological study on the peanut inoculated with spontaneous avirulent mutant of *Pseudomonas solanacearum.* Oil Crops of China 1: 38-40.

Liao, B. S., Duan, N. X., Tan, Y. J., Li, D., and Tang G. Y. 1994. Cytoplasmic effect for resistance to bacterial wilt in Chinese dragon groundnut (Abstract). ACIAR Bacterial Wilt Newsletter 10:2.

Liao, B. S., Jiang, R. W., Tan, Y. J., Rao, R. H., and Tang, G. Y. 1992. A study on characters of nitrogen fixation in some groundnut lines resistant to bacterial wilt Oil Crops of China 4: 34-37.

Liao, B. S., Li, W. R., and Sun, D. R. 1986. A study on inheritance of resistance to Pseudomonas solanacearum E.F. Smith in *Arachis hypogaea* L. Oil Crops of China 3:1-8.

Mehan, V. K., Liao, B. S., Tan, Y. J., Robinson-Smith, A., McDonald, D., and Hayward, A. C.1994. Bacterial wilt of groundnut. Information Bulletin no. 35. Patancheru 502 324, Andhra Pradesh, India: International Crops Research Institute for the Semi-Arid Tropics. 28 pp.

Tan, Y. J., Duan, N. X., Liao, B. S., and Xu, Z. Y. 1993. A summary of research on groundnut bacterial wilt at OCRI in 1990-1992. ACIAR Bacterial Wilt Newsletter 9:10.

Tan, Y. J., and Liao, B. S. 1990. A review of groundnut bacterial wilt research in the world. Oil Crops of China 4: 87-90.

Yen, M. E, Fu, Q. G., Li, X. Y, and Zheng, G. R. 1991. Groundnut varietal trials for resistance to bacterial wilt in south China. Guangdong Agricultural Sciences 3:14-16.

Zhang, Y. X., Hua, J. Y., and He, L. Y. 1993. Effect of infected groundnut seeds on transmission of *Pseudomonas solanacearum.* ACIAR Bacterial Wilt Newsletter 9: 9-10.

Discussion

J G Ryan: Can you clarify the data on adoption of wilt-resistant cultivars in China? Do the data refer to current levels of adoption or are they cumulative figures since the year of release? What was the extent of wilt incidence in the varieties that were replaced by the resistant varieties?

Y J Tan: The data refer to the total area under each cultivar since its release. The incidence of bacterial wilt in the varieties that were replaced by the resistant varieties ranged from 30-70%.

R Black: Is the effect of crop rotation with rice due to flooding or is it because rice is a nonhost?

Y J Tan: It can be due to both.

M Machmud: I agree with the comments of Dr Tan. Wilt incidence is more in groundnut that follows rice in uplands than in that which follows irrigated rice.

Status of groundnut bacterial wilt research in Indonesia

M Machmud and S A Rais[1]

Abstract

Bacterial wilt is still a major constraint to groundnut production in Indonesia. Field surveys indicated that strains of the wilt pathogen Pseudomonas solanacearum were of race 1 and biovar 3. New weed hosts susceptible to the disease were identified. Several resistant and moderately resistant local and introduced groundnut genotypes were identified in field and nursery tests. Integrated disease management, by growing resistant cultivars and rotation with nonhost crops or with rice, was very effective. Breeders and pathologists are working together to combine good agronomic characteristics with multiple disease resistance. Collaborative research with national and international institutions to develop new control strategies for the disease is described.

青枯病至今仍然是印度尼西亚花生生产的一个主要限制因素。田间调查表明该国的病原菌为 *Pseudomonas solanacearum* 小种 1 号生物型 3。鉴定出了一些新的感病杂草。一些高抗和中抗的地方品种和引进的材料在田间和病圃作了鉴定。利用抗病品种并结合轮作措施的综合防治非常有效。病理学家和育种家正在协同培育兼抗型花生品种。关于病害防治的国内外合作研究状况作了阐述。

Introduction

Groundnut is the second most important food legume crop in Indonesia. It is grown mostly at low elevations (up to 500 m above sea level), and usually as a monocrop, though sometimes it is intercropped with maize, cassava, or such vegetables as pepper and eggplant. In some areas, groundnut is also grown as a cash crop in between such estate crops as rubber, coconut, and oil palm. The area under groundnut is relatively stable at about 500 000 ha, with annual production of 550 000 t. Due to the government policy to encourage soybean, there has been some shift in groundnut production areas from upland to irrigated lowland, where it follows rice.

1. Bogor Research Institute for Food Crops, J1, Tentara Pelajar 3A, Bogor 16111, Indonesia.

Machmud, M., and Rais, S.A. 1994. Status of groundnut bacterial wilt research in Indonesia. Pages 115-119 in Groundnut bacterial wilt in Asia: proceedings of the Third Working Group meeting, 4-5 Jul 1994, Oil Crops Research Institute, Wuhan, China (Mehan, V. K. and McDonald, D., eds.). Patancheru 502 324, Andhra Pradesh, India: International Crops Research Institute for the Semi-Arid Tropics.

Bacterial wilt caused by *Pseudomonas solanacearum* (Smith) Smith is a major constraint to groundnut production, and efforts are being made to develop effective integrated control measures for the disease.

Distribution, severity, and yield losses

Bacterial wilt is widely distributed in Indonesia, New isolates have been obtained recently from different localities. This is probably due to the presence of indigenous strains or introduction of infected seed into new areas. Some factors that favor the spread of the disease in field are (1) insufficient supplies of seeds of resistant cultivars, (2) cultivation of local varieties to meet the demand of the home food industry, and (3) low inputs and low adoption of proper technology Disease severity, in terms of plant mortality, varies from 5 to 65%, depending upon the variety, location, soil type, and cultural practices.

Annual yield losses are estimated at 50 000 to 150 000 t, valued at US$50 million to 150 million. Losses are generally lower in irrigated lowlands than in dry uplands.

Occurrence of races and biovars of *Pseudomonas solanacearum*

During the period 1992 to 1994, 264 isolates of P. solanacearum were obtained from soils, weeds, and groundnut plant samples collected from different groundnut-growing areas in Java. Thirty-two other isolates were obtained from eggplant, pepper, potato, and tomato in the vicinity of groundnut crops and from high elevation areas (> 1000 m). Based on host range and biochemical reactions, all the isolates (from soil, groundnut plants, weeds, and other hosts) were identified as belonging to race 1 biovar 3. Earlier collection contained only one or two isolates, from Irian Jaya, that belonged to race 1 biovar 4; these were probably introduced into Indonesia (Machmud 1993). Most of the isolates from high elevation areas, particularly those from potato and tomato, belonged to race 3 biovar 2, although one isolate was of race 1 biovar 3.

Research on disease management strategies and constraints to their adoption

Research on groundnut bacterial wilt from 1992 to 1994 concentrated on (1) evaluation of groundnut germplasm for resistance to bacterial wilt, (2) combining bacterial wilt resistance with resistance to such other diseases as late leaf spot and rust, (3) strain identification, (4) seed transmission, and (5) disease management.

A total of 655 germplasm accessions, breeding lines, and local and improved cultivars were evaluated for their resistance to bacterial wilt both in greenhouse and field screening. Sixteen lines were identified as resistant: ICG Nos.5990, 7200, 7230, 7883, 7886, 7888, 7893, 9294,10916,10918,10920,10928, 10931,10939,10978, and 11073.

Table 1. Resistance/susceptibility of various progenies of breeding lines to bacterial wilt, 1992-1993, BORIF, Bogor, Indonesia[1]

Cross/generation	No. of progenies[2]			
	R	MR	MS	S
Gajah/Lokal Ponorogo (F6)	55	-	1	-
Kelinci/ICGS 62 (F6)	-	-	6	1
Kelinci/ICG(c)5 (F7)	29	8	6	4
Macan/L. Majalengka (F6)	36	5	2	-
Macan/L. Majalengka (F7)	41	5	2	7
Macan/850/1 (F7)	29	17	5	6
Macan/850/1 (F8)	4	-	-	-
Kidang/L. Garut (F6)	28	6	7	12
Tupai/L. Cianjur (F7)	26	15	6	1
Gajah/L. Bengkulu (F6)	2	2	-	-

1. These results are based on artificial inoculation in greenhouse, and on natural infection in a field heavily infested with bacterial wilt.
2. R = resistant (0-15% wilt incidence); MR = moderately resistant (16-25% wilt incidence); MS = moderately susceptible (26-35% wilt incidence); S = susceptible (>35% wilt incidence)

Several advanced lines with resistance to bacterial wilt were obtained (Table 1), and further selections will be made from these to develop new cultivars.

