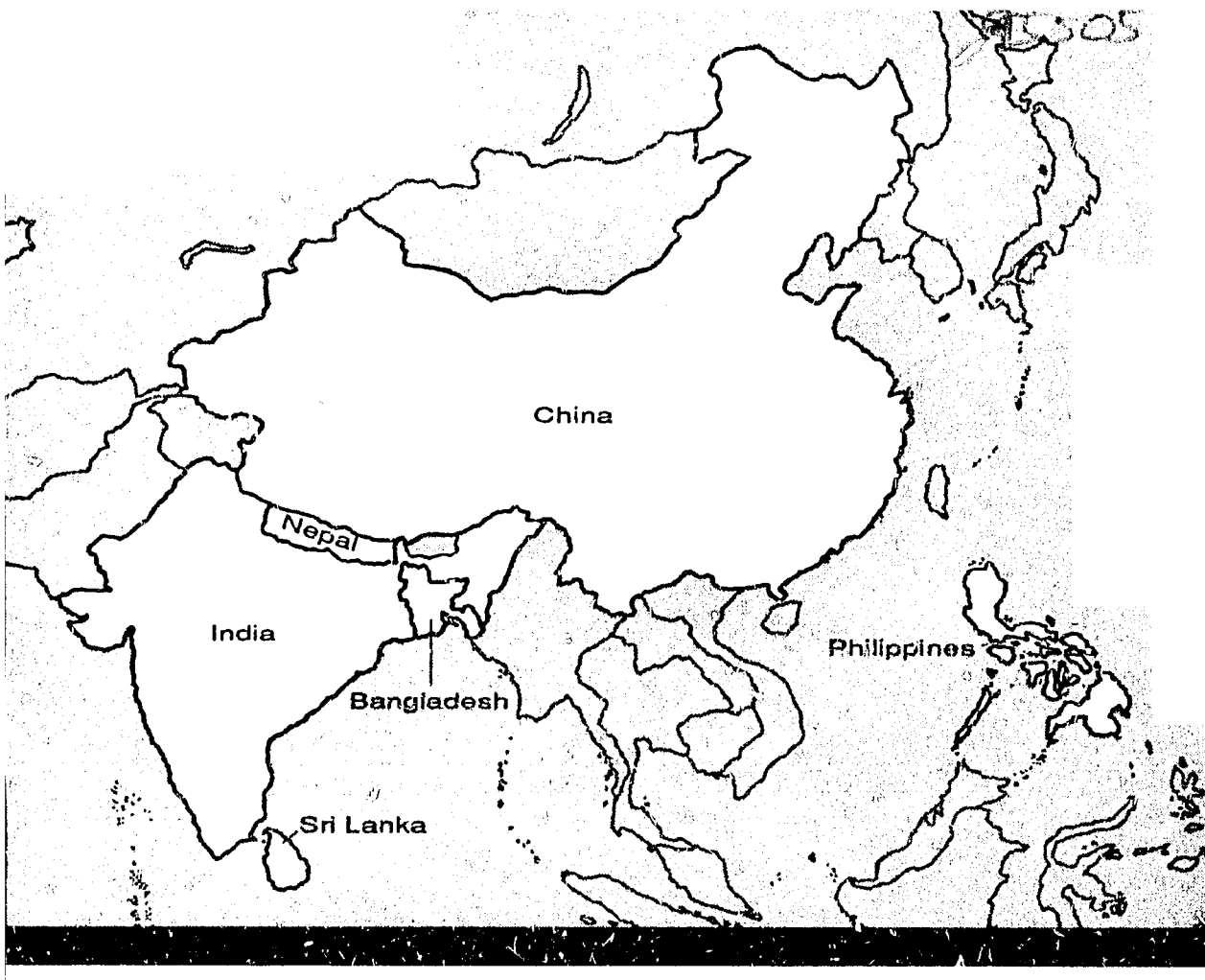
Integrated Management of Bacterial Wilt



Edited by
Bill Hardy
Edward R. French

INTERNATIONAL POTATO CENTER (CIP)

Integrated Management of Bacterial Wilt

Proceedings of an international workshop held in New Delhi, India, October 11-16, 1993

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Bill Hardy

Edward R. French

In collaboration with the
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Foreword

Potato, along with rice, wheat, and maize, has become one of the four major food crops of the world. It can be used together with cereals in intensive, diversified land use patterns, and it is often cultivated by low-income farmers to improve cash flow. Potato is a high-value crop that can also be used for weed and pest control. The potential benefits of potato as a commodity that can contribute to technological change come from its short growth period and high productivity per unit of time and area. It is an excellent food source, and provides high energy and protein production per unit of cultivated area and time, as well as important vitamins and minerals. Potato's adaptability is increased by the fact that planting materials can be multiplied in a variety of ways. Potatoes can be grown from true potato seed, stem cuttings, minitubers, or whole or cut seed tubers.

Since the early 1960s, potato production has expanded more rapidly than that of any other major root or tuber crop in Asia, Africa, and Latin America. As its availability has grown, its dietary role in many countries has significantly broadened. Although consumption trends show that potato is a popular food in the developing world, greater consumption is constrained by high prices. Losses caused by potato diseases and pests are responsible for a substantial part of this problem. If the potato is to be grown more extensively in developing countries, these losses must be dramatically reduced.

This international workshop focuses on one of the most important diseases of potato: bacterial wilt. This disease is second only to late blight in worldwide importance. CIP has begun a vigorous campaign to develop and promote the use of integrated management of bacterial wilt. CIP's approach involves individual and group training (including a series of workshops around the world) and collaborative research with institutions in developing and industrialized countries. Key elements in integrated management are crop rotation and healthy seed, which are prominently covered in several of the papers presented during this workshop and thus

published in these proceedings. But more than a dozen additional measures can be part of an integrated control program.

These proceedings include information that clearly illustrates the importance of fundamental research that has led to breakthroughs in understanding the bacterium *Pseudomonas solanacearum*, and thus to better approaches to control it. Molecular aspects of modern biotechnology are also analyzed and their potential judged. Socioeconomic considerations in designing integrated control practices are also shown to be essential in some instances.

It is apparent, therefore, that integration is needed: not only of many control measures but also of several scientific disciplines. Furthermore, the diversity of contributions to the total knowledge about bacterial wilt from participants from different institutions representing many countries stresses the need for further collaboration on a regional and even a worldwide basis.

Peter Gregory
Deputy Director General for Research
International Potato Center

Inaugural Address

Dr. K.L. Chadha
Deputy Director General (Horticulture)
Indian Council of Agricultural Research,
New Delhi, India

It is my great pleasure to be with a galaxy of bacterial wilt scientists this morning. This is the first international workshop on bacterial wilt organized in India by the International Potato Center (CIP) in collaboration with the Central Potato Research Institute (CPRI) in Shimla. Bacterial wilt of potato is a destructive disease. Its importance is evident from the priority it has received in both national and international programs in various countries.

If we look at trends in area, production, and productivity of potato during 1970-1989 in developed and developing countries, it is clear that these three factors in developing countries have increased by 42.5, 110.6, and 47.6%, respectively. These figures are substantially higher than those of developed countries, where productivity has increased 21.7% in Europe and 27.3% in North America. Area and production have declined by 39.3 and 26.2% in Europe. In North America, area has also declined by 5.5% and production has increased by only 27%. Potato in developing countries is thus gaining ground. This change is welcome. Potato is a nutritional gold mine and can supply more nutritious food faster, with less land and under harsh climate, than any other major food crop. Potato produces more calories and high-quality protein per hectare per day than any other major food plant. With almost no fat, it has better quality protein, which is nearly as nourishing as milk protein, and is rich in vitamins and minerals. The potato comes close to being a perfect source of nourishment. As a result of its natural nutritional goodness, agronomic adaptability, and culinary versatility combined with human ingenuity, it is rapidly joining the fight against world hunger, and is becoming a vital source of nutrition around the world. Potato is thus a potential solution to world hunger, even more so in developing countries.

Potato productivity is much higher in Europe and the USA. The Netherlands tops the list, with 41.3 t/ha. India, with an average yield of 16 t/ha, just equals the world's average potato yield. Other Asian countries have much lower yields—in the range of 4.2 to 11.3 t/ha. A comparison of yields obtained in India with those obtained in developed countries, or

even in experimental fields in India, shows a big gap between potential yield and yields actually obtained by farmers. This gap needs to be bridged. To keep up the pace of potato production and productivity in developing countries, we need to tackle the problems that arise. One such problem is bacterial wilt.

Pseudomonas solanacearum, the causal organism of this disease, is a unique pathogen. It has a wide host range and can infect plants belonging to several families. Its hosts include all types of plants, i.e., herbs, shrubs, and trees. The bacterium can produce all types of symptoms known for plant bacterial diseases. The ability of the bacterium to infect a large number of hosts makes its control rather difficult. In India alone, 117 hosts of the pathogen have been recorded. The bacterium causes yield losses in potato due to premature wilting of plants, and rotting of tubers in the field as well as in stores. Losses as high as 20-70% have been reported in India and other countries.

To my knowledge, the International Potato Center has done a good job in developing potato varieties that are resistant to bacterial wilt. However, the resistance available is strain specific and is not effective in warm climates like those that prevail in India and other Asian countries. The Indian potato program has identified certain agronomic practices that are recommended for management of the disease. The impact of these practices is quite evident in several areas, and average disease incidence has been reduced to 0.1-5%, compared with 10-30% found earlier. One of the most important components of disease management seems to be the assured supply of healthy seed through a scientific seed production program. For example, in India prior to the establishment of a seed production program, the disease was prevalent in more than 60% of potato area, with an average incidence of 5-50% in different areas. A scientific seed production program for potatoes was organized in India in 1966, through which disease-free seed is produced in high hills, in Northwestern and north-central regions, and parts of the eastern plains. The seed production program now meets about 55% of the country's requirements. Efforts are under way to increase seed production by involving state agricultural universities and employing rapid multiplication and micropropagation techniques. True potato seed technology is also expected to supplement current seed production. We expect that by the year 2000, 80-100% of our seed requirements will be met. With the increase in seed production and adoption of agronomic practices, disease incidence is expected to decline further.

Part of this workshop's agenda is to develop ways to set up an Asian network for research, technology transfer, and manpower development for management of bacterial wilt of potato. India's program on bacterial

wilt of potato is quite sound on the ecology of the pathogen, pathogen detection methods, and disease management practices, including biocontrol. Asian countries can take advantage of the expertise available in the Indian program through the network we propose to develop.

I am happy to see that several Asian countries—Bangladesh, Bhutan, Indonesia, Nepal, Philippines, Sri Lanka, Thailand, China, and Vietnam—are participating in this workshop. Eminent research scientists have also come from CIP (Lima, Peru), Australia, and the U.K. to cover newer aspects of pathogen ecology and detection, disease epidemiology, host resistance, and genetic variation in the pathogen. I am also happy to note that out of 29 participants, 11 are from India, mainly from CPRI. To that extent, India is proud to host this workshop. I am sure that deliberations in this workshop will go a long way toward identifying more durable resistance sources, integrated disease management programs, better pathogen detection methods, and a better understanding of pathogen ecology and disease epidemiology. An important goal is to establish a network for information exchange, technology transfer, and manpower development.

With these words, I inaugurate the workshop and wish you all great success in your deliberations.

Chairman's Remarks

J.S. Grewal
Director, Central Potato Research Institute, Shimla, India

Dr. K.L. Chadha, Deputy Director General (Horticulture) of the ICAR, Dr. French, Dr. Holloway, Dr. Upadhya, and distinguished scientists. It gives me great pleasure to be with potato scientists specialized in bacterial wilt from various countries.

Among major crops grown in India, the potato ranks 24th in area (970,000 ha) and is fourth in production (15.7 million tons) after sugarcane, rice, and wheat. Cropped area under potato is about 8.6%, compared with about 27% under paddy, 16% under wheat, and 4% under maize. India ranks fifth on the world potato map in terms of production. Over the past four decades, cropped area has increased by 303%, production by 889%, and yield by 146%, with annual compound growth rates of 3.6, 6.2, and 2.5%, respectively.

With existing growth rates, the area under potato will increase to 1.4 million ha, yield to 22.0 t/ha, and production to 30.9 million tons by the end of the century. Thus, the National Commission on Agriculture's target of 30 million tons of potato by the year 2000 will be achieved.

Unlike European countries, 80% of the potatoes in India grow under short-day conditions as a short-duration crop, i.e., 70-90 days. Tubers are exposed to high temperatures during planting (September-October) and harvesting (February-May). The crop is irrigated and suffers mid-day water stress. The breakthrough in potato production came as a result of indigenous research and development efforts. The main achievements included:

- Development of varieties that could give economic yields under short days and a short growing season in the plains.
- Development of varieties that resist the devastating late blight disease.
- Development of a "seed plot technique" that made it possible to produce potato seed in northern India's subtropical plains and thereby provide continuous sources of seed in addition to what comes from traditional hill areas.