More isolates of *P. solanacearum* from different hosts and localities in Java are being collected. Besides race and biovar identification, a new approach to identify strains using molecular techniques was initiated through collaboration with the Australian Centre for International Agricultural Research (ACIAR) in Canberra. Increased emphasis is being placed on integrated disease management through the use of resistant cultivars and cultural practices. Groundnut intercropping and rotation with nonhost crops have been studied in greater detail. The shifting of groundnut cultivation from upland to irrigated lowland after rice considerably reduced the disease in the field.

Studies on seed transmission indicated two possible routes by which the seed is infected. In one, the pathogen infects the pod shell directly. The other route is an indirect one: the pathogen enters the plant through roots, travels upwards to the stem, and then gains access to the pod through the peg. Greenhouse studies showed that seedlings raised from infected seeds expressed the symptoms one to two weeks after sowing. This highlights the possibility that infected seed is an important source of disease in the field. A field study indicated that sources of disease in the field are (1) infected seed, (2) weed hosts, and (3) volunteer plants. Host range studies indicated that *Ageratum conyzoides, Crassocephalum crepidioides,* and *Croton hirtus,* weeds commonly found in groundnut fields, were very susceptible to bacterial wilt Also, as reported earlier, *Portulacca oleracea* is a symptomless carrier of the bacterium. These weeds can help to maintain high populations of the bacterium in the soil from season to season.

Present staffing and resources available for research

A small group of scientists, consisting of five pathologists and five breeders, is currently involved in research on groundnut bacterial wilt. Two pathologists are at the Bogor Research Institute for Food Crops (BORIF), Bogor; two at the Malang Research Institute for Food Crops (MARIF), Malang; and one at the Sukarami Research Institute for Food Crops (SARIF), Sukarami. Two groundnut breeders are at BORIF, one at SARIF, and two at MARIF. With the current reorganization of our research institutions, there will be slight changes in research strategies and priorities.

Workplan and collaborative research

Our research is in collaboration with ICRISAT Asia Center and ACIAR, Australia. ICRISAT has supplied 115 groundnut germplasm lines to be evaluated for resistance to bacterial wilt. The collaboration with ACIAR, under a 3-year (1992 to 1994) agreement, on new approaches to control of bacterial wilt involved four countries and seven institutions in the region. The main objectives of the collaboration are (1) training scientists from developing countries in molecular techniques, (2) applying molecular techniques to bacterial detection and strain identification, and (3) conducting short training courses on molecular genetics for agricultural scientists. It is expected that the collaboration will be extended to another 3-year period.

Releases of wilt-resistant varieties and their impact

Bacterial wilt resistance has been a high priority objective in the Indonesian groundnut breeding program, and all cultivars released to farmers have some degree of resistance to bacterial wilt. Since 1989, nine improved wilt-resistant cultivars (Badak, Biawak, Jepara, Komodo, Landak, Mahesa, Simpai, Trenggiling, and Zebra) have been released.

Although improved cultivars with resistance to bacterial wilt are available, the disease continues to be a problem in many areas. Only 29% of improved cultivars are currently grown by farmers. Insufficient supply of seed and preference for local cultivars that meet the specific demands of consumers have restricted the area under the resistant cultivars. The government can meet only one-fifth of the demand for seed at present.

The current groundnut varietal improvement program at BORIF includes the following activities:

- Incorporation of wilt resistance in short-duration high-yielding cultivars.
- Combining wilt resistance with resistance to rust and late leaf spot, particularly in Gajah, Kidang, and Pelanduk cultivars.
- Incorporating agronomically desirable traits into the wilt-resistant lines.

Future research plan

Research on groundnut bacterial wilt will be concentrated at BORIF and MARIF. Scientists at BORIF will conduct research on molecular and basic aspects of the

host and the pathogen, and those at MARIF will conduct research on disease control measures.

The following activities will be pursued:

- Further evaluation of groundnut germplasm for resistance to bacterial wilt.
- Development of improved varieties with multiple disease resistance, i.e., to bacterial wilt, late leaf spot, and rust.
- Strain differentiation, using conventional and molecular techniques.
- Integrated management of groundnut bacterial wilt using host-plant resistance and cultural practices, particularly crop rotation and sanitation.

Reference

Machmud, M. 1993. Present status of groundnut bacterial wilt research in Indonesia. Pages 15-25 in Groundnut bacterial wilt: proceedings of the Second Working Group Meeting, 2 Nov 1992, Tainan, Taiwan (Mehan, V. K. and Hayward, A. C., eds.). Patancheru 502 324, Andhra Pradesh, India: International Crops Research Institute for the Semi-Arid Tropics.

Discussion

V K Mehan Are you using the name 'New Kelinci' for the improved resistant cultivar Kelinci?

M Machmud Not yet. We are still using the name 'Kelinci' for the improved resistant cultivar.

V K Mehan You have listed the accession ICG 7893 under two heads, 'Resistant' and 'Moderately resistant'. Could you care to explain the difference?

M Machmud That is a mistake. ICG 7893 should be considered resistant to bacterial wilt in the field.

Y L Nene I should like to know if you are planning to carry out studies on eradicating the seed-borne pathogen. That would allow free international movement of seed.

M Machmud I have not thought of this line of approach yet.

V K Mehan Did you compare the virulence of biovars 3 and 4 on groundnut? What is the frequency of biovar 4 isolates in Indonesia?

M Machmud Biovar 3 isolates are more virulent than biovar 4 isolates. Biovar 4 isolates are occasionally associated with groundnut in Indonesia.

Status of groundnut bacterial wilt research in Malaysia

K Y Lum and S Hamidah[1]

Abstract

Bacterial wilt caused by Pseudomonas solanacearum is a serious disease of groundnut in the states of Kelantan and Terengganu. Disease surveys conducted in 1993 indicate that wilt incidence ranged from 0 to 20% at different locations in the two states. A new weed host, Acalypha sp, was consistently found affected by P solanacearum in groundnut fields. Two hundred P. solanacearam isolates from wilt-affected plants were collected and identified. Preliminary results indicate that most of the isolates belong to biovar 3. Studies on genetic diversity of the isolates are now under way. Fifteen percent of the seeds from wilt-affected plants were found infected. Fifteen cultivars/lines were screened for resistance to bacterial wilt Three lines (Tae Kwang Tangkong, Cina, and Dhaka 1) showed very low susceptibility at two locations (Serdang and Bukit Tangga). Future research will focus on strain differentiation and distribution, and on developing cultural and biological approaches to disease management.

由 *Pseudomonas solanacearum* 引起的花生青枯病是 Kelantan 和 Terengganu 州的重要病害。1993 年的调查表明，该病害的发病率为 0－20%。已发现一种花生田感青枯病的杂草"*Acalypha sp*"。从病区收集到了 200 个菌株，初步研究表明多数菌株属生物型 3。关于菌原遗传分化的研究正在进行之中。发病植株上有 15%的种子受到感染。作了 15 个品种的抗性鉴定，3 个表现较好。进一步的研究将集中在菌系分化和分布，建立栽培防治和生物防治方法。

1. Malaysian Agricultural Research and Development Institute, P 0 Box 12301,50774 Kuala Lumpur, Malaysia.

Lum, K. Y., and Hamidah, S. 1994. Status of groundnut bacterial wilt research in Malaysia. Pages 121-124 in Groundnut bacterial wilt in Asia: proceedings of the Third Working Group meeting, 4-5 Jul 1994, Oil Crops Research Institute, Wuhan, China (Mehan, V. K. and McDonald, D., eds.). Patancheru 502 324, Andhra Pradesh, India; International Crops Research Institute for the Semi-Arid Tropics.