- Development of packages of cultural practices including nutrient management and disease and pest management.
- Development of potato seed production and certification programs.
- Development of cold storage for the subtropics and tropics.
- Partial mechanization of the potato crop in regions such as Punjab.

Indigenous technological advances made it possible to extend potato cultivation to almost all parts of the country except the warm plains of Tamil Nadu, Kerala, and Andhra Pradesh. But several constraints to increasing potato production remain, and one of them is bacterial wilt. The disease is favored by tropical climates and is important not only in India but in most Asian countries. The Central Potato Research Institute (CPRI) in Shimla has done intensive research on bacterial wilt since 1972. During the two decades that followed, an excellent understanding of the pathogen and disease in different agroclimates has been achieved.

Major achievements include:

1. Identification of agronomic practices such as crop rotations and application of stable bleaching powder for management of the disease.
2. Mapping of races and biovars prevalent in the country.
3. Biocontrol for the pathogen, which is now being tested in multilocational trials.
4. Standardization of methods for detection of the pathogen.
5. Information on basic aspects such as ecology and virulence shifts in the pathogen.
6. Pathogen survival, especially in potato tuber lenticels.

We urgently need better and more economical management of the disease, resistant varieties, and more efficient methods for pathogen detection for seed certification. The International Potato Center in Lima, Peru, and the CPRI in India have made headway. But resistance available so far is not adequate for subtropical and tropical areas. We hope that efforts under way at CIP to pool resistance from different sources will solve the problem.

I thank the International Potato Center, especially Drs. M.D. Upadhya and E.R. French, for choosing India as this workshop's venue. I am sure that the participating countries will benefit from the exchange of information and ideas during the deliberations.

I extend a hearty welcome to all participants to visit the Central Potato Research Institute in Shimla on October 15, 1993, as scheduled. We will try our best to make your visit to the Institute fruitful.

CHAPTER 1

Bacterial Wilt of Potato in Bangladesh

M.S. Ali*

Introduction

Bacterial wilt (*Pseudomonas solanacearum* E.F. Smith) of potato has been occurring in Bangladesh for a long time, but its incidence and severity increased markedly after 1982, posing a threat to seed potato production. For the past few years, commercial potato crops have also been affected by the disease, even in fields that remain flooded for 3-4 months. Limited work has been done on the disease for occurrence, distribution, severity, and screening of varieties for resistance. The information gathered so far is preliminary and inconclusive. Studies on bacterial wilt of crops, including potato, need to be done in Bangladesh by the National Agricultural Research System (NARS), in cooperation with international agencies.

Distribution of the Disease

During 1990-1993, the incidence of bacterial wilt and brown rot was determined in different regions of the country (Table 1).

Data were gathered on prone areas, devastating occurrences, and incidence in seed potato crops (Figure 1; Tables 2 and 3). Bacterial wilt was severe in farmers' commercial crops in Comilla during 1990-1991.

Sources of Primary Inoculum

A survey revealed that the sources of primary inoculum of the disease were seed, an alternate host, and infested soil. The Bangladesh Agricultural Development Corporation (BADC) is the only government agency for the multiplication of certified seeds, but it covers only about 5% of the

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Table 1. Occurrence of bacterial wilt of potato in Bangladesh (1990-1993).

Region	Average disease incidence		Crop plants affected	Estimated losses
	Wilted plants (%)	Tuber infection (%)		(%)
Northwest Bogra, Dinajpur, Pabna, Rajshahi, Rangpur	8.0	14	Tomato, brinjal, potato, tobacco	16.0
Central Comilla, Dhaka, Faridpur, Kustia, Tangail	12.0	19	Tomato, brinjal, tobacco, potato	20.0
Southern Barisal, Chittagong, Ctg Hill tracts, Jessore, Khulna, Noakhali, Patuakhali	2.5	4	Tomato, potato, brinjal	5.5
Northeast Jamalpur, Kishoreganj, Mymensingh, Sylhet	9.3	14	Tomato, potato, brinjal	15.0
Average	7.95	12.75		14.1

country's overall requirement. About 95% of the seeds are produced by the farmers themselves. But farmers do not use proper sanitation practices, rogue wilted plants, or sort tubers. So there is a chance for latent infection of seeds. Alternate hosts such as tomato, brinjal, and tobacco are grown extensively throughout the country, especially in the highlands. These crops are often grown adjacent to potato crops, without maintaining proper isolation distances. In Bangladesh, potato is a profitable crop for farmers. Therefore, crops are grown year after year in the same fields, even after the detection of bacterial wilt. This results in a buildup of soil inoculum potential.

The following factors affect disease incidence:

1. Farmers lack knowledge about it.
2. Central distribution of certified seed by BADC.

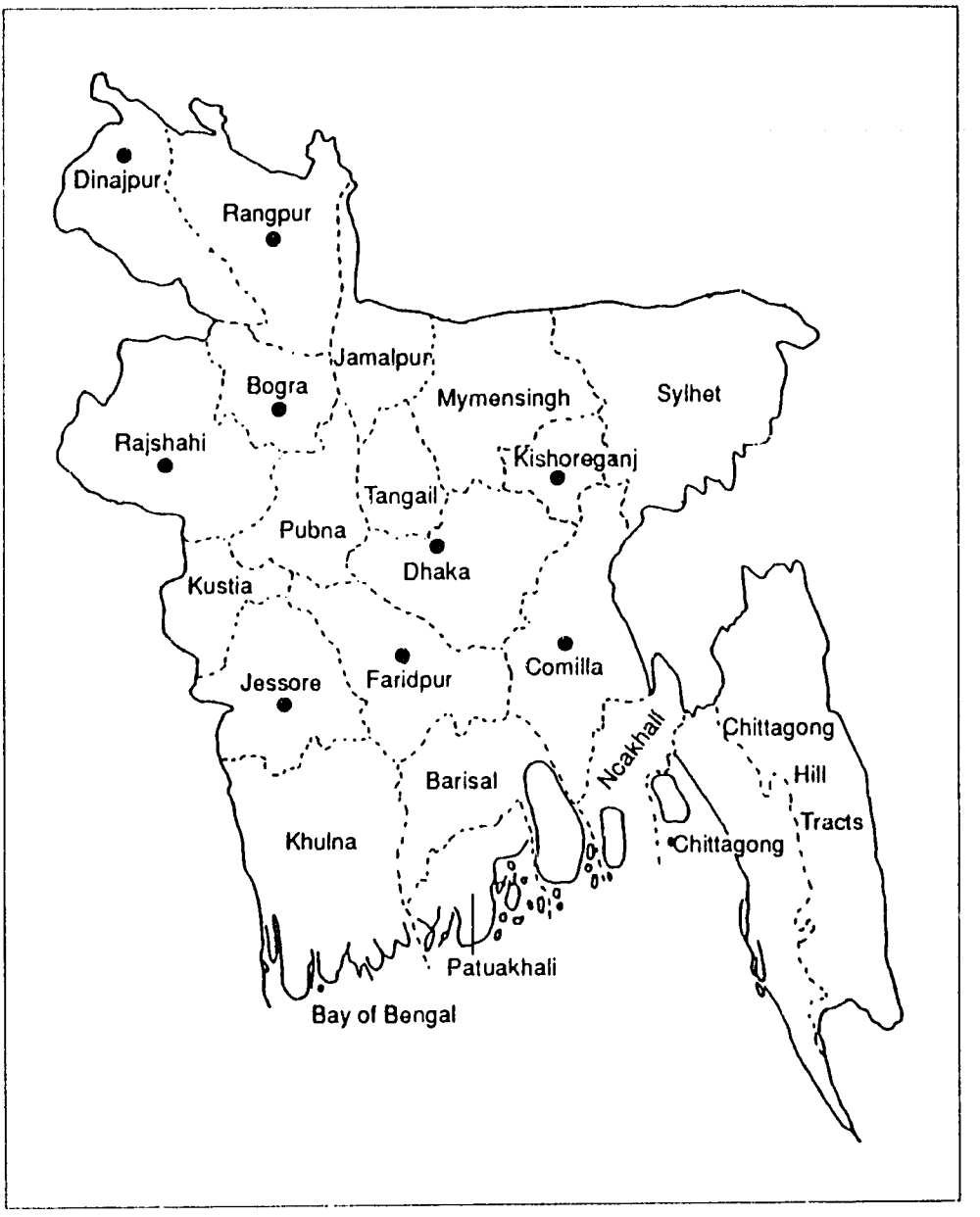

Figure 1. Areas prone to bacterial wilt (●) in Bangladesh.

Table 2. Bacterial wilt of potato, showing prone areas and years of devastating occurrence in Bangladesh.

Prone areas	Severe years
Highland (nonflooded)	
Rangpur	1982-83, 1985-88
Gazipur	1983-89
Bogra	1984-85, 1987-88
Rajshahi	1987-88
Kishoreganj	1988-89
Thakurgaon	1988-89
Jessore	1991-82
Lowland (flooded)	
Faridpur	1990-91
Comilla	1989-93
Munshiganj	1991-93

Table 3. Incidence of bacterial wilt in seed potato crops of the Bangladesh Agricultural Development Corporation during 1990-1991.

Area	Block	Variety	Severity
Faridpur	Khalpar	Cardinal	Major
	Miapara	Cardinal	Minor
Jessore	Nangarpur	Diamant	Minor
Rangpur	Ashmkhai	Patrones	Minor
	Guyarkhan	Diamant	Minor
	Shannashi	Diamant	Minor
Kishoreganj	Bashardia	Diamant	Minor
	Kaliachapra	Diamant	Minor
Kashimpur	Jarunbari	Diamant	Minor
Bogra	RDA	Cardinal	Minor

3. Heavy rain and surface irrigation.
4. Cultivation of potato in homestead areas.
5. Cultivation of two consecutive potato crops in the same field.
6. Crop is vulnerable to the disease in post-tuberization stage when temperature is higher.

Identification of the Disease

The organisms that cause brown rot affect both the growing plants and the tubers. The disease in the field was identified by the characteristic symptoms, the water test (Sequeira and Averre, 1961), and the KOH test (Gregersen, 1978).

Symptoms

Initially, leaf drooping occurs, followed by wilting. Wilted leaves may turn yellow and plants become stunted. Wilting may be expressed in one side of a leaf or branch. But, in most cases, sudden wilting predominates without yellowing. The vascular region of the stem base becomes discolored. Ooze comes out if the cut stem end is pressed. Plant symptoms are generally followed by tuber symptoms. Soil adheres to the eyes of some infected tubers as a result of oozing. Ooze is shiny and white to light cream in color. Distinct browning of the vascular tissue of the tubers is evident. The roots and stolons may also show brown vascular discoloration. Infected plants may be localized or sparsely distributed in the field.

Water test

With a blade, we cut a 2-4-cm piece from the base of the infected potato stems. The stem piece was then suspended vertically with string in clear water in a glass container. The container was kept undisturbed. Within 2-5 minutes, milky white thread-like bacterial streaming occurs at the cut end of the stem and descends toward the bottom of the container.

Potassium hydroxide (KOH) test

One or two drops of 3% KOH were put on a glass slide. Fresh ooze collected from infected tubers or stems was then placed on the KOH solution with a toothpick and mixed together for 5-10 seconds. The suspension became viscous. On drawing it with the toothpick, it formed a fine viscous thread (1½ to 3 cm in length), indicating the presence of *P. solanacearum*. This test was used in the field to distinguish between *P. solanacearum* (Gram-negative) and *Clavibacter michiganensis* ssp. *sepedonicus* (Gram-positive), as the latter does not produce the thread.

Tetrazolium chloride (TZC) medium

Isolates of *P. solanacearum* were collected from a wilted potato crop, maintained in sterile water, and then cultured on a TZC medium for separation of *P. solanacearum* by characteristic colony color.

Disease Management Practices

We advocate the following measures for potato growers to minimize bacterial wilt:

1. Use disease-free seeds.
2. Use crop rotations, mainly with cereals.
3. Rogue infected plants properly and remove surrounding soil.
4. Do not grow potato in fields where tomato, brinjal, and tobacco were grown in the past three years.
5. If possible, do not cut seed tubers.
6. Do not grow potato in homestead areas.
7. Manage irrigation properly.
8. Sort tubers properly.
9. Do not keep seed tubers coming from infected fields.

The BADC and the Breeders' Seed Production Unit of the Tuber Crops Research Centre (TCRC) are strictly following these measures for seed production.

Research Program

Recently, a research program on bacterial wilt of potato began in Bangladesh. In spite of some limitations, especially the lack of skilled manpower, a program has begun on potato, tomato, brinjal, and chili for surveys, screening of varieties, and cultural practices such as soil amendments, time of planting, grafting, integrated control, etc.

The current research program on potato includes:

1. Identification of species, races, and biovars of the causal organism of bacterial wilt of potato.
2. Integrated control of potato bacterial wilt.

3. Screening of potato varieties/lines against potato bacterial wilt.
4. A survey of bacterial wilt of potato in farmers' fields and seed production units.