Introduction

In Malaysia, groundnut is cultivated mainly by small farmers, along sandy river banks in the states of Kelantan and Terengganu, on rehabilitated tin-tailings in the state of Perak, and, on a smaller scale, in inland areas in the state of Pahang. In eastern Malaysia, the crop is grown around Kuching, Serian, and Sibu. Bacterial wilt is a serious disease of groundnut in the major production areas of Kelantan and Terengganu.

Disease survey

A survey was conducted in April 1993 to assess the status of groundnut cultivation and the incidence of bacterial wilt in Malaysia, The results of the survey indicate that groundnut continues to be cultivated in riverine areas of Kelantan and Terengganu in peninsular Malaysia and Sarawak in eastern Malaysia. It is also cultivated in inland areas in Pahang. However, the area under groundnut in Kedah, Perlis, and Perak has significantly declined.

The incidence of bacterial wilt in the fields surveyed varied from 0 to 20%. The other most commonly encountered disease was collar rot. In most groundnut-growing areas, the smallholders normally rotate groundnut with maize.

In the course of the survey, an additional alternative host for the pathogen was identified. Plants of *Acalypha* sp, growing as a weed, were consistently found wilted in groundnut fields in Kelantan and Terengganu; the pathogen was successfully isolated from these plants.

Characterization of isolates

Two hundred isolates of the wilt pathogen, *Pseudomonas solanacearum* (Smith) Smith, were obtained from wilt-affected plants from many locations in the country. The genetic diversity of the isolates within and among locations is being studied. Initial tests indicate that most of the isolates belong to biovar 3.

Detection of latent infections by polymerase chain reaction (PCR)

Seeds from diseased plants were tested for latent infection. Extracts of single seeds were used to detect the wilt pathogen, using the PCR technique. Initial results indicate that approximately 15% of the seeds carried the pathogen. However, PCR failed to detect the pathogen in any of 25 samples of clean seed collected as seed stock.

Germplasm evaluation

Gertnplasm accessions obtained from the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) were screened for resistance to

natural bacterial wilt infection at two locations, Serdang, Selangor and Bukit Tangga, Kedah. The mortality (% incidence) levels for the tested lines at these two locations are given in Table 1.

Table 1. Incidence of wilt in selected groundnut lines in response to natural infection with *Pseudomonas solanacearum* at Serdang and Bukit Tangga, Malaysia, 1993.

	Mortality (% wilt incidence)	
Cultivar/line	Bukit Tangga	Serdang
Gajah	0	40
	0	80
Macan	10	30
Matjam	30	70
MKT1	20	100
Mahesa	100	100
Tae Kwang Tangkong	0	0
K. Honheah 60-1	80	60
Kidang	10	40
ACC 12	50	50
Cina	0	0
Pelanduk	50	20
Khonhaeh 60-2	70	80
Dhaka 1	0	10
GH 467	80	80

Planned activities

Although there are indications that groundnut cultivation in the country attracts little interest in the face of cheaper imports, research on bacterial wilt will continue to receive emphasis as the disease affects a wide range of economically important crop plants. Research will focus on

- ELISA and PCR techniques for the detection of the pathogen;
- strain differentiation and distribution in relation to host type and locality; and
- integration of cultural and biological approaches to manage the disease.

Discussion

A C Hayward What restriction enzyme did you use in the pulse field gel electrophoresis work?

K Y Lum XbAI.

V K Mehan The *P. solanacearum* seed infection level presented by you seems to be high. Did you conduct seed transmission tests?

K Y Lum We only detected the wilt pathogen in seed using the PCR technique. No seed transmission tests were done.

C L L Gowda Are there desiccation-resistant resting stages in *P solanacearum?*

A C Hayward There is no known desiccation-resisting stage in *P. solanacearum.* We do not know whether there are viable non-culturable stages of *P. solanacearum* as described for marine bacteria.

Status of groundnut bacterial wilt research in the Philippines

MP Natural[1]

Abstract

Bacterial wilt caused by Pseudomonas solanacearum has recently been observed in several parts of the Philippines. In 1994, the disease was reported in a seed production farm in Isabela, where it affected about 30% of the crop. All isolates of P. solanacearum obtained from wilt-affected groundnut plants from different locations in the Philippines belong to race 1 biovar 3. Breeding lines are routinely tested for their reactions to bacterial wilt at disease hot-spots and/or in the greenhouse. Some advanced breeding lines showed little or no incidence of wilt. Research is under way to develop techniques to detect P. solanacearum in soil and plant debris.

最近在菲律宾的一些地区发现了由 *Pseudomonas solanacearum* 引起的青枯病。1994 年，在伊萨贝拉(Isabela)的一个生产种子的农场发现了这一病害，花生发病率达 30%。从菲律宾各地收集到 菌株均属于小种 1 号，生物型 3。育种材料正在重病地和温室进行青枯病抗性的鉴定，一些高世代材料表现出极低的发病率。目前正在研究建立检测土壤和植株中青枯菌的技术方法。

Status of the disease

No systematic surveys have been conducted for groundnut bacterial wilt in the Philippines. The disease was reported in January 1994 in a seed production farm in Isabela, where it affected about 30% of the crop. Sporadic outbreaks of bacterial wilt were observed in other groundnut-growing areas.

Losses in yield due to the disease have not been assessed, but could be as high as 30% since they are linearly correlated with the extent of disease incidence.

Races and biovars of Pseudomonas solanacearum

All isolates of *P. solanacearum* collected from naturally infected groundnut plants were of race 1, biovar 3.

1. Department of Plant Pathology, University of the Philippines, Los Banos, Laguna 4031, the Philippines.

Natural, M. P. 1994. Status of groundnut bacterial wilt research in the Philippines. Pages 125-127 in Groundnut bacterial wilt in Asia: proceedings of the Third Working Group meeting, 4-5 Jul 1994, Oil Crops Research Institute, Wuhan, China (Mehan, V. K. and McDonald, D., eds.). Patancheru 502 324, Andhra Pradesh, India: International Crops Research Institute for the Semi-Arid Tropics.

Table 1. Reactions of groundnut breeding lines to bacterial wilt under greenhouse conditions, Laguna, the Philippines, 1993.

Line	Wilt incidence (%)	Disease reaction[1]
IPB Pn 82-71-32	0	R
82-70-37	0	R
82-71-33	0	R
82-71-59	0	R
82-71-15	13	MR
82-71-29	0	R
48-75	13	MR
82-70-71	0	R
82-68-94	0	R
82-71-19	0	R
48-90	13	MR
42-14	13	MR
80-72-2	0	R
82-70-51	0	R
82-71-64	0	R
LPL Pn 4(check)	46	S

1. R = Resistant, MR = Moderately resistant, S = Susceptible

Research on disease management

Many breeding lines of groundnut have been evaluated for resistance to *P. solanacearum*. Some advanced breeding lines showed little or no incidence of wilt (Table 1). Reactions of other important breeding lines are shown in Tables 2,3, and 4.

Present staffing and resources available for research

Very limited resources are available for research on groundnut bacterial wilt; the staff at the Institute of Plant Breeding, Los Banos, are mainly involved with

Table 2. New groundnut accessions resistant to bacterial wilt.

Line	Pedigree
F 87609	Marc 1
F87514	GFA x NDBC
F 1109	NC 18230 x NC 2-25
F 1107	NC 17721 x NC 182229-16B
F1004	NC 17721 x NC 182229-16A
F 1212	Laos
F 1097	Faizpur x Tainan 9
F1018	Ah7223xSK38
F1230	Southern runner
F 81206-2	Ar-1 x Tainan 9

Table 3. New groundnut breeding lines susceptible to bacterial wilt, Los Banos, the Philippines, 1993.

F1104
F 851033
F 623
GK 53
Local collection Aurora
BC2 (JL 24 x PI 298115) x JL 24
Sunbelt Runner x NDBC
UF 71513 x Tainan 9
PI 337409 x Tainan 9
Agusan del Sur collection
Georgia red
NC 180168 x NC2-30
JL 24xPI 298115 x JL 24
JL 24xPI 25947x JL24

Table 4. Reactions of eight groundnut lines to bacterial wilt in a trial under shade conditions, Los Bafios, the Philippines, 1993.