Bacterial wilt caused by *Pseudomonas solanacearum* E.F. Smith is not new in the area that is now Bangladesh. Toward the beginning of the 20th century, the disease was reported in tobacco from Rangpur (Hutchinson, 1913; Butler, 1918). Hedayetullah and Saha (1941) recorded bacterial wilt in tomato from Dhaka. Studies on bacterial wilt have been done on a limited scale in Bangladesh for brinjal, tomato, and tobacco on screening for resistant varieties, soil treatment with chemicals, the use of nitrogen and trace elements, and manipulating the time of planting to control the disease (Talukdar, 1974; Ahmed and Talukdar, 1978; Ali et al., 1980; Hoque et al., 1981; Rahman and Hoque, 1988; Miah and Hoque, 1987, 1991; Mondal and Khan, 1991). The role of nematodes in bacterial wilt of tomato (BARI, 1988, 1990; Miah and Hoque, 1991) and the effect of graft culture in controlling bacterial wilt of eggplant and tomato (Mondal and Khan, 1992) were also studied.

Talukdar (1974) reported this disease in potato as a minor problem in Bangladesh, but it has now become a devastating disease, second only to late blight. The inspection team responsible for checking the quality of seed potato crops raised by the BADC has been reporting the disease since 1982 from different sites in Bangladesh (Hossain and Luitjen, 1988; Ali and Khan, 1990; Turkensteen, 1990, 1992).

Five potato varieties were inoculated artificially with *P. solanacearum* using the scissors method. All these varieties were found susceptible to wilt, with incidence between 60% and 100%. Disease symptoms became visible within 10 days after inoculation.

To control bacterial wilt of potato, two experiments in farmers' fields (previously severely infested by wilt) were conducted with the following treatments:

T_1 = Planting apparently healthy seed (farmers' own seed)

T_2 = T_1 + Full earthing at planting

T_3 = T_1 + Bleaching powder (12 kg/ha)

T_4 = T_1 + Bleaching powder (25 kg/ha)

T_5 = T_1 + Bleaching powder (50 kg/ha)

T_6 = T_1 + Bleaching powder (0.2%) and drenching at emergence

T_7 = T_1 + Bleaching powder (0.2%) and drenching at emergence and again 30 days after planting

$T_8 = T_3$ + Full earthing at planting

$T_9 = T_4$ + Full earthing at planting

$T_{10} = T_5$ + Full earthing at planting

Results indicate that all the treatments significantly reduced bacterial wilt incidence over the control (Table 4). Maximum disease control was obtained in T_9 (apparently healthy seed + bleaching powder at 25 kg/ha + full earthing at planting).

The infection potential of potato soil inoculum (*P. solanacearum*) on brinjal was studied by collecting soil samples from potato fields of Jessore, Faridpur, and Comilla representing nonflooded, long-term flooded, and short-term flooded areas, respectively. It appeared that bacteria from nonflooded and short-term flooded areas have infection potential to a certain extent. Long-term flooded soil (Faridpur) did not show any inoculum potential (Table 5). We suspect that race 1 of the pathogen prevailed in Comilla and Jessore soils while race 3 prevailed in Faridpur soil. We need studies to confirm this.

Several isolates of *P. solanacearum* collected from different potato-growing areas of the country are awaiting further study.

Table 4. Effect of integrated practices on the incidence of bacterial wilt of potato.

Treatment[a]	Wilted plants (%)		
	Farmer 1[b]	Farmer 2	Mean
1	37.08 a	15.00 a	26.04
2	22.50 b	5.41 b	13.95
3	17.50 bcd	2.08 b	9.79
4	10.41 cd	0.83 b	5.62
5	11.66 cd	1.66 b	6.66
6	15.41 bcd	5.41 b	10.41
7	13.33 bcd	2.50 b	7.91
8	12.50 bcd	0.41 b	6.45
9	8.33 d	0.41 b	4.37
10	10.41 cd	0.83 b	5.62

a. See the last section of this chapter ("Research Program") for an explanation of the treatments.
b. Means with the same letter within the same column do not differ significantly at the 1% level using Duncan's Multiple Range Test.

Table 5. Inoculum potential of different soils planted with brinjal.

Source of soil (area)	Days to wilting after transplanting	Wilted seedlings (%)
Comilla	25-30	10
Jessore	33-34	30
Faridpur	No wilt	No wilt

Bacterial wilt is a serious threat to potato production in Bangladesh. But only some limited, preliminary work has been done in the country. Therefore, detailed studies on the disease, covering factors that favor it, resistance sources, etc., need to be done in cooperation with CIP and other national and international agencies.

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CHAPTER 2

Current Status of Research on Bacterial Wilt of Potato in China

Liyuan He*

Occurrence and Spread of Bacterial Wilt in Potatoes

Occurrence of bacterial wilt (BW) in potato in China was first observed in 1965 in the Shanghai suburbs, although its damage in peanuts and other crops was recorded in the early 1930s. In the 1970s, BW in potato was limited to the southern part of China, mainly along the Yangtze river valley and its lowlands surrounding big cities. But in the 1980s, this disease moved toward high latitudes and high altitudes of low latitude regions, and it has now spread northward to the Yellow river valley and even over the Great Wall to 41° N. In southern China, it has moved to hilly potato-producing areas (He and Hua, 1985; He et al., 1987) (Table 1). Bacterial wilt of potato has been found in at least 17 provinces or regions of the country.

Table 1. Major climatic factors in potato-growing regions and severity of bacterial wilt.

Region	Range of annual temp. (°C)	Range of temp.in hottest month (°C)	Range of annual sunshine time (hr)	Range of annual rainfall (mm)	Severity of BW[a]
Northern single cropping	-4-10	10-24	2000-3000	50-1000	+
Central double cropping	10-18	22-28	1500-2750	500-1750	+
Southern double cropping	18-24	28-32	500-2500	1000-3000	+ + +
Southwestern single and double mixed	8-12	>28	1250-2750	500-1500	+ + +

a. Number of pluses (+) indicates degree of severity.

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Strains of *Pseudomonas solanacearum* in Potatoes

Seventy-four isolates of *P. solanacearum* were collected from potatoes in different locations around the country. They were identified as biovars 2, 3, and 4 based upon oxidation of three disaccharides and three hexose alcohols according to Hayward's classification scheme (Hayward, 1964, 1988). Of these, 69 isolates belonged to biovar 2, four to biovar 3, and one to biovar 4 (Table 2). Biovar 2 is considered to be a predominant strain in all potato-cropping areas of the country, including the subtropical lowlands (Table 3). A biovar 2 strain was isolated from naturally infected eggplants. Some isolates of biovar 2 were examined for pathogenicity on several common host plants. Results demonstrated that they could infect potato, eggplant, and tomato but had no or very weak virulence in peanut and tobacco.

Evaluation and Screening of Germplasm for BW Resistance

Introduction of BW-resistant germplasm

Ninety-two clones from two sets of BW-resistant germplasm were introduced from CIP-Lima in 1984 and 1985. Of these, however, only 69 clones were multiplied into a sufficient number of populations for evaluating use. An additional 8 CIP BW-resistant clones and 16 TPS tuber families were obtained in 1987 through the Vegetable Research Institute (VRI) of CAAS. In the spring of 1987, CIP provided 37 TPS progenies combining BW-resistant parents. In 1989, four sets of 64 TPS progenies containing BW-resistant genes were obtained from CIP scientists in Manila and Lima. All introduced tuber materials were multiplied in the Bashang Agricultural Institute (BSAI), Zhangbei County, Hebei Province, where the climate is good for potato growing, with less degeneration, and there is no bacterial wilt problem. After propagations of sufficient tuber seeds, the germplasm was delivered to three places: the Institute of Plant Protection (IPP), CAAS, Beijing; the Crop Research Institute (CRI), SCAAS, Chendu; and the Potato Center of South China (PCSC), Enshi, Hubei, for BW evaluation.

Establishment of BW-infected nurseries

In order to evaluate potato germplasm for resistance to different local strains of *P. solanacearum* and for adaptation to environments, three screening nurseries were established at the Penxian experimental base of CRI, CAAS, in Chendu; at the PCSC at Enshi, Hubei Province; and at the IPP of CAAS in Beijing in 1985, 1986, and 1987, respectively. An additional screening nursery representing the southern double potato-cropping

Table 2. Biovars of *P. solanacearum* in potato in China.

Carbohydrate	Biovar 2[a]	Biovar 3	Biovar 4
Maltose	+-	+	-
Lactose	+	+	-
Cellobiose	+	+	-
Mannitol	-	+	+
Dulcitol	-	+	+
Sorbitol	-	+	+
Isolates included	Po 2-8, Po 8-12, Po 15-36, Po 38-74	Po 1, Po 7, Po 13, Po 37	Po 14

a. + = oxidation reaction positive, - = oxidation reaction negative.

Table 3. Distribution of *P. solanacearum* strains in potatoes in China.

Strain	Number of isolates	Location
Biovar 2	69	12 provinces
Biovar 3	4	3 provinces (Shandong, Fujian, and Sichuan)
Biovar 4	1	1 province (Hunan)

region was established in the Agricultural Institute at Haihua of Hunan Province in 1989. The screening nurseries, except for the Beijing one, were originally BW-diseased potato fields, but they were then also inoculated to obtain uniformity in high populations of the pathogen. The Enshi nursery has 0.2 ha and is good for long-term use in BW resistance evaluation.

Results from evaluation of BW resistance

Results from evaluation of 69 clones from first two sets. Sixty-nine clones introduced from CIP in 1984-85 were evaluated for BW-resistance at three sites: Penxian of Sichuan Province, Enshi of Hubei Province, and Beijing for three to five years (He et al., 1989). Five promising clones were identified: 800928 (MS-42.3), 800935 (MS-1c.2), 377852.2, 381064.8, and 800938 (AVRDC-1287.19). MS-42.3 and MS-1c.2 showed good resistance to race 3 strains. MS-42.3 also appeared to be highly resistant to race 1 (strain Po 1). These two clones are considered to be good sources of BW resistance in breeding. CIP 377852.2 showed moderate resistance to race 3 in Penxian and Beijing, but not in Enshi, whereas 381064.8 showed moderate

resistance to the Enshi strain (race 3) and to Shangdong strain Po I (race 1) (Table 4). These two clones possess high yield potential, with some good agronomic qualities, and could therefore be used in BW resistance breeding as well as in potato production in some regions (Table 5). AVRDC-1287.19 seemed to be a source of resistance to race 1, although it was not resistant to race 3 strains at the three experimental sites (Table 4).

Results from CIP TPS progenies. Evaluation of the 98 CIP TPS progenies for BW resistance at three locations indicated that a large portion of them produced a high percentage of resistant plants, which showed good resistance to race 3 at all three experimental sites. During three years of continuous screening and evaluation in BW-infected

Table 4. Response of some CIP clones to bacterial wilt pathogen strains in different locations and years.

Clone	Penxian (race 3)[a]				Beijing (race 3)		Beijing (race 1)		Enshi (race 3)		
	'85	'86	'87	'88	'87	'88	'88	'89	'87	'88	'89
MS-42.3	MR	R	R	R	R	R	R	R	MS	MS	MR
MS-1c.2	MR	MR	-	R	-	R	MR	MR	R	R	MR
377852.2	-	MR	MR	MR	MR	MR	MR	-	R	MS	MS
381064.3	-	MS	MS	-	-	R	R	R	S	R	R
AVRDC-1287.19	MS	MS	-	-	S	S	R	R	-	S	-
800222	MS	MS	-	-	S	S	S	MR	MR	MR	-
800223	MR	-	-	-	MR	-	-	MR	MR	S	-
Check	-	S	S	-	S	S	S	MR	S	S	S

a. R = resistant, MR = moderately resistant, MS = moderately susceptible, S = susceptible.

Table 5. Yield of five selected bacterial-wilt resistant clones in BW-free fields at Zhangbei, Hebei Province (in g/plant).

Clone	1984	1985	1986	1987
MS-42.3	100	370	368	250
MS-1c.2	50	346	361	560
377852.2	-	965	1056	1390
381064.3	-	1965	1244	778
AVRDC-1287.19	500	388	542	880

nurseries, 17 BW-resistant clones with some good agronomic characteristics have been selected. Of these, clones BP 88098-7 and BP 88170-1 showed high resistance to bacterial wilt and late blight and good yield potential, and have been recommended for breeding or use in production (Table 6).

Use of CIP BW-resistant germplasm evaluated. In 1987, four clones (MS-42.3, MS-1c.2, CIP 377852, and CIP 358282) were crossed with good local cultivars Mira, Chuangfengshou, 676-4, and 694-11 by the Southern China Potato Center (PCSC). The TPS progenies received were planted

Table 6. Evaluation of new clones for resistance to both bacterial wilt and late blight in 1993.