Line	Wilt incidence (%)	Disease reaction[1]
HYQ S-28	7	R
HYQS-30	0	R
IPB Pn 42-14	13	R
HYQS-40	20	MR
82-68-140	40	S
82-68-97	46	S
82-68-196	40	S
12-12	53	HS
Check (LPL Pn4)	66	HS

1. R = resistant, MR = Moderately resistant, S = Susceptible, HS = Highly susceptible

other research problems and breeding for host-plant resistance to constraints to legumes production.

Action on work plan and collaborative research

- Continue evaluating advanced breeding lines for their reactions to *P. solanacearum*.
- Determine biovars or races of isolates of the wilt pathogen from various locations.

Future research plans

- Continue evaluating breeding lines for resistance to bacterial wilt.
- Develop techniques to detect P. solanacearum in soil and plant debris.

Status of groundnut bacterial wilt research in Thailand

W Butranu, S Yinasawapun, and W Srithongchai[1]

Abstract

Bacterial wilt caused by Pseudomonas solanacearum is one of the serious diseases of groundnut in Thailand. First observed in 1987 in the southern parts of the country, it now occurs in many areas of the southern, central, and northeastern provinces. The disease is generally severe in rich soils, especially in areas where groundnut is grown every year. Local groundnut cultivars are highly susceptible to bacterial wilt compared to such recommended cultivars as Tainan and Khon Kaen 604. Research indicated that the wilt pathogen belonged to race 1. The groundnut isolate of P. solanacearum was found highly virulent on bitter gourd, cucumber, potato, ridge gourd, and water melon. Eggplant, ginger, sesame, tobacco, and tomato showed varying levels of susceptibility to this isolate. Eighteen lines/cultivars were tested for resistance to bacterial wilt under natural disease pressure. Local cultivars showed high disease incidence (>50%), and the infected plants died 4 weeks after emergence. The cultivars Khon Kaen 604 and Tainan showed tolerance to the disease.

由 *Pseudomonas solanacearum* 引起的青枯病是泰国花生重要的病害之一。该国 1987 年首次在南部发现花生青枯病，现在在南部、中部和东北部的许多省份均有发生。青枯病一般在肥沃的土壤，尤其在连作花生的土壤上很严重，相对于"台南"和"Khon Kaen 60－1"等品种而言，地方花生品种高感青枯病。研究表明，侵染花生的青枯菌为小种 1 号，花生菌株对包括苦瓜、黄瓜、西瓜、马铃薯在内的一些作物有很强的致病力，芝麻、蕃茄、烟草、姜也有不同程度的感染。在自然条件下作了 18 个品种的抗性比较试验，出苗后 4 周，地方品种的发病率超过 50%，而"Khon Kaen 60－1"和"台南"表现出一定的耐病性。

Introduction

Bacterial wilt disease of groundnut, caused by Pseudomonas solanacearum (Smith) Smith, is becoming an increasingly serious disease of groundnut in Thailand. The disease was first observed in 1987 in some groundnut-growing

1. Khon Kaen Field Crops Research Center, Field Crops Research Institute, Department of Agriculture, Khon Kaen 40060, Thailand.

Butranu, W., Yinasawapun, S., and Srithongchai, W. 1994. Status of groundnut bacterial wilt research in Thailand. Pages 129-133 in Groundnut bacterial wilt in Asia: proceedings of the Third Working Group meeting, 4-5 Jul 1994, Oil Crops Research Institute, Wuhan, China (Mehan, V K. and McDonald, D., eds.). Patancheru 502 324, Andhra Pradesh, India: International Crops Research Institute for the Semi-Arid Tropics.

areas in southern Thailand; more recently, it has been found in many places in the southern provinces and in some places in the central and northeastern provinces. Yield losses vary with location, being influenced by genotype, climate, and cultural practices.

Research on groundnut bacterial wilt

Disease surveys conducted during 1990/91 indicated that groundnut bacterial wilt was generally severe on crops grown in rich soils, especially in areas where groundnut has been grown continually, year after year. It was rarely observed on crops grown in sandy loam or sandy soils. The disease caused more severe damage to local cultivars than to the recommended cultivars Tainan and Khon Kaen 60-1.

Comparison of Pseudomonas solanacearum isolates

Thirty-eight isolates of *P. solanacearum* from groundnut were compared with standard isolates in many physiological and biochemical tests (Tables 1 and 2).

Table 1. Comparison of physiological and biochemical characteristics of a groundnut isolate of Pseudomonas solanacearum with other reported isolates.

	P. solanacearum							
Test	Groundnut isolate[1]	1	2	3 & 4[2]		H[3]	T[4]	U[5]
Gram reaction	—	-	-	-		-	-	-
Oxidase	+	+	+	+		+	+	+
Catalase	+	+	+	+		+	+	+
Motility	+					+	+	+
Fluorescent pigment								
Nitrate reduction	+					+	+	+
Arginine dihydrolase								
Poly-hydroxy butyrate accumulation	+	+	+	+		+	+	+
Polyacetate degradation	+					+		+
Growth at 41 mC								
Levan production	V[6]							V
Hypersensitivity test	+							+

1. Highly virulent isolate selected from among the 38 groundnut isolates tested.
2. Sands et al.(1980).
3. Hayward (1964).
4. Titatan and Tananuson (1981).
5. Uematsu et al.(1983).
6. Variable.

Sources: Samerchai and Duangjai (1989), Samerchai et al. (1987).

Table 2. Utilization of organic compounds by a groundnut isolate of *Pseudomonas solanacearum* compared to that by other reported isolates.

		P. solanacearum					
Organic compound	Groundnut isolate[1]	1	2	3 & 4[2]	H[3]	T[4]	U[5]
Mannitol	+	+	+	+	+	+	+
Benzoate	-	-	-	-		-	-
Sucrose	+	+	+	+	+	+	+
Arabinose	V-[6]	-	-	-	-	-	-
B-alanine	V+7	V	V	V			V+
Erythritol	-						-
Adonitol	-						-
Lactose	+				-	+	-
Cellobiose	v+	-	-	-	-		V-
Trehalose	v+	+	-	+		+	+
L-rhamnose	-	-	-	-	-	-	-
Glucose	+	+	+	+		+	+
Maltose	v-				-	+	V-
Sorbitol	v+	-	-	+	-	+	

1. Highly virulent isolate selected from among the 38 groundnut isolates tested.
2. Sands et al. (1980).
3. Hayward (1964).
4. Titatan and Tananuson (1981).
5. Uematsu et al. (1983).
6. Variable; 90% negative.
7. Variable; 90% positive.

Sources: Samerchai and Duangjai (1989), Samerchai et al. (1987).

The results indicated that the groundnut isolates belonged to race 1. The colonies were usually slightly opaque, darkening in their later stages, and were relatively small, ranging from 1 to 1.5 cm in diameter.

Pathogenicity and host range studies

Twenty-six plant species were tested for their reactions to groundnut isolates of *P. solanacearum,* using the stem inoculation method. *Pseudotnonas solanacearum* was consistently highly virulent on bitter gourd, cucumber, potato, ridge gourd, and water melon (Table 3); all the plants died within five days of inoculation. Eggplant, ginger, sesame, tobacco, and tomato showed varying degrees of susceptibility.

Of the several inoculation techniques tested, clipping the petiole close to the base with scissors dipped in bacterial suspension was found to be the most effective for resistance screening. With this technique, wilt symptoms appeared within five days of inoculation of groundnut plants (cv Tainan 9); the disease incidence was over 70%. Root inoculation and soil drenching also resulted in high levels of infection but the symptoms appeared slightly later compared to the petiole-clipping method.

Table 3. Reactions of some crop plants to a groundnut isolate of *Pseudomonas solanacearum*.