Clone no.	Pedigree	Disease incidence		Resistance[a]		Yield
		BW (%)	LB	BW	LB	(kg/mu)[b]
BP88098-6	BR-63.5 x 676098	7.8	++++	R	S	1031
8920008	Huton x 377852.2	17.8	++++	MR	S	589
891027	Huton x AVRDC-1287.19	8.9	++++	R	S	1111
3884414-11	A29.10 x BPHM	3.3	++++	R	S	780
BP88096-1	BR-63.5 x 676098	0	+++	R	MS	1202
BP80098-3	BR-63.5 x 676098	2.2	+++	R	MS	1053
898006	Mira x MS-42.3	5.6	+	R	R	1427
890073	76-5-156 x AVRDC-1287.19	3.3	++	R	MR	1304
BP88176-1	P-7 x BW13	2.2	++	R	MR	1427
BP88105-4	BR-63.5 x P-5	3.3	+	R	R	927
BP88098-7	BR-63.5 x 676098	0	+	R	R	2313
Check (552)		43.9	+	MS	R	1379

a. R = resistant, MR = moderately resistant, MS = moderately susceptible, S = susceptible.
b. 1 mu = 1/15 hectare.

and screened in a BW-infected nursery for three years, and one advanced clone (87TS-1) has been selected. In 1988-89, CIP clones MS-42.3, MS-1c.2, 377852.2, 381064.8, and AVRDC-1287.19 served as either males or females to cross separately or in bulk with good-quality, high-yielding local varieties 78-27, Epoka, Zigan, Mira, Favorita, Hutou, Bashu No. 10, and 76-5-156 by IPP, BSAI, and PCSC. TPS progenies from the crosses were evaluated and screened for BW resistance in BW-infected nurseries in Penxian, Enshi, and Beijing. During three-four years of continuous screening and evaluation in BW-infected nurseries, 13 resistant clones were selected in 1993. Of these, clones 898006 and 897003 are highly resistant to bacterial

wilt and late blight, with high yield potential, and will therefore be recommended for breeding or use in production (Table 6).

Evaluation of local potato varieties and application of BW-tolerant cultivars. A total of 494 national potato varieties were tentatively evaluated in BW-infected nurseries at PCSC (Enshi) and IPP (Beijing). These varieties and their parents were mostly introduced from Europe and have certain good agronomic characteristics. None of them showed the desired resistance to bacterial wilt. But, in a comparative trial, a newly bred variety—783-1—had a disease index of 40, whereas the disease index of the susceptible check variety Escorte reached 100 (Table 7). As a result, this variety is considered tolerant of BW and promising for use in BW-epidemic regions of southwestern China.

Cultural Control Practices

Rotation

Rotation of potato with flooded rice is quite common in the southern potato double-cropping region, and proves to significantly reduce bacterial wilt. But this disease is still an important problem because a suitable rotation system has not been established and infection sources from tuber seeds, irrigation water, other plant hosts, and manure are ignored.

Table 7. A comparison of some potato varieties (lines) for bacterial wilt resistance at Enshi, Hubei, in 1988.[a]

Variety name	Disease index (0-100) (according to DAP)			Resistance[b]
	178	203	215	
MS-1c.2	0	0	6	R
MS-42.3	0	0	73	MS
783-1	0	0	40	MR
802-552	0	40	93	S
8302-81	0	83	87	S
827-26	4	45	90	S
7914-33 (Check)	7	69	100	S
Escorte (Check)	9	85	100	S

a. We examined 210 plants in 3 replications of each variety.
b. R = resistant, MR = moderately resistant, MS = moderately susceptible, S = susceptible.

Use of small whole tubers for seeds

In double potato-cropping regions of southern China, farmers usually use tuber cuttings as seeds for the autumn potato crop to save tubers and treat them for rapid emergence by heating, which greatly increases bacterial wilt infection. The use of short-term dormant varieties and small whole tubers as seeds has proved to be an effective measure for reducing bacterial wilt and increasing yield (Li, 1989) (Table 8). Use of this method in Xinhuang County of Hunan Province has restricted BW epidemics in recent years.

Table 8. Effects of use of small whole tubers as seeds.[a]

Treatment	Emergence rate (%)	Bacterial wilt infection (%)	Yield (t/acre)
Tuber cuttings	42	37	9.2
Whole tubers	99	2	25.6
Increase (%)	57	35	278

a. Data from W.X. Li, 1989.

Intercropping

Intercropping has proved to effectively reduce bacterial wilt in potato (Persley et al., 1988). In southwestern and southeastern parts of China, farmers usually intercrop potatoes with maize, soybean, cabbage, and other crops. But these intercropping systems are not well designed and are therefore not as effective in controlling bacterial wilt as expected.

Establishment of a seed production base

Tuber seeds with latent infection have proved to be the most important source of infection in BW-free areas. Importing tuber seeds from the northern single-cropping BW-free region is an effective way to reduce bacterial wilt in southern potato-growing regions. But this cannot be done in hilly southern potato-producing areas because of transportation problems. Establishing seed bases in BW-free locations or rice-growing areas is the best way to solve this problem. This work began in Hubei, Sichuan, and Guangdong provinces a few years ago.

Biological Control

Two avirulent bacteriocin-producing strains—AP7 and JF1—were selected from 82 *Pseudomonas solanacearum* and fluorescent *Pseudomonas*

strains and were tested as a treatment for potato tuber seeds to control bacterial wilt. These two agents showed 58% and 50% control efficiency, respectively, in the fifth week after planting in a greenhouse (Table 9). Further experiments in fields did not show the desired effectiveness in control of potato bacterial wilt. In recent years, we isolated more than 700 bacterial strains from rhizosphere soils and roots of potatoes and other crops. Of these, 120 strains showed some antagonism to *P. solanacearum*. Results from greenhouse trials demonstrated that several of the antagonistic bacteria had significant growth-promoting effects on potato, tomato, and other crops. Tentative experiments with three bacterial strains in the field at Enshi, Hubei Province, and in the western suburbs of Beijing revealed good effectiveness in reducing BW incidence and increasing potato yield (Tables 10 and 11).

Development of Monoclonal Antibodies for Detection of BW

Five hybridoma cell lines secreting monoclonal antibodies to *P. solanacearum* were established by fusing Sp2/0-Ag14 myeloma cells with spleen cells of Balb/c mice immunized by potato strains Po 41 and Po 1 (Xie and He, 1989). Glycoprotein extracts were used as immunogens and as antigens in indirect ELISA for screening specific hybridomas. Monoclonal antibodies produced by these hybridomas were examined separately for specificity against 38 strains of *P. solanacearum* from different host plants (Table 12), and 11 other species of pathovars of phytopathogenic bacteria. Monoclonal antibodies produced by five hybridoma cell lines did not react with 11 representative phytopathogenic species. Two of them, McAb3 and McAb7, reacted strongly with all 36 strains of *P. solanacearum* tested; two others, McAb1 and McAb2, reacted strongly with most strains tested; and

Table 9. Effects of tuber treatment by AP7 and JF1 on bacterial wilt of potato.

Treatment by	Days after planting				
	14	21	28	35	42
AP7 disease index	0	21	31	42	58
Control efficiency (%)	100	66	62	58	42
JF1 disease index	15	39	43	50	69
Control efficiency (%)	65	35	47	50	31
Check (water) disease index	43	60	81	100	100

Table 10. Yield-increasing effect (YIE) of antagonistic strains of bacteria on potato.

Treatment	Number of plants	Total yield (kg)	Average yield (g)	YIE (%)
C2-2	80	29.3	365 a	0.4
BP7-13	83	30.5	365 a	40.4
Check	81	21.0	260 b	-

Table 11 Effect of antagonistic bacterial strains on the control of bacterial wilt of potato in the field at Enshi.

Antagonistic	BW strain plants (%)	Control infection (%)	Yield effect (kg/mu)	Yield increase (%)
C2-2	0	100	1287	9
BD-12	0	100	1467	24
Check	17	75	1364	15
Check	87	-	1181	-

the remaining one, McAb4, reacted strongly with most strains tested except for strains Po 7, Po 41, and Po 45 from potato, and Tm7 from tomato (Table 13). The indirect immunofluorescent antibody staining procedure (IFAS) was used to detect bacterial wilt infection in potato plants and tuber seeds with McAb3. The sensitivity of IFAS was approximately 2×10^3 to 2×10^4 cells/ml. Cross reactions were not observed when using McAb3 to examine *Erwinia carotovora* ssp. *carotovora*, *E.c.* ssp. *atroseptica*, and *Clavibacter michiganensis* ssp. *sepedonicus* in potato.

Studies on Virulence Genes of the Pathogen

In order to study the virulence of *P. solanacearum* and mechanisms of pathogenesis in potato, a preliminary analysis was made of the involvement in pathogenicity of extracellular polysaccharide (EPS), pectinase, and cellulase secreted by a potato isolate of the pathogen (Mao et al., 1991). Thirty-five deficient mutants were screened from 8,000 transconjugants by mating potato strain Po 41 with Tn5 transposon. Of these, 16 mutants are deficient in EPS, 11 are deficient in

Table 12. ELISA reactions of McAbs with representative strains of *P. solanacearum*.

P. solanacearum		Reaction of				
Strain	Race	McAb1	McAb2	McAb3	McAb4	McAb7
Po 1	1	A2+[a]	2+	4+	3+	3+
Po 3	3	-	-	4+	2+	4+
Po 7	1	-	1+	3+	-	3+
Po 14	1	-	-	4+	3+	2+
Po 22	3	1+	-	4+	2+	3+
Po 37	1	1+	3+	4+	2+	4+
Po 41	3	-	-	3+	-	3+
Po 45	3	-	-	4+	-	3+
82	1	-	-	4+	2+	3+
Tm1	1	-	1+	4+	3+	4+
Tm2	1	1+	-	5+	4+	4+
Tm4	1	1+	2+	4+	3+	4+
Tm6	1	-	-	4+	3+	1+
Tm7	3	-	-	3+	-	2+
Tm9	1	-	1+	4+	4+	4+
C1	1	1+	-	4+	3+	3+
C2	1	2+	2+	5+	4+	4+
C3	1	1+	1+	4+	4+	4+
P1	1	-	-	5+	4+	3+
P6	1	1+	-	4+	3+	3+
P7	1	1+	2+	4+	4+	4+
M2	4	-	-	3+	2+	2+
M5	4	-	-	3+	2+	2+
M6	1	-	-	4+	3+	2+
B2	1	-	-	4+	3+	1+
B4	1	-	-	5+	3+	3+
Z2	1	1+	1+	5+	4+	3+
Z3	1	-	1+	4+	4+	4+
O2	1	-	-	4+	3+	3+
O3	1	-	-	4+	3+	3+
SS1	1	-	-	4+	3+	3+
Sn1	1	-	-	3+	2+	1+
E1	1	2+	2+	5+	4+	4+
Pe1	1	2+	2+	5+	4+	3+
Bn1	1	2+	1+	4+	3+	3+
Tb2	1	-	-	5+	4+	4+

a. Absorbance 490 nm; 0.00-0.05 (-), 0-05-0.10 (1+), 0.10-0.20 (2+), 0.02-0.5 (3+), 0.5-1.00 (4+), 1.00-1.80 (5+).

Table 13. Use of McAb3 to detect bacterial wilt infection in tuber seeds by IFAS.

Sample	Cultivar	No. of tubers with suspected symptoms /25 tubers sampled	IF cells/ ml (average)
1	Huaishu no. 6	5	>26
2	Hubei Yang shu	2	<5
3	Hubei Yang shu	0	0
4	Huai shu no. 6	6	>50
5	Zhongzhai huang pi	3	>26
6	Zhongzhai huang pi	0	0
7	Zhongzhai huang pi	0	>6
8	Huai shu no. 6	2	>50
9	Xiang 864-25	4	>50
10	MS-42.3	0	0
11	Xiang 8334-10	0	0
12	Feng shoubal x MS42.3	0	0
13	Xiang 8015-15	0	0
14	1-3	0	0
15	Xiang 8334-10	1	<5
16	Xiang 8334-10	2	>6
17	III-57	2	<6
18	III-56	0	>6
19	Huai shu no. 6	0	0
20	Xiangyun 864-25	0	0

polygalacturonase (PG) and polymethylgalacturonase (PMG), 4 are deficient in endoglucase (EG, or cellulase cx), and 4 are deficient in PG, PMG, and EG. Pathogenicity tests demonstrated that either EPS, PG, PMG, or EG was important in pathogenicity to potato, but not absolutely necessary. Dot blot assays confirmed that the above-mentioned mutants resulted from Tn5 transposition into the chromosome of potato strain Po 41. A gene library of this strain, containing 2,000 cosmid clones with 20-30 kb DNA fragments, was constructed. The gene encoding EPS was successfully cloned from the Po 41 gene library. Recently, some mutants with Tn5 insertions were defective in extracellular enzymes, PG, EG, and PMG, and were characterized and identified. Four cosmids containing out genes have been successfully cloned from the Po 41 gene library.