Crop plant	Reaction
Bitter gourd (*Momordica charantia*)	+ (6)[1]
Cassava (*Manihot esculenta*)	-
Chili (*Capsicum frutescens*)	-
Chrysanthemum (*Chrysanthemum sp*)	-
Cucumber (*Cucumis sativus*)	+ (5)
Eggplant (*Solatium melongena*)	+ (3)
Ginger (*Zingiber officinale*)	+ (10)
Leaf mustard (*Brassica juncea*)	-
Maize (*Zea mays*)	-
Mulberry (*Morus alba*)	-
Mung bean (*Vigna radiata*)	-
Potato (*Solanum tuberosum*)	+ (5-10)
Ridge gourd (*Luffa acutangula*)	+ (6)
Sesame (*Sesamum indicum*)	+ (5)
Soybean (*Glycine max*)	-
Sweet pepper (*Capsicum annuum*)	-
Sweet pea (*Pisum sativum*)	-
Sweet potato (*Ipomea batatas*)	-
Tobacco (*Nicotiana tabacum*)	+ (3)
Tomato (*Lycopersicon esculentum*)	+ (5)
Water melon (*Citrullus vulgaris*)	+ (3)
Yard long bean (*Vigna sesquipedalis*)	-

1. Numbers in parentheses indicate how many days the plants took to die after inoculation.

Source: Samerchai et al. (1987).

Host resistance

Eighteen groundnut lines/cultivars were tested for resistance to bacterial wilt under field conditions. Local cultivars showed 50% wilt incidence, and the plants died as early as 4 weeks after emergence. The cultivar Khon Kaen 60-2 was highly susceptible whereas Khon Kaen 60-1 and Tainan 9 were tolerant.

References

Hayward, A. C. 1964. Characteristics of *Pseudomonas solanacearum.* Journal of Applied Bacteriology 27: 265-277.

Samerchai, C, and Duangjai, C 1989. Bacterial wilt of groundnut in southern Thailand. Warasan Rok Phut [Thai Phytopathology] 9(2-4): 54-58.

Samerchai, C., Suttirack, L., Wallapa, K., and Daungjai, C. 1987. Study on bacterial wilt of groundnut in southern Thailand. Pages 189-193 in Proceedings of the 8th Peanut Meeting, 3-5 May 1987, Roi-Et province, Thailand.

Sands, D. C., Schroth, M. N., and Hildebrand, D. G 1980. Pseudomonas. Pages 36-44 *in* Laboratory guide for identification of plant pathogenic bacteria

(Schaad, N. W., ed.). St. Paul, Minnesota, USA: The American Phytopathological Society.

Uematsu, T., Chuenchitt, S., Karnjanarat, S., Vivithajinda, S., Nabheerong, N., Benjathikul, S., Nilmanee, S., Dhirabhava, W., and Buangsuwon, D. 1983. Bacterial diseases on economic crops in Thailand. Tokyo, Japan: Tropical Agriculture Research Center, Ministry of Agriculture, Forestry and Fishery and Bangkok, Thailand: Department of Agriculture, Ministry of Agriculture and Cooperatives. 266 pp.

Discussion

Y W Kang Are *P. solanacearum* isolates from groundnut virulent on cucumber and watermelon?

W Butranu *Pseudomonas solanacearum* isolated from groundnut proved to be virulent on bitter gourd, cucumber, potato, and water melon; disease symptoms were observed five days after inoculation.

M T Vinh Did you investigate soil inoculum in the area where the disease occurred?

W Butranu Yes, we did. We used the wilt-infested soil in greenhouse experiments.

Status of groundnut bacterial wilt research in Vietnam

N X Hong[1], V K Mehan[2], N T Ly[3], and M T Vinh[4]

Abstract

Groundnut bacterial wilt is widespread in the major groundnut-growing areas of both northern and southern Vietnam. Significant yield losses have been observed in some areas of Ha Bac, Long An, Nghe An, Tay Ninh, and Thanh Hoa provinces. The disease is most severe in dryland cropping systems, especially on sandy soils along river banks, and on uplands. In general, rotation of groundnut with rice in low-lying areas is effective in reducing disease incidence and severity. The disease is more severe in autumn (July-November) than in spring (January-June) crops. All groundnut isolates of Pseudomonas solanacearum obtained from wilt-affected plants from many areas in northern Vietnam belonged to race 1. Research is now under way to determine the biovars of the wilt pathogen from many disease-affected areas of Vietnam. Preliminary research indicated seed-transmission of groundnut bacterial wilt to the extent of 4-8%. Most of the local groundnut cultivars and breeding lines are found to be susceptible to the disease. Some groundnut lines reported resistant in Indonesia and China have shown resistance to the disease in several hot-spot locations The major research thrust at the National Institute of Agricultural Sciences (INSA) is to identify wilt-resistant germplasm and breeding lines in the Spanish type. Extensive disease surveys are needed to assess yield losses, particularly in southern and central Vietnam. Research to breed stable wilt-resistant cultivars, and to incorporate resistances to other important diseases (late leaf spot and rust) and drought tolerance will be given high priority.

花生青枯病在越南北部和南部的花生主产区普遍发生，在许多省份均发现该病引起显著减产。该病在沙性土壤，河滩地和高坡地很严重。在地势低洼的地方，花生与水稻轮作可以有效地减轻发病。该病害在秋季比春季严重。从越南北方收集的所有花生菌株均为 *Pseudomonas solanacearum* 小种 1 号，目前正在进行生物型的鉴定。初步研究表明，花生种传率为 4－8%。多数地方品种高度感病，印尼和中国的品种表现抗病。国家农业科学研究所主要致力于西班牙型花生抗性资源的筛选和育种。需要更广泛的调查以估计中部和南部的产量损失。选育抗青枯病和兼抗性品种将是研究的重点内容。

1. National Institute of Agricultural Sciences (INSA), Hanoi, Vietnam.
2. ICRISAT Asia Center, Patancheru 502 324, Andhra Pradesh, India.
3. Plant Protection Research Institute (PPRI), Hanoi, Vietnam.
4. Institute of Agricultural Sciences for South Vietnam, Ho Chi Minn City, Vietnam.

ICRISAT Conference Paper no. CP 908.

Hong, N. X., Mehan, V. K., Ly, N. T., and Vinh, M. T. 1994. Status of groundnut bacterial wilt research in Vietnam. Pages 135-141 in Groundnut bacterial wilt in Asia: proceedings of the Third Working Group meeting, 4-5 Jul 1994, Oil Crops Research Institute, Wuhan, China (Mehan, V. K. and McDonald, D., eds.). Patancheru 502 324, Andhra Pradesh, India: International Crops Research Institute for the Semi-Arid Tropics.

Introduction

Groundnut is the most important food legume crop in Vietnam. The area under groundnut increased from 200 000 ha in 1991 to 230 000 ha in 1994, and a further increase is expected in the near future since the trend is to grow groundnut to break rice monoculture, improve soil fertility, and to secure additional income through the export of groundnut and its products. Although soils and environmental conditions in Vietnam are favorable for groundnut production, the average pod yield is low (about 11 ha^{-1}). Bacterial wilt, caused by *Pseudomonas solanacearum* (Smith) Smith, is one of the major biotic constraints to groundnut production. The disease has undoubtedly been present on groundnut in the country for many years, although it has been documented only recently (Mehan et al. 1991). Bacterial wilt is becoming increasingly serious in the major groundnut-growing areas, and is now considered to be a major threat to groundnut production in both northern and southern Vietnam.

Disease distribution and economic importance

Groundnut bacterial wilt was first identified in 1991; it was found to be widespread in Nghe An (formerly Nghe Tinh) and Ha Bac provinces. Regular surveys carried out since the 1991 spring season (last week of January to first week of June) indicate that the disease is widely distributed in Vietnam. Approximately 105 000 ha are estimated to be infested with the wilt pathogen; some regions are heavily infested.

The disease is most severe in Bac Thai, Ha Bac, Hatinh, Nghe An, and Thanh Hoa provinces of northern Vietnam and in Long An and Tay Ninh provinces of southern Vietnam. In Nghe An, it is particularly severe in Nam Dan and Nghia Dan districts (the two major groundnut-producing areas) while it is destructive in Hatrung and Hoang Hoa districts of Thanh Hoa. In Ha Bac, it is serious in Lang Giang and Viet Yen districts. In these areas, plant mortality due to bacterial wilt commonly ranges from 15 to 45%, and can reach 70-80%. In southern Vietnam, 20-30% plant mortality is common in Long An and Tay Ninh provinces, and in some areas can reach 80%, particularly in fields where groundnut is grown season after season.

Disease incidence is moderate (5-20%) in Bac Thai and Hatinh provinces, but is rarely observed in newly cultivated areas in Bac Thai, Ha Bac, and Hatinh provinces.

Bacterial wilt also occurs in Hai Hung, Hanoi, Nam Ha, Ninh Binh, and Vinh Phu provinces in northern Vietnam and in Dong Nai, Ho Chi Minh, Quang Nam, and Thuan Hai provinces in southern Vietnam; disease incidence varies greatly from place to place, ranging from 5 to 40% depending on cropping patterns and soil conditions.

Disease severity in different production systems

The disease is severe in upland areas, particularly in light sandy soils along river banks where only dryland crops, e.g., groundnut, maize, and sweet potato, and such vegetable crops as eggplant and tomato, are grown. The disease is becoming more severe every year, and plant mortality is estimated at 40-50%. In low-lying fields, the disease incidence is very high and the disease is severe. Many farmers in such areas (e.g., Nam Dan and Nghia Dan districts in Nghe An, and Hatrung district in Thanh Hoa) now reckon this disease to be a serious threat to groundnut production, as they are being forced to replace groundnut with such less profitable crops as sweet potato. It is noteworthy that many upland areas are being used for seed multiplication in autumn (July-November).

In rice-growing areas, bacterial wilt incidence generally ranges from 5 to 15%, but can be as high as 25% in heavily infested fields. Groundnut-rice rotation is common in Vietnam, but as rice is edged out and the land cropped season after season with groundnut, bacterial wilt is becoming more prevalent. Rotating groundnut with such vegetable crops as eggplant and tomato is even worse, as disease incidence is increased in the groundnut crop that follows them.

In fields where groundnuts are intercropped and/or mixed with maize and mung bean, the disease incidence is moderate. In some areas of Thanh Hoa province, bacterial wilt has been found to be serious on groundnuts intercropped with orange or pineapple.

In general, the disease is more prevalent in soils of low fertility. Disease incidence and severity are high in acidic soils (pH 5.0-5.5).

The disease incidence in general is the highest at peak flowering and podding stages, although the disease can appear early in the season (about 30 days after sowing). In fields with a history of heavy infestation, the disease appears much earlier than in other areas. Bacterial wilt develops most rapidly if the soil moisture and temperature are high during early crop growth. Heavy rains followed by a few sunny, hot days result in severe wilting. In general, the disease is more severe in the autumn crop than in the spring crop; high soil moisture and temperatures in the autumn favor disease development.

Research activities

Research on groundnut bacterial wilt is being conducted mainly by the National Institute of Agricultural Sciences (INSA), Hanoi. The Plant Protection Research Institute (PPRI), Hanoi; INSA; and the Oil Plant Research Institute (OPRI), Ho Chi Mirth City have participated in disease surveys carried out during 1991-94. Disease surveys have been carried out jointly by Vietnamese and ICRISAT scientists. At INSA, two scientists and two research scholars are currently working on several aspects of groundnut bacterial wilt. Research on the disease is given high priority. INSA has developed strong collaborative research plans with ICRISAT. Some research on host plant resistance is being

carried out at OPRI. Resources including greenhouse research facilities are very limited.

Strains of *Pseudomonas solanacearum* and their virulence

At INSA, over 50 isolates of *P. solanacearum* from wilt-affected groundnut plants collected from several groundnut-growing regions in northern Vietnam were tested and found to be highly pathogenic to groundnut. Several isolates from disease hot-spots in Bo Ha State Farm (Ha Bac), Hatrung State Farm (Thanh Hoa), and Hong Long (Nghe An) were found to be highly virulent on groundnut, and most were virulent on eggplant, pepper, potato, and tomato, but less virulent on tobacco. Tests indicated that all isolates collected belonged to race 1.

Recently (April 1994), 50 isolates of the wilt pathogen were collected from different agroecological regions with various cropping systems in major groundnut-growing areas of northern Vietnam. Research is now in progress to determine the biovars, their virulence, and host ranges. Some isolates from eggplant, potato, tobacco, and tomato are also being tested at INSA for virulence on groundnut.

Several naturally infected weed hosts in groundnut fields have also been identified.

Seed transmission

Groundnut bacterial wilt has been reported to be seed-transmitted. As this is important in relation to safe movement of seed within the country and integrated disease management, seed transmission of the disease has been investigated. In the 1993 autumn season, groundnut pods of two cultivars (ICG 3704 and Su Tuyen) were collected from plants that showed typical bacterial wilt symptoms at the Bo Ha State Farm, and the INSA Legumes Research Station at Ha Bac—areas known to be heavily infested with *P. solanacearum.* The pods showed discoloration of the funiculus, pod shell, and seed coat. *Pseudomonas solanacearum* was isolated from different parts of pod and seed. When discolored seeds from freshly harvested pods were sown in the greenhouse, 4-8% of the plants wilted within 4 weeks. Research is now in progress to determine the extent to which *P. solanacearum* can be transmitted in both freshly harvested and well-dried seed.

Host-plant resistance

Identification of wilt-resistant groundnut germplasm and breeding lines is the major research thrust at INSA and OPRI. Most of the local varieties are susceptible, but some varieties (e.g., Sen Lai and Tram Xuyen) have shown less than average susceptibility (<25% plant mortality) under high disease pressure.

Several disease hot-spots identified in Ha Bac, Nghe An, and Thanh Hoa provinces are being used to screen germplasm and breeding lines. A 600-m^2

wilt-sick plot has now been developed at the INSA Legumes Research Station at Ha Bac for screening selected lines for wilt resistance. The sick plot was developed over several seasons by (1) continually growing a highly susceptible cultivar in the same field; (2) incorporating wilt-affected plants collected from farmers' fields in Ha Bac province into the soil; and (3) using inoculated seeds. The disease incidence and severity are also enhanced by sowing the crop late in the spring season.

Over 500 germplasm and breeding lines have been screened in disease host-spots and/or in the wilt-sick plot. These included 16 lines reported resistant in Indonesia and China. Most of the lines tested were susceptible to bacterial wilt. Disease reactions of selected lines are shown in Table 1.

Some lines that had been reported resistant in Indonesia [Gajah, Kidang (ICG 5276), Matjan, and Schwarz 21 (ICG 1609 and ICG 7968)1 showed resistance in the spring of 1992 and 1993. However, some accessions of Kidang

Table 1. Incidence of bacterial wilt in 18 germplasm and breeding lines grown at different locations in northern Vietnam.

		Wilt incidence (%)		
		Ha Bac		Nghe An
Cultivar/Line	Accession[1]	1992	1993	1993
Schwarz 21	ICG 1609	0.0	0.0	-[2]
Schwarz 21	ICG 7968	3.2	0.0	9.0
Schwarz 21	ICG 8666	1.3	12.0	17.5
Schwarz 21	ICG 7343	17.2	0.0	-
Kidang	ICG 5276	2.0	0.0	-
Kidang	ICG 11210	25.4	23.5	7.9
Gajah	ICG 5272	4.0	7.7	-
Matjan	ICG 5273	2.4	0.0	-
NC Ac 171273	ICG 1703	1.4	5.3	3.3
NC Ac 171293	ICG 1704	4.0	0.0	12.4
NC Ac 171303	ICG 1705	4.3	0.0	-
PI 393531[3]	ICG 7893	0.0	18.2	8.9
PI 393641[3]	ICG 7894	2.4	0.0	10.0
-	ICGV 87165	3.2	0.0	-
-	ICGV 87206	14.2	25.0	14.4
-	ICGV 88252	0.0	0.0	4.4
-	ICGV 88271	9.2	0.0	0.0
-	ICGV 88274	14.5	8.3	28.5
Do Ba Giang[4]	-	26.9	29.0	-
Sen Lai[4]	-	9.6	4.2	-
U 4-47-7[5]	ICG 3263	68.3	100.0	20.6

1. ICG = ICRISAT Groundnut Accession Number and ICGV = ICRISAT Groundnut Variety
2. - - Not tested
3. Originated in Peru
4. Local variety
5. Originated in Uganda

(ICG 11210) and Schwarz 21 (ICG 7343 and ICG 8666) showed considerable wilt incidence at one location or the other. Some lines resistant to rust and late leaf spot (NC Ac 17127, NC Ac 17130, and PI 393641) showed high levels of resistance to bacterial wilt at nearly all locations in both the years. Three breeding lines (ICG 87165, ICG 88252, and ICG 88271) were also found resistant (<10% wilt incidence). These lines are also resistant to rust and moderately resistant to late leaf spot. In northern Vietnam, these lines have given pod yields of 2-2.5 t ha^{-1}. All accessions of Schwarz 21 and some breeding lines (e.g., ICGV 87206 and ICGV 88271) have shown low to moderate susceptibility to bacterial wilt in artificial inoculation tests in the greenhouse (Hong and Mehan 1993). In the 1994 spring season, all the above-mentioned promising cultivars/lines are being evaluated for wilt resistance and agronomic characters at several disease hot-spots in northern Vietnam. Some 77 genotypes are being evaluated in the Cu Chi district of southern Vietnam.