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CHAPTER 3

Bacterial Wilt of Potato in India

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India is a land of diversity. Geographically, it is situated in the northern hemisphere, and the mainland extends between 8°4' and 37°6' N, and 68°7' and 97°25' E. Potato was introduced in this ancient land perhaps in the 16th or early 17th century by the Portuguese or the British. It is now being cultivated in about 0.53% of total cultivated area, under different agroecological settings. India produces about 5.4% (16.2 million tons) of total world production and follows the former USSR, Poland, China, and the USA in total potato production. The National Commission on Agriculture has set a target production of 30 million tons by the end of this century, which is needed to meet the growing population pressure. The country seems to have reached its limits in cultivation, as the proportion of cultivated area to the total is 45.6%, which is the highest in the world (Shekhawat et al., 1992a). Therefore, we have to increase average productivity, which is now around 16.2 tons/ha, only marginally higher than the world average. This can be achieved by either further improving productivity in the most stable land or by increasing it in areas where land is highly subdivided and farmers still practice subsistence agriculture. But bacterial wilt/brown rot of potato (caused by *Pseudomonas solanacearum*) is a major limiting factor in such areas. Therefore, more attention and resources are needed to achieve suitable management practices for bacterial wilt in endemic areas, particularly in the fragile land areas such as Himachal Pradesh, the hills of Uttar Pradesh, the northeastern states, the Chota Nagpur plateau, and the Nilgiri hills.

Distribution of Bacterial Wilt

In India, bacterial wilt of potato was first observed in 1892 by Cappel (Cappel, 1892), four years before Smith described the disease and its causal agent (Smith, 1896). The bacterial nature of this disease was established by Coleman in 1909 (Coleman, 1909). Since then, it has been reported in

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almost the entire length and breadth of the country, wherever potato is grown, though subsequently it was found not to be endemic in all areas (Shekhawat et al., 1992b). The relative importance of bacterial wilt in different zones of the country changes with time and space. The latest surveys (Shekhawat et al., 1978; Singh et al., 1986; Ram, 1989; AICPIP, 1989 and 1990; Gadewar et al., 1991) showed that the disease is endemic in the west coast from Thiruvananthapuram in Kerala to Kher in Gujarat; Karnataka; western Maharashtra and Madhya Pradesh; the eastern plains of Assam, Orissa, and West Bengal; the Chota Nagpur plateau; and Andaman and Nicobar Islands. It is also endemic in the northwestern hills up to 2200 m.a.s.l.; the eastern hills of West Bengal, Meghalaya, Manipur, Mizoram, Nagaland, Tripura, and Arunachal Pradesh; and in the Nilgiris, Annamalai, and Palni hills of Tamil Nadu (Figure 1).

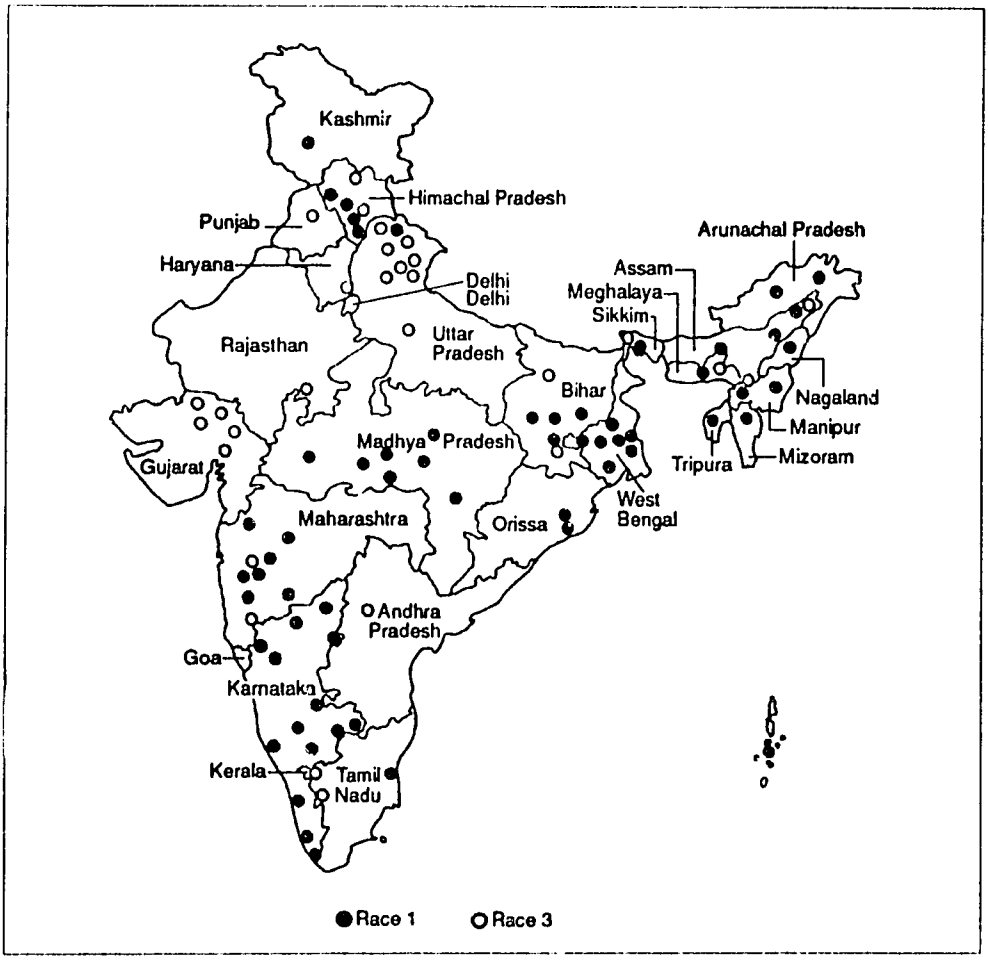

Figure 1. Distribution of *Pseudomonas solanacearum* in India.

The incidence of bacterial wilt changes with changing farming practices in a region. In the past, bacterial wilt was a limiting factor for potato cultivation in several parts of India. But lately, its incidence is gradually declining in some areas. For example, in the Pune region of Maharashtra, bacterial wilt was a severe problem during the first half of this century (Butler, 1918; Mann and Nagpurkar, 1920). Later surveys, however, revealed only a negligible level of wilt incidence (Patel et al., 1952; Bhide, 1959). The likely reasons for these changes in the bacterial wilt scenario in India will be discussed in a separate article for this workshop.

The major crop plants affected by *P. solanacearum* in India are potato, tomato, eggplant, and chilies. A recent compilation revealed that nearly 115 different plant species representing 37 different families are infected by *P. solanacearum*, with or without visible symptoms (Shekhawat et al., 1992b). Nutmeg (*Myristica fragrans* L.) and mulberry (*Morus alba* L.) were recently found to be natural hosts of *P. solanacearum* (Mathew et al., 1993a and 1993b). Potato is mainly affected by race 3/biovar 1 in the hills and race 1/biovars 3 and 4 in the plains (Shekhawat et al., 1978). Race 1 and race 3 occur in mid-hills and the northeastern hills (Kishore et al., 1991; Singh, 1993). Bacterial wilt of tomato, chilies, potato, eggplant, sesame, and peanut has also been reported in the Andaman and Nicobar Islands even in some fields newly cleared for cultivation (Ramesh and Bandyopadhyay, 1993). Whether the bacterium has been introduced in these islands and, if so, how it could get established in a jungle ecosystem have yet to be investigated. Moko disease of banana was first reported in West Bengal (Chattopadhyay and Mukhopadhyay, 1968), but its incidence and status have never been confirmed. In the meantime, race 2/biovar 1 causing moko wilt of triploid bananas has been observed in Tamil Nadu (Gnanamanickam et al., 1979; Sivamani and Gnanamanickam, 1987). Race 1/biovar 1, not encountered previously, was also recently found to be endemic in Nagaland and affects crops such as rapeseed (*Brassica napus* L.), cabbage, and tomato (Singh, 1993). Indian strains so far studied are nonpathogenic to tobacco and wilt of peanuts occurs only in pockets.

The extent of losses depends on the age of the crop affected. Premature wilting before tuber setting results in 100% loss. Wilt in later stages gives rise to infected produce, which is likely to rot in stores. In some areas, cropping pattern had to be changed due to high wilt incidence (Ghufran and Chakravarti, 1960). Losses reported are up to 55% in the Kumaon hills (Hari Kishore and Pushkarnath, 1963), around 30% in the Mani district of Himachal Pradesh (Sood and Singh, 1993), 33-40% in Maharashtra (Paharia, 1963), 20-25% in Hyderabad (Nath et al., 1958), and more than 75% in some localities of Karnataka (Gadewar et al., 1991).

Source of Inoculum and Its Perpetuation

Infected seed tuber is the most effective inoculum source for short- and long-distance dispersal of the disease (Patel and Kulkarni, 1953; Shekhawat et al., 1982). Tubers may carry the pathogen in three ways: in vascular tissues, on the tuber surface, and in lenticels (Shekhawat, 1983; Sunaina et al., 1989). In the past, epidemics of the disease in several nonendemic areas have occurred due to infected seed potatoes (Dutt, 1979). Tomato, brinjal, and chili seedlings grown in infested areas are also important inoculum sources for the disease (Keshwal, 1980). It is generally believed that *P. solanacearum* is primarily a root inhabitant and cannot survive long free in soil. In soil, plant parts affected by brown rot decay and release masses of bacteria along with copious slime. These slimy bacterial masses, when adhered to soil, may form small pellets that enhance survival of the bacterium (Shekhawat et al., 1986). Sheltered sites such as leftover plant debris (Nagaich et al., 1974), rhizospheres of nonhost plants, and annual and perennial weed hosts also facilitate long-term field survival, particularly where intensive agriculture is practiced. Recent studies suggest that the pathogen can also survive symptomlessly in the roots of weed hosts or in presumed nonhost plants (Shekhawat et al., 1990). The long-term field suvival of this bacterium is, therefore, assisted by several nonhost plants and weed hosts.

Disease Diagnosis

Detection of bacterial wilt and its causal organism is now being done by the following methods:

1. Ooze test from stem (Martin and French, 1985).
2. Ooze test in tubers. Shiny white exudates in the eyes of severely infected tubers can be observed with the naked eye. The cut surface of infected tubers, when slightly pressed, exudes milky white droplets from the vascular bundles. Apparently, healthy infected tubers may show bacterial ooze in the eyes after incubation at 28-30°C for seven days.
3. Potassium hydroxide test (Suslow et al., 1982).
4. Oxidase test (Fucikovsky, 1980).
5. Serodiagnosis. Rabbit antisera have been produced against formalin-killed whole cells, glycoprotein, and the lipopolysaccharide fraction of a race 1 isolate with 1280, 160, and 100 titers, respectively. A DAS-ELISA protocol has been

standardized, which could detect about 10^5 cfu/ml of *P. solanacearum* (Chakrabarti et al., 1993).

Management Practices

Detailed management practices being advocated will be discussed in a separate paper. In brief, management practices recommended by the Central Potato Research Institute, Shimla, follow:

1. Use only healthy, disease-free seeds. It is safer to obtain seed from known disease-free areas like the northwestern and central plains and western high hills (above 2200 m altitude). Do not cut seed tubers.
2. Follow a 2-3-year crop rotation with crops such as maize, finger millet, cereals, garlic, lupin, onion, and cole.
3. Restrict postemergence tillage to a minimum and practice full earthing-up immediately after planting. Tillage after plant emergence causes injuries to roots and stolons through which the pathogen may enter the host.
4. Collect and burn diseased plant materials and tubers after harvesting.
5. Plough soil and leave it exposed to hot summers in the plains and peninsular India, and to cold winters in the hills. Ploughing will also check weeds, which otherwise harbor the pathogen.
6. Apply stable bleaching powder at 12 kg/ha with fertilizer in furrows at planting.
7. Plant crops early, in February, to significantly reduce wilt incidence in the northeastern and northwestern hills. In the eastern and Nilgiri hills, planting the autumm crop in September reduces wilt incidence by 98%.

Research Programs

Current research programs include:

1. Identification and distribution of races and biovars of *P. solanacearum* in different agroclimatic zones.

2. Role of revertant afluidal colony types as primary source of inoculum and survival of the pathogen.
3. Identification of weed hosts and their role in epidemiology.
4. Identification of potato germplasm with bacterial wilt tolerance for potential use in breeding.
5. Standardization of serological methods for detection of *P. solanacearum* in latently infected tubers, stems, and soil.
6. Designing integrated management practices that include host resistance, agronomic practices, and biocontrol.

Research findings include:

1. Reversion of afluidal colony types to fluidal, virulent wild types was observed in a few Indian isolates. The rate of reversion increased in the presence of antibiotics (oxytetracycline and tetracycline) and higher temperature.
2. *P. solanacearum* can infect parenchymatous tissue of potato through lenticels and cause localized decay lesions. It can also survive in lenticels latently.
3. The bacterium causes local lesions in roots of several nonhost plants.
4. Biocontrol of bacterial wilt/brown rot is possible by treating seed tubers with *Bacillus* sp., *Pseudomonas fluorescens*, and actinomycetes.
5. Crops such as wheat, barley, maize, finger millet, sorghum, onion, garlic, cabbage, cauliflower, knol-khol, carrot, and horse gram can reduce the *P. solanacearum* population in the soil by 70-90% and wilt incidence by 80-90%.
6. ELISA for detection of *P. solanacearum* has been standardized.
7. The distribution of races and biovars in different agroclimatic zones has been determined.