In general, in most lines, wilt intensity was higher in the autumn than in the spring. These results emphasize the need to evaluate the resistance over time and across locations. A drought-tolerant line (ICG 3704) has been found to be highly susceptible to bacterial wilt, and is now regularly used as a susceptible control cultivar in screening trials.

It is interesting to note that a commercial Indian cultivar, JL 24, showed low susceptibility (10-15% wilt incidence) at a disease hot-spot location, where the susceptible control cultivars showed 90-100% wilt incidence. INSA is now multiplying the seed of this cultivar for possible release in areas where the disease is endemic. This variety is being tested in the national varietal trials in northern Vietnam.

No wilt-resistant cultivar has yet been released in Vietnam.

Disease management strategies

The need for integrated disease management is emphasized. High priority is given to host-plant resistance and appropriate cultural practices. Crop rotation, especially with rice, is considered important in disease management. However, it is not possible to adopt such crop rotations in upland and marginal lands where groundnuts are grown under rainfed conditions. The use of seed from disease-free areas is advocated.

Future research priorities and plans

Intensive surveys will be carried out to assess more accurately the distribution and economic importance of groundnut bacterial wilt, particularly in southern Vietnam. Research into stable genetic resistance to bacterial wilt will be given high priority. More systematic research will be undertaken to understand the influence of different cropping systems and crop management practices on disease incidence and severity. Increased emphasis will be given to investigation of seed transmission.

We shall also collaborate more closely with ICRISAT on the bacterial wilt disease problem, particularly on host-plant resistance. It is intended to establish links with researchers, especially in China, Indonesia, and Malaysia, through active participation in the Groundnut Bacterial Wilt Working Group. High priority will be given to conducting the proposed International Groundnut Bacterial Wilt Disease Nursery in hot-spot locations in northern and southern Vietnam,

References

Hong, N. X., and Mehan, V. K. 1993. Research on bacterial wilt of groundnut in Vietnam. Pages 219-220 *in* Bacterial wilt: proceedings of an International Conference, 28-31 Oct 1992, Kaohsiung, Taiwan (Hartman, G.L. and Hayward, A.C., eds.). ACIAR Proceedings no. 45. Canberra, Australia: Australian Centre for International Agricultural Research.

Mehan, V. K., Hong, N. X., and Ly, N. T. 1991. On the identification of pathogens causing the so-called groundnut wilt disease in north Vietnam. Pages 105-110 *in* Improved cultivation techniques for groundnut and other food legumes in Vietnam (Dan, N.T. and Gowda, C.L.L., eds.). Hanoi, Vietnam: Ministry of Agriculture and Food Industry, Vietnam and Agricultural Publishing House.

Discussion

W Butranu Did you check the soil inoculum of the wilt pathogen before conducting seed transmission tests? Did you also assess the soil inoculum in areas where the disease occurred?

N T Ly We used sterile soil for seed transmission studies in the greenhouse. Wilt was not detected in any of the plants raised from seed of healthy plants. We also tested soil samples from several infested areas; bacterial populations were high in these soil samples.

W Butranu Has any wilt-resistant cultivar been released in Vietnam?

N T Ly No. We are multiplying the seeds of some promising lines for multilocational tests for resistance to bacterial wilt.

Recommendations

Recommendations

Following intensive discussions, the Working Group agreed upon the following recommendations.

- Increased emphasis should be placed on producing highly specific monoclonal antibodies to detect the individual biovars and pathotypes of the wilt pathogen in seed, plant tissues, and soil. Efforts should be made to develop more sensitive ELISA techniques for detecting the pathogen. Research along these lines, initiated at the Rothamsted Experimental Station, UK, should be encouraged.
- Molecular methods, particularly polymerase chain reaction techniques, should be developed to characterize various strains/pathotypes of the pathogen. Research on this aspect at the Rothamsted Experimental Station and Malaysian Agricultural Research and Development Institute (MARDI), Malaysia, should be strengthened.
- All strains reported as highly virulent to groundnut should be sent to the Rothamsted Experimental Station for maintenance and documentation. Priority should be given to collecting strains from South and Southeast Asia. Efforts should be made to elucidate the molecular/genetic basis of high and low virulence of the strains.
- Training courses should be organized for scientists and technicians engaged in bacterial wilt research, focusing on serological and molecular methods for detecting the pathogen (biovars/strains) and on methods of screening groundnut germplasm for resistance to the disease.
- An international groundnut bacterial wilt nursery should be established to determine the stability of wilt resistance through multilocational testing (in China, Indonesia, Malaysia, Vietnam, and, possibly, Thailand). ICRISAT should coordinate this nursery and facilitate the exchange of wilt-resistant germplasm and breeding lines.
- Specific wilt-resistant germplasm identified in China, Indonesia, the Philippines, and Thailand should be supplied to ICRISAT for multiplication and distribution to interested national programs.
- High priority should be given to determining the possible pathotypes of the wilt pathogen, particularly in China and Indonesia. It is imperative to use a set of differentials (indicator groundnut cultivars) for this activity. Standard protocols for pathotype determination should be used.
- Host-plant resistance is an important component of disease management. More coordinated research is needed to evaluate wilt resistance. Landraces originating from humid areas in the centers of crop diversity should be

evaluated for wilt resistance at disease hot-spots in China, Indonesia, and Vietnam. Integration of resistance genes from different genetic backgrounds should receive more attention.

- Concerted efforts should be made to understand the mechanisms and components of wilt resistance.
- High priority should be given to breeding high-yielding cultivars combining resistances to bacterial wilt and major foliar fungal diseases (rust and late leaf spot), which are serious constraints to groundnut production in warm, humid areas.
- Increased emphasis should be given to elucidating the role of improved farming practices in disease management. High priority should be given to on-farm research to demonstrate the impact of crop rotation and other cultural practices on disease incidence and severity.
- Integrated disease management systems should be developed for specific production systems. Primarily, it should be the responsibility of the national agricultural research systems in China and Indonesia to conduct research in this area.
- Seed transmission of groundnut bacterial wilt should be verified by further research. It is of particular importance where seed from the previous crop is used to sow the next crop, and also when the moisture content of seed exceeds 9%.
- External funding is imperative, and should be sought, to support various activities of the Working Group, to promote bacterial wilt research in several countries of Southeast and East Asia, and to expand strategic research in advanced laboratories, particularly in the UK (Rothamsted Experimental Station and the Natural Resources Institute).