In addition to current research programs, investigation on the following aspects will be undertaken in the future.

1. Occurrence of phenotypic reversion and its extent in Indian isolates (races 1 and 3).
2. Molecular genetic analysis of reversion observed in Indian isolates.
3. Standardization of NCM-ELISA for detection of *P. solanacearum*.
4. Possibilities of virulence modulation of *P. solanacearum* in plants.
5. Mechanisms of wilt tolerance in potato.

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CHAPTER 4

Bacterial Wilt of Potatoes in the Northeastern Hills of India

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The northeastern hill region, comprising the states of Arunachal Pradesh, Assam, Manipur, Meghalaya, Mizoram, Nagaland, and Tripura, lies between 21.5° and 29.5° N and 89.4° and 97.25° E. The region occupies 255,090 km^2. Its climate varies from tropical to temperate. Altitude ranges from 73 to 5000 m.a.s.l., thus representing climates of unique diversity. The total area under potato in the region is 88,000 ha (2.42% of the net cropped area). The average potato yield in the region is only 6.7 t/ha compared with India's overall average of 16 t/ha. This shows that there is still much room for improving yield. One problem is bacterial wilt, which occurs throughout the region, with incidence ranging from 0.5 to 25%. However, little was known about pathogen survival. Therefore, a study was begun in 1985 at the Central Potato Research Station located in the Khasi hills of Meghalaya, at 1600 m.a.s.l.

Materials and Methods

Field incidence and occurrence of races and biovars

Surveys in the east Khasi hills were conducted during six years (1985-1990) and wilt incidence was periodically studied in potato and other solanaceous crops. Five localities within a radius of 40 km from Shillong were surveyed by random selection in five plots each having 45 to 50 plants per plot. Pure cultures of the pathogen from different localities of this region were established from potato, tomato, brinjal, and chilies and maintained on tetrazolium chloride agar (Kelman, 1954). Isolates from different hosts/localities were characterized into races and biovars according to the system of Hayward (1964) and Buddenhagen et al. (1962).

Population of *P. solanacearum* in the soil

The population was determined in two infested plots. In one plot,

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potato (cv. Kufri Jyoti) was cultivated for two years in the summer and autumn seasons. The second plot was fallow for two years. Soil samples were taken in February, 30 days after emergence, at harvest of the summer crop (July), and at harvest of the autumn crop (November), each time from nine random spots up to 30 cm depth per plot. The samples were mixed to obtain a composite sample and were processed as described by Skekhawat and Perombelon (1991) for estimating the *P. solanacearum* population.

Latent infection in tubers and lenticels

Three thousand apparently healthy tubers were collected from a summer crop grown from disease-free seed in an infested field and stored in baskets at temperatures ranging from 5 to 27°C. They were cut after six months to record vascular infection. Samples of 200 tubers were also collected from a potato crop harvested on five different dates (Table 1) in the summer and autumn season and were stored in a country store at room temperature. Five lenticels from each tuber were excised every month with the help of a sterilized needle and were macerated in sterilized water with a mortar and pestle. The macerate was plated on crystal violet tetrazolium agar (Granada and Sequeira, 1983).

Latent infection in symptomless weed hosts

Examination was made for the presence of *P. solanacearum* during 1989-1990 in symptomless weeds growing in and around fields affected by bacterial wilt in various localities. Root segments of the weeds were thoroughly surface sterilized with 90% alcohol and washed with sterilized water. Macerates of root segments were plated on potato dextrose crystal violet tetrazolium agar.

Results

Field incidence of the disease and occurrence of races and biovars

In the Khasi hills, wilt incidence in potato raised under low-lying but well-drained paddy fields in the spring season did not exceed 0.5%. In the summer crop grown under raised beds and "Jhum" cultivation (a local practice of soil burning), incidence did not exceed 10% and 1%, respectively. But tuber rot was higher than wilt incidence. In an autumn crop planted after harvesting the summer crop, wilt incidence was more than 11% and in some cases was as high as 35-45% (Table 2). A crop raised on flat land suffered more wilt than one grown on raised beds. Two hundred sixty-one isolates of *P. solanacearum* from different plants collected from the northeastern region belong to race 1 and biovar 3.

Table 1. Populations of *Pseudomonas solanacearum* in lenticels of potato tubers as affected by date of harvesting.

Tuber number	Populations of *Pseudomonas solanacearum* (cfu x 10^3/lenticel) according to date of harvesting														
	A 4-VI-89			B 24-VI-89			C 14-VII-89			D 9-VIII-89			E 15-I-90		
	1	3	5	1	3	5	1	3	5	1	3	5	1	3	5
1	6.8	1.7	0.0	8.5	5.1	0.0	8.5	5.1	0.0	15.3	6.8	0.0	0.0	0.0	0.0
2	5.1	3.4	0.0	8.5	3.4	1.7	8.5	11.9	1.7	17.0	15.3	0.0	0.0	0.0	0.0
3	8.5	3.4	0.0	6.8	5.1	0.0	17.0	10.2	1.7	17.0	15.3	1.7	0.0	0.0	0.0
4	3.4	1.7	1.7	5.1	8.5	0.0	15.3	17.0	3.4	18.7	10.2	1.7	0.0	0.0	0.0
5	5.1	0.0	1.7	6.8	3.4	1.7	22.1	10.2	0.0	17.0	10.2	0.0	0.0	0.0	0.0
6	3.4	0.0	0.0	8.5	3.4	0.0	20.4	6.8	0.0	15.3	5.1	0.0	0.0	0.0	0.0
7	3.4	0.0	0.0	15.3	11.9	1.7	17.0	11.9	1.7	17.0	5.1	1.7	0.0	0.0	0.0
8	1.7	0.0	0.0	6.8	0.0	0.0	10.2	8.5	0.0	20.4	10.2	0.0	0.0	0.0	0.0
9	0.0	8.5	1.7	8.5	8.5	0.0	15.3	5.1	0.0	8.5	10.2	1.7	0.0	0.0	0.0
10	0.0	0.0	0.0	8.5	10.2	0.0	18.7	5.1	0.0	15.3	15.3	0.0	0.0	0.0	0.0
Mean	3.7	1.9	0.5	8.3	6.0	0.5	15.3	9.1	0.8	16.1	10.3	0.6	0.0	0.0	0.0
	80	50	30	100	90	30	100	100	40	100	100	40			
	Percentage of tubers carrying inoculum														

a. A, B, C, D, and E are samples collected on different dates; 1, 3, and 5 are months after harvesting.

Table 2. Percentage incidence of bacterial wilt in potato and other cultivated crops in the east Khasi hills.

Year	Potato			Cultivated crops				
	Spring	Summer	Autumn	Ginger	Peanut	Brinjal	Chili	Tomato
1985	0.0-0.5	3-5	16-31	0.3-11	—	3-7	5-9	3-11
1986	0.3-0.5	4-10	11-19	0.5-7	—	5-13	3-7	9-15
1987	0.0-0.3	3-10	30-35	1-7	—	3-9	4-10	7-13
1988	0.0-0.3	3-8	13-45	1-3	—	1-5	1.7	3-15
1989	0.1-0.5	5-10	14-26	2-3	—	2-1	13.7	4-13
1990	0.1-0.5	5-8	18-24	5-17	2-5	7-15	1-5	3-17
1991	0.4-0.5	6-10	19-31	3-11	7-11	17-26	3-7	8-12
Mean	0.12-0.4	4.1-8.7	17.4-30.3	1.8-9.0	4.5-8.0	5.4-12.2	2.8-7.4	5.2-13.7

All the isolates were pathogenic to potato, chili, tomato, and brinjal and used lactose and maltose, and oxidized mannitol and sorbitol (Tables 3 and 4). Hence, it appears that in this region the solanaceous race is predominant.

Population of *P. solanacearum* in the soil

The initial population of *P. solanacearum* in the plot cultivated with potato and in the other kept fallow was the same, i.e., 2.4 x 10^2/cfu/g of soil (Table 5). In the plot with potato, the increase in population during the cropping season was more (maximum 8.0 x 10^5/g of soil) compared with

Table 3. Identification of biovars from various isolates of *Pseudomonas solanacearum*.

Host	Locality	No. of isolates analyzed	Biovar no.
Potato	Meghalaya, Mizoram, Assam, Arunachal	132	3
Tomato	Maghalaya	29	3
Pepper	Meghalaya	20	3
Brinjal	Meghalaya	21	3
Ginger	Meghalaya	11	3
Peanut	Meghalaya	12	3
Polygonum hydropiper	Meghalaya	35	3
Solanum	Meghalaya	1	3

Table 4. Cross inoculation of isolates for the identification of races of *Pseudomonas solanacearum*.

Inoculated plants	Isolates from:				
	Potato	Chili	Tomato	Datura	Tobacco
Potato	+	+	+	+	*a
Tomato	+	+	+	*	*
Chili	+	+	+	*	*
Brinjal	+	+	+	*	*
Polygonum hydropiper	+	+	+	*	*

a. * = Clear symptoms did not appear. The plants recovered after initial wilting.

Table 5. Populations of *Pseudomonas solanacearum* in the soil.

Treatment	Soil condition			Soil bacterial population			
	Texture	pH	Wilt (%)	Before planting (Feb-March)	30 days after germination in May	At harvest (July)	After harvest (Aug-Nov)
Plot under potato crop (Kufri Jyoti)	Sandy loam	6.2	21	2.5×10^2	8.0×10^5	8.0×10^7	1.6×10^4
Plot under fallow	Sandy loam	6.2	—	2.4×10^2	9.6×10^2	8.0×10^5	2.4×10^5

the fallow plot (maximum 9.6×10^2/g). In both plots, the population reached the maximum in July. A decrease in population was noticed in November. Probably the low temperatures prevalent from November to March (minimum 4.7-10°C, maximum 17.8-21°C) were responsible for the decrease in bacterial population.

Survival in tubers and lenticels

Visible and latent vascular infection in tubers was 9.3% and 6.1%, respectively. Analysis of tubers harvested on four different dates revealed that 80-100% of tubers from the summer season carried a bacterial population in the lenticels (Table 1). A long delay in harvesting the summer crop results in a high percentage of infected lenticels containing a higher bacterial population. Lenticel colonization occurred in tubers harvested from June 4 to July 14. The bacterium survived up to 150 days in the lenticels, though the population and the number of tubers carrying lenticel infection decreased. About 2% of tubers develop visible lesions due to lenticel infection during storage. Tubers from the autumn season crop harvested on January 15, 1990, did not contain a bacterial population in the lenticels. This may be due to moisture stress or low temperature or a combination of both.

Latent infection in symptomless weed hosts

About 25% of plants of *Polygonum hydropiper*, a common weed, carried *P. solanacearum* symptomlessly. The other six common weeds (*Ambrosia artimisofolia, Galinsuga parviflora, Cosmos bipinatus, Paspaltum* sp., *Anaphalis contorta*, and *Spergula arvensis*) did not show latent/visible infection.

Discussion

Bacterial wilt of potato is a problem throughout the northeastern hills and is widespread in mid-hill elevations of Aranachal Pradesh, Mizoram, Meghalaya, the plains of Assam, and valleys of Manipur, where it survives

in virulent form on cultivated crops such as potato, tomato, chilies, capsicum, brinjal, ginger, and peanut, and locally produced potato seed tubers.

Pseudomonas solanacearum is sensitive to high temperature and cannot withstand exposure to soil temperature above 43°C (Shekhawat et al., 1978). Meteorological data recorded at the CPRI research station at Shillong indicated that mean monthly temperature ranged from 5.2-17°C minimum to 17.0-23.7°C maximum, respectively. These temperatures impose no stress on the survival of *P. solanacearum*. The presence of high soil moisture, favorable temperatures, abundant vegetation, and the practice of growing three potato crops in a year favor survival of the pathogen in fields. Infected seed tubers are the most efficient source of dispersal of the pathogen (Patel and Kulkarni 1953; Shaekhawat et al., 1982).

Several researchers have reported the role of vascular, lenticular, and surface contamination as a source of inoculum (Shekhawat et al., 1983; Sunaina et al., 1989). Our studies revealed that 9.36% and 6.1% of tubers carried vascular and lenticular infection. Analysis of tubers harvested on different dates also revealed detectable bacterial populations in lenticels up to 150 days. Weed hosts and crop plants have been reported to carry latent infection with or without symptom expression (Sunaina et al., 1989; Shekhawat et al., 1990). These hosts include potato, tomato, chilies, capsicum, brinjal, ginger, groundnut, and many weeds. *Polygonum hydropiper*, a widespread perennial weed, was found to be a potential host of latent infection in the Khasi hills. Thus, *P. solanacearum* survives in weed hosts and seed tubers.

Acknowledgments

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CHAPTER 5

Bacterial Wilt of Potato in Nepal

S.P. Dhital*

Nepal occupies an area of 141,577 km², and lies between 80°15' and 88°10' E and 26°20' and 30°10' N.

Agroclimatically, the country is mainly divided into three regions (Figure 1): the Terai/Inner-Terai/Plain (less than 300 m.a.s.l.), the mid hills (300-1800 m), and the high hills (above 1800 m), covering 17, 68, and 15% of cultivated area, respectively.

Nepal has a monsoon climate, with an average of 1600 mm rain, of which about 80-90% occurs from June through September.

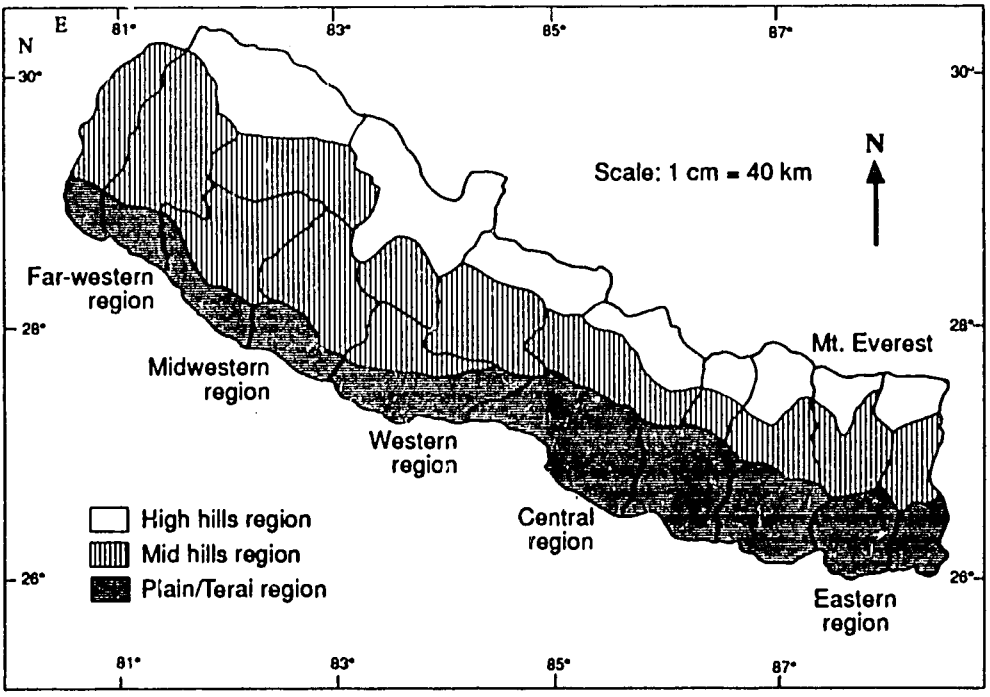

Figure 1. Map of Nepal.

* Assistant Plant Pathologist, Potato Research Programme, Nepal Agricultural Research Council, Kathamandu, Nepal.

The Nepalese economy depends largely on agriculture and related industries. Rice, maize, wheat, millet, and potato are the main staple crops. Potato ranks fifth in area and fourth in total production, and is the second most important cash crop after oil seed. The potato crop is grown from the Terai/Plain at 70 m.a.s.l. up to 4800 m. However, major potato-growing areas lie in the mid hills. High-hill areas are well known for virus-free, high-quality seed production; therefore, these areas have been a traditional seed source for low-elevation potato areas.

In 1991-1992, total area under potato was around 85,000 ha, which is about 2.5% of the country's total cultivated land (Figure 2). The estimated average potato yield is around 8.63 tons/ha. During the eighth five-year plan period (1992/1993-1996/1997), potato productivity has been estimated to increase from 8.68 to 10.87 tons/ha, at the expense of other crops. To achieve this productivity, we have outlined a policy to develop disease-resistant, high-yielding varieties, and improved pest management and production technologies.

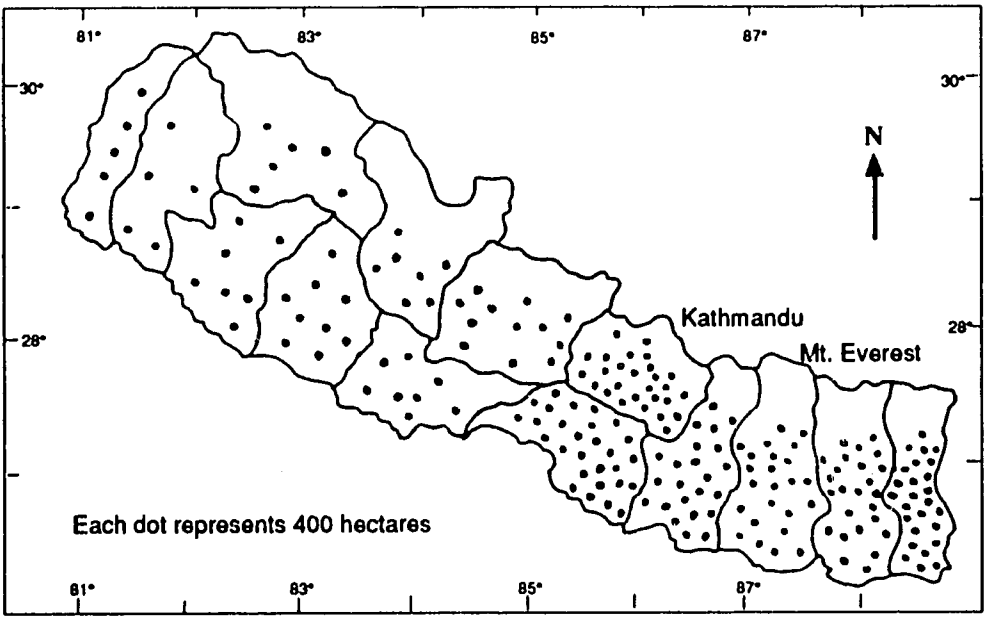

Figure 2. Distribution of potato-growing areas in Nepal.

Several factors limit potato production. Among these, diseases are probably the most important. Seventeen important potato diseases have been reported so far (Shrestha, 1978). Of these, nine are fungal, two are bacterial, and six are viral.

Bacterial wilt, the major potato disease caused by *Pseudomonas solanacearum* E.F. Smith, is commonly known as "Khaire Pipachakke Rog"

by Nepalese farmers. It is a serious problem in major potato-growing areas of the mid to high hills. It produces minor infection in the plains (tropical) area. The disease was first reported by Pushkar Nath in 1963 (Shrestha, 1988). In 1977, Shrestha also confirmed *P. solanacearum* as the causal organism of wilted potato plants by performing physical, biological, and host-range tests (Shrestha, 1977a, 1988).

Distribution of the Disease

Region or agroclimatic zone

In 1977 and 1979, Shrestha (1977a, 1977b, 1978, 1988) made a detailed survey of bacterial wilt and identified its presence in several areas: Palung and Kathmandu valleys of the mid hills, Illam, Nuwakot, Rasuwa, Sindhupalchok, and Dolakha in high-hill districts of the central region. Shrestha (1988) reported that bacterial wilt of potato was also observed at Sikle (high hills) and Dhampus of Kaski and Palpa (mid hills) districts of the Western Development Region.

Bacterial wilt was also observed on some government farms and stations at Kakani (1500 m), Kitripur (1350 m), Daman (2500 m), and Nigale (2500 m) of the central hills, and Pokhara (900 m) of the western hills. At these farms and stations, the disease might have spread through a seed multiplication scheme (Figure 3) and seed used in experiments (Shrestha, 1977a, 1979, 1988).

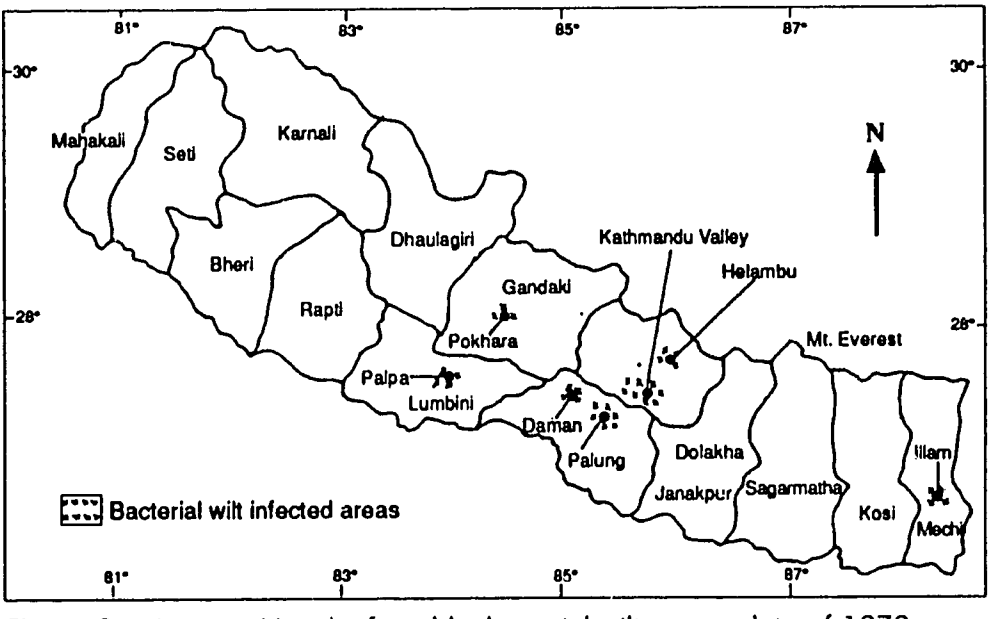

Figure 3a. Areas in Nepal infested by bacterial wilt, survey data of 1979.

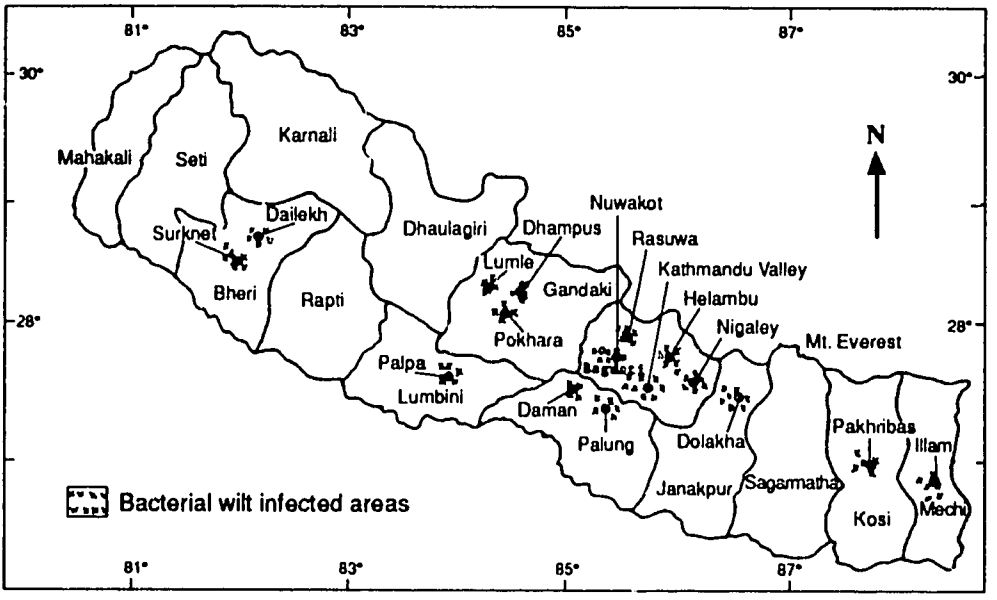

Figure 3b. Areas in Nepal infested by bacterial wilt, survey data of 1990.

The disease was also observed at two British Government Agricultural Research Stations: Pakhribas (eastern mid hills) in 1980 and Lumle (western mid hills) in 1986 (LRARC, 1991; Shrestha, 1988). This indicates that bacterial wilt of potato was severe in the subtropical to cool highlands ranging from 900 to 2500 m (Shrestha, 1978) (Table 1).

Later reports from the National Program and other institutions show that bacterial wilt spread to other potato-growing areas of the mid to high hills such as Dhading, Lamjung, Gorkha, Tanahun, Surkhet, and Dailekh districts (Elphinstone, 1992; Shrestha, 1988).

Average disease incidence

Reports indicate that crop infection increased from 5-40% in 1977 to 25-80% in 1980 and tuber rotting in stores ranged from 10 to 20% in Nuwakot, Rasuwa, Sindhupalchok, Dolakha, Kaski, Makwanpur, and Palpa districts, where the disease was identified earlier (LRARC, 1990; Shrestha, 1977a, 1977b, 1979, 1988).

Few wilted potato plants were observed on the Nucleus Seed Potato Farm, Nigale, in the month of May and 5-10% infection was recorded in surrounding farmers' fields. After two months, wilt incidence reached 75% in farmers' fields and tubers showed about 50% rotting in stores due to this disease (Shrestha, 1988). In general, disease incidence was higher in the high hills, followed by the mid hills and lowland areas.

Table 1. Distribution of bacterial wilt disease of potato in Nepal.

Agroclimotic zone	District	Location	Altitude (m.a.s.l.)	Average disease incidence in crop (%)	Crop plants affected	Races/ biovors of pothogen	Losses coused (%)
Subtropicol (Plain/Terai)	Sorlahi	Sarlahi	100	low	Tomato	-	low
Subtropical to temperate (mid hills)	Dhonkuta	Pakhribas	1,740	-	Potato	R 3, b 2	-
	Kavre	Nola	1,350	-	Potato	R 3, b 2	-
	Kathmondu	Jorpati	1,330	30	Potato	R 3, b 2	-
		Baneswore	1,320	5-20	Potato	R 3, b 2	-
		Balaju	1,320	20	Potato	R 3, b 2	-
		Shankhu	1,320	-	Potato	R 3, b 2	-
		Kirtipur Farm	1,350	-	Potato	R 3, b 2	-
	Bhaktapur	Borde	1,320	30	Potato	R 3, b 2	-
		Thimi	1,320	5-10	Potato	R 3, b 2	-
		Suryabi-nayak	1,350	5-10	Potato	R 3, b 2	-
	Nuwakot	Kakani Farm	1,500	-	Potato	-	
	Makwanpur	Polung valley	1,760	5-30	Potato	-	
	Dhading and Tanahu	Lowland area	-	-	Tomato & eggplant	Race 1	-
	Kaski	Dhampus	1,600	-	Potato	R 3, b 2	-
		Pokhara Farm	900	10-30	Potato	R 3, b 2	
		Lumle	1,675	-	Potato	R 3, b 2	
	Palpa	Kasemi	1,050	10-30	Potato	R 3, b 2	-
Temperote to cool temperate (high hills)	Illam	Jasbire, Maipokhari, Raukshe	2,000	-	Potato	R 3, b 2	20
	Dolakha	Chagu	1,820	3-5	Potato	R 3, b 2	-
		Bigu	2,430	3-5	Potato	R 3, b 2	-
		Sailung	2,000	5-50	Potato	R 3, b 2	20
	Sindhupal-chok	Helambu	2,000	10-40	Potato	R 3, b 2	-
		Dharamtheli	2,280	40	Potato	R 3, b 2	-
		Nigale Farm	2,500	75	Potato	R 3, b 2	50
	Rasuwa/ Nuwakot	Bungtang, Kimtang, Bhelche	2,000	30	Potato	R 3, b 2	10-15
	Makwanpur	Daman Farm	2,500	-	Potato	R 3, b 2	-
	Kaski	Ghandruk	2,000	25-80	Potato	R 3, b 2	-
		Sickles	2,200	-	Potato	R 3, b 2	-
		Mauja, Ulleri, Jhilebarang	1,900	20-30	Potato	R 3, b 2	>30
	Surkhet	Channa	2,000	-	Potato	R 3, b 2	-
	Dailekh	Ranimatta	-	-	Potato	R 3, b 2	-

Crop plants affected

Bacterial wilt has also been reported in tomato and eggplant in the mid hills, besides potato (Shrestha, 1977a). Shrestha (1988) reported that tomato was also infected by this pathogen to some extent in the plains area (100 m.a.s.l.). More research is needed to identify the host range of this pathogen.

Races/biovars of pathogens

Typical of the bacterium present in the mid- to high-hill regions of Nepal was race 3/biovar 2 (Shrestha, 1977a), considered to be one of the most virulent types causing bacterial wilt in potato. Race 1 has been found associated with the wilting of tomato and eggplant from the mid-hill (lowland) areas of Dhading and Tanahun districts (Elphinstone, 1992).

Losses caused

A detailed study on losses caused by this disease in Nepal is not available. However, in some pockets, average losses caused by this disease in the field reached 50% (Shrestha, 1988), whereas in general storage conditions it was 2-50% in the high hills of the Sindhupalchok district, depending upon the potato clones/varieties (Bhomi et al., 1992; Shrestha, 1976) (Table 1).

Source of Primary Inoculum

Bacterial wilt of potato was observed for the first time in the early 1960s on the Horticulture Farm, Kirtipur (Shrestha, 1988), where some new clones/ varieties from different sources were introduced. Due to lack of knowledge in disease management, bacterial wilt spread in farmers' fields of the Kathmandu and Palung valleys, mainly through infected planting material (Shrestha, 1988). Volunteer potato plants also played a significant role in survival of the pathogen in fields for a long time.

Disease was disseminated through the use of latently infected seed tubers, and was aggravated by inappropriate cultural and management practices. Studies on the alternate host range of bacterial wilt are still lacking in Nepal. The role of soil-borne inoculum and its localized transmission through irrigation water, floods, and farm equipment is of lesser importance.

Disease/Pathogen Detection Method Being Used

Several methods have been used to detect bacterial wilt disease/pathogen of potatoes in Nepal. Visual observation of infected plants and their examination for bacterial oozing is a common method used to detect the disease in the field. Tuber indexing (Florida test method) has been used regularly in the glasshouse to detect the level of infection in seed lots. A serological (NCM-ELISA) test is being initiated in collaboration with CIP.

Disease Management Practices

Production and distribution of clean seeds

Because infected seeds are an important source for spreading this disease, clean seed has been multiplied in areas free of bacterial wilt. Regular supervision and assessment of bacterial wilt incidence are carried out in seed-growing areas to standardize seed health and quality.

Bacterial-wilt-free seeds are produced through the following steps:

- Supply of initial planting material produced in the glasshouse using disease-tested in vitro plantlets. Selection of bacterial-wilt-free areas for further multiplication of basic seeds.
- Continuous supply of initial clean planting materials in seed-growing areas, including traditional seed production areas in high-elevation regions.
- Roguing of volunteer potato plants from the field.
- Use of whole seed tubers for seed production.
- Tuber indexing of seed lot after harvest.

Field management

Farmers in irrigated areas of the mid hills and plains are advised to use a crop rotation with paddy for at least two seasons and use clean seed (Hoger and Shrestha, 1982).

Farmers are also advised to use recommended cultural practices and sanitation of field and equipment. Exposing the field after ploughing for 3-5 weeks (if possible) in the hot summer is also recommended, together with the use of clean planting materials.

Extension, training, and publicity

- Different kinds of posters, bulletins, and articles (in Nepali and other local dialects) on bacterial wilt control have been published for technicians and farmers.

- The Potato Research Program (PRP) has designed a training module and materials on potato production for technicians and farmers, emphasizing management of this disease.

Research Programs

Current research

Most research on bacterial wilt is focused on integrated disease management.

1. A three-year crop rotation with finger millet, barley, upland rice, and mustard in upland areas of the mid hills.
2. A study on integrated management of bacterial wilt that includes weeding, roguing, and application of lime.
3. Fifteen tolerant clones are being screened.

Research findings

1. Races 1 and 3 of *P. solanacearum* have been identified in lower altitudes and the mid to high hills of the country, respectively (Elphinstone, 1992).
2. Disease-infected areas in major potato-growing regions have been identified.
3. A crop rotation with paddy for at least two seasons and the use of clean seed potatoes are recommended in irrigated areas of the mid hills and plains (Hoger and Shrestha, 1982).
4. Potato genotypes evaluated so far have not been found resistant to this disease, but two lines—MS 35-9 and BR 63-65—show some tolerance (Shrestha, 1988).
5. Results obtained from Nigale show that in the high hills inoculum pressure in the soil can be minimized by a crop rotation with a wheat-potato-fallow pattern (Lama, 1987).

Future research strategies

Future research in this area is not yet clear at the national level. The PRP is trying to begin a collaborative research program in various aspects of this disease with different national and international research institutions. Germplasm screening against potato bacterial wilt has been outlined in a future research program. Nepal is also hoping for further collaboration in conducting other important research activities for bacterial wilt, such as:

1. Identification of collateral weed hosts.
2. Possible crop rotation to minimize bacterial wilt in the high hills.
3. Determination of survivability of *P. solanacearum* in soils of different agroclimatic zones.

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CHAPTER 6

Evaluation of Potato Genetic Material for Resistance to *Pseudomonas solanacearum* in Bukidnon, Mindanao, Philippines

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E. Chujoy,[2]
and L. Ramos*

Introduction

Bacterial wilt (BW) caused by *Pseudomonas solanacearum* is an important disease in Bukidnon, Mindanao, considered a mid-elevated area of the Philippines. In spite of large areas suitable for potato production and favorable climatic conditions, only 110 ha have been planted with potato, with an average yield of 7-8 tons/ha (Kloos and Fernández, 1985).

A project in Mindanao started about 10 years ago through collaboration between CIP and the Department of Agriculture. This project aimed to screen different germplasm for *Pseudomonas* resistance in the region. The experimental site is located in Bukidnon, Mindanao, where bacterial wilt occurs in farmers' fields and is considered a severe constraint to increasing potato production. During the project's first year, experiments were conducted that resulted in the development of a BW nursery and field procedures for screening BW resistance.

Materials and Methods

Experiments were conducted in 2 locations—Intavas (1200 m.a.s.l.) and Dalwangan (800 m.a.s.l.)—in Bukidnon, Mindanao, Philippines. Yield and adaptation trials were conducted during the dry and wet seasons on fields with no record of potato or a solanaceous crop cultivated during the previous 3 years at Intavas. Field screening of selected clones for resistance to BW was conducted in an infected BW nursery at Dalwangan during the wet season. Race 3, biovar 2, of *P. solanacearum* is known to exist here.

Most experiments were conducted in randomized complete block designs with 4 or 6 replications. The experimental unit was a double-row plot with 10, 16, or 20 hills. Harvesting was 85 to 90 days after planting.

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Data on plant wilt were collected following French (1982). A water test was conducted during the growing period and when plants started to wilt. At harvest, all tubers were examined for external disease symptoms. Two or three tubers were cut and examined from each healthy looking hill.

Results

From 1983 to 1984, most of the genetic materials introduced derived their resistance from various sources of resistance, predominantly from *Solanum phureja* (Table 1).

Table 1. Best clones from field screening at Dalwangan.

Clone	Source of resistance
MS-35-22	*Solanum phureja*
MS-IC-2	*S. phureja*
Cruza 148	Unknown
BR-63.74	*S. phureja*
381064.10	*S. phureja, S. chacoense, S. raphanifolium*

In 1985, introductions included those with pedigrees involving *S. phureja* and wild species *S. chacoense* and *S. raphanifolium*. Clone AVRDC 1287.19 was the source of wild species resistance. Several selections from these introductions—384012.10, 384403.3, 384408.5, 384011.3, 384558.10, and 384559.6—the so-called Group IV of the CIP breeding program, proved to be adapted and resistant.

Three sets of TPS progeny evaluations were conducted at Intavas and Lantapan. The best progenies were Greta x 800938, Serrana x LT-7, and AVRDC 1287.19 x MS-35-22.

We also did experiments on cropping systems for sloping land infested with bacterial wilt to determine the effect of different cropping systems on BW incidence and whether contouring or terracing was better for long-term continuous farming. Results showed that terracing and contouring have almost the same effect on BW incidence and pathogen buildup in the soil. Monoculture potato increased the pathogen population in the soil as well as BW incidence at 3 locations. Maize and beans were the most effective crops for reducing BW population in the soil.

From 1988 to 1990, 10 clones were selected, and Table 2 shows the best five. Their pedigree indicates that they had diverse genetic origins. They are cleaned for seed potato production and for on-farm trials.

Tuber latent infection may cause high BW incidence and thus needs to be closely monitored. Blackleg and soft rot caused by *Erwinia* spp. resulted in extensive losses, but *Erwinia* is believed to be an opportunistic pathogen acting as a secondary invader.

Table 2. Best clones for high yield and low susceptibility to bacterial wilt.

Code	Parentage
BBP87022.4	384015.24 x LT-7
387585.3	HP-118 x 84.194.30
387585.5	HP-118 x 84.194.30
388014.3	(P5 x BR112-113)
BP88080.1	(AVRDC 1287.19 OP)

Issues for Future Research on Bacterial Wilt of Potato

Some issues that resulted from this and other projects include:

1. More clones have been selected for resistance to bacterial wilt in the regions. We need to verify their resistance at different locations around the world. We need to determine the level of resistance of diverse genetic materials with different sources of resistance. A concerted effort needs to be made in this direction.
2. It has been mentioned that resistance to bacterial wilt is location-specific for pathogen variability and growing conditions. Will this mean that breeding for resistance needs to be done in the regions, including the early stages of screening of germplasm of wild species?
3. Elimination or avoidance of tuber latent infection, for example through clean seed, is recognized as an effective measure for reducing or eradicating bacterial wilt disease. We have observed that some selections with a lesser degree of susceptibility have latent infection, which renders them unsuitable for further testing. We need ways to eliminate latent infection.
4. Further research on agronomic management to control bacterial wilt will be emphasized, because this disease is a major constraint in the region. We need to give more support to linkages with either private research institutions in the Philippines or those other countries interested in conducting research on bacterial wilt with CIP.

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CHAPTER 7

Bacterial Wilt in Sri Lanka

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Potato is susceptible to several bacterial diseases: bacterial wilt (*Pseudomonas solanacearum*), soft rot of stem and tubers (*Erwinia carotovora*), common scab (*Streptomyces* spp