Participants

Participants

Australia

A C Hayward
Reader and Acting Head
Department of Microbiology
University of Queensland
Brisbane, Queensland 4072

China

Xiao Daren
Deputy Chief of Administrative Department of Sciences
Oil Crops Research Institute, Wuhan 430062

N X Duan
Head of Plant Germplasm Unit
Oil Crops Research Institute, Wuhan 430062

R M Fan
Agronomist
Agricultural Bureau of Tonghi County, Henan 47700

J P Hu
Associate Professor and Director of Legumes Laboratory
Institute of Crop Germplasm Resources, Beijing 100081

J H Huang
Assistant Director
International Corporate Office
Chinese Academy of Agricultural Sciences, Beijing 100094

Y W Rang
Plant Pathologist
Plant Protection Institute, Beijing 100094

Liang Keyong
Vice-President
Chinese Academy of Agricultural Sciences, Beijing 100094

S L Li
Plant Pathologist
Guangdong Academy of Agricultural Sciences
Guangzhou, Guangdong 564000

X Q Liang
Research Associate
Guangdong Academy of Agricultural Sciences
Guangzhou, Guangdong 564000

B S Liao
Groundnut Breeder
Oil Crops Research Institute, Wuhan 430062

G Z Mao
Research Associate
Plant Protection Institute, Beijing 100094

Guo Qingyuan
Director
Oil Crops Research Institute, Wuhan 430062

X S Song
Plant Pathologist
Shandong Peanut Research Institute, Laixi,
Shandong 266001

D R Sun
Senior Scientist
Oil Crops Research Institute, Wuhan 430062

Y J Tan
Plant Pathologist
Oil Crops Research Institute, Wuhan 430062

R H Tang
Research Associate
Guangxi Academy of Agricultural Sciences, Nanning,
Guangxi 530007

Y D Tang
Research Assistant
Plant Protection Institute, Beijing 100094

Y Y Wang
Principal Groundnut Breeder
Oil Crops Research Institute, Wuhan 430062

Zhan Xianhe
Chief Administrative Officer for Research
Oil Crops Research Institute, Wuhan 430062

Z Y Xu
Plant Pathologist
Oil Crops Research Institute, Wuhan 430062

X F Yang
Groundnut Breeder
Nanchong Agricultural Research Institute, Nanchong,
Sichuan 63700

Yan Yang
Senior Programme Officer
Division of International Cooperation
Chinese Academy of Agricultural Sciences, Beijing 100094

Wu Yong
Agronomist
Agricultural Bureau of Hong An County, Hong An,
Hubei 431500

Q G Zhang
Research Associate
Institute of Plant Protection, Nanchang, Jiangxi 330200

X Y Zhang
Groundnut Breeder
Cash Crops Research Institute
Henan Academy of Agricultural Sciences, Zhengzhou,
Henan 45002

Xu Zhide
Research Associate
Institute of Plant Protection
Hunan Academy of Agricultural Sciences, Changsha,
Hunan 41025

Indonesia

M Machmud
Bacteriologist
Bogor Research Institute for Food Crops
Jalan Tentara Pelajar 3A, Bogor 16111

Malaysia

S Hamidah
Plant Pathologist
Malaysian Agricultural Research
and Development Institute (MARDI)
PO Box 12301,50774 Kuala Lumpur

K Y Lum
Bacteriologist
Malaysian Agricultural Research
and Development Institute (MARDI)
PO Box 12301, 50774 Kuala Lumpur

Philippines

M P Natural
Plant Pathologist
Department of Plant Pathology
University of the Philippines, Los Banos, Laguna 4031

Thailand

W Butranu
Researcher (Pathology)
Khon Kaen Field Crops Research Center
Field Crops Research Institute, Department of Agriculture,
Khon Kaen 40060

United Kingdom

R Black
Bacteriologist
Natural Resources Institute
Central Avenue, Chatham Maritime
Kent ME4 4TB

A Robinson-Smith
Bacteriologist
Rothamsted Experimental Station
Harpenden, Hertfordshire, AL5 2JQ

S Seal
Bacteriologist
Natural Resources Institute
Central Avenue, Chatham Maritime
Kent ME4 4TB

Vietnam

N T Ly
Researcher
Plant Protection Research Institute
Hanoi

M T Vinh
Researcher
Institute of Agricultural Science
for South Vietnam
Ho Chi Minh City

International institutes

FAO

C Y Shen
Regional Plant Protection Officer
Food and Agriculture Organization of the United Nations
Regional Office for Asia and the Pacific
Bangkok 10200, Thailand

IPGRI

Zhou Mingde
Coordinator
Office for East Asia
International Plant Genetic Resources Institute
Beijing 100081, China

ICRISAT

C L L Gowda
CLAN Coordinator

V K Mehan
Senior Scientist (Pathology)

Y L Nene
Deputy Director General

S N Nigam

Principal Breeder

J G Ryan

Director General

International Crops Research Institute for the Semi-Arid Tropics

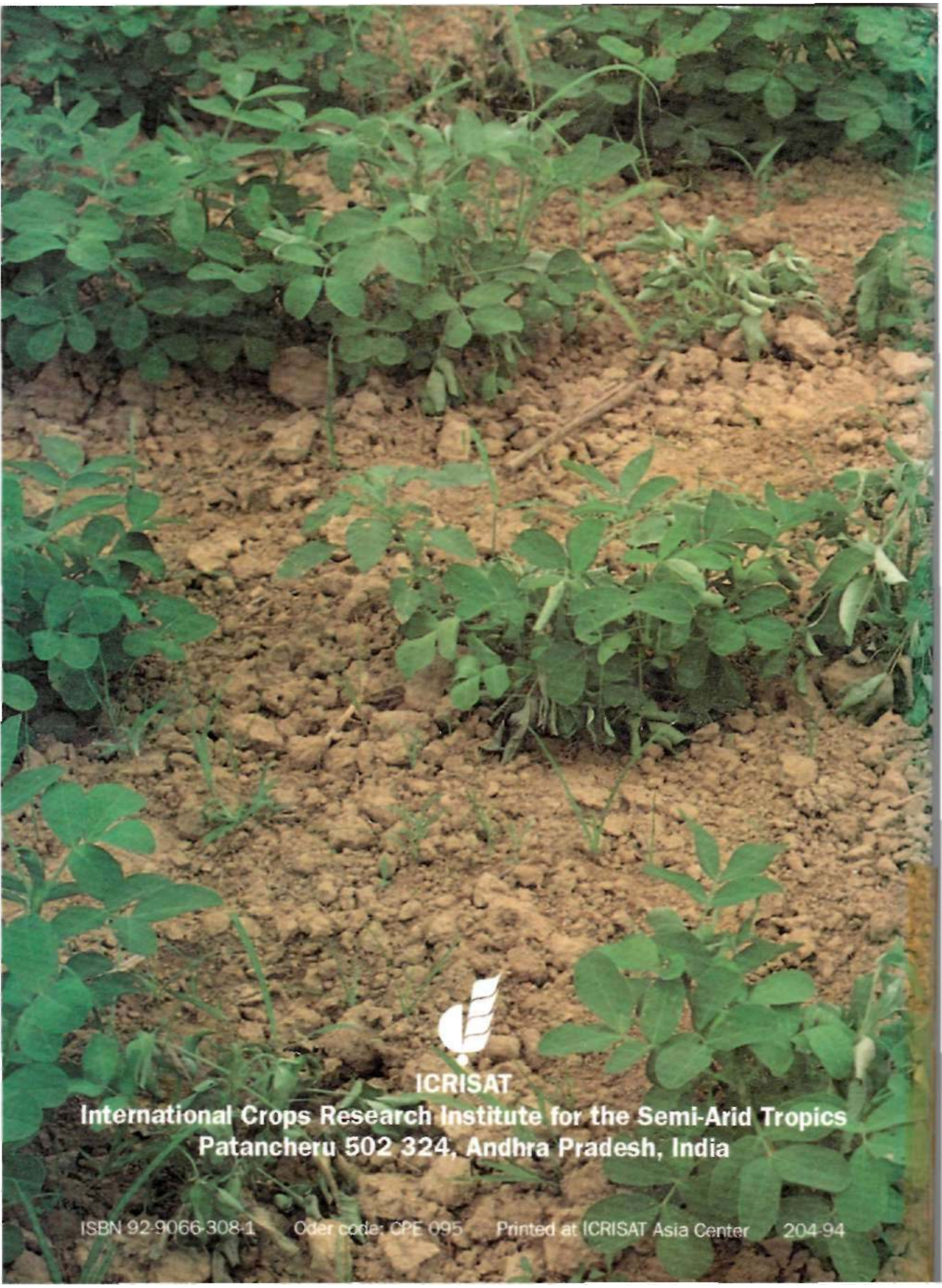
Patancheru 502 324, Andhra Pradesh, India

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.

ICRISATs 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. ICRISATs 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).



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

ISBN 92-9066-308-1 Oder code: CPE 095 Printed at ICRISAT Asia Center 204-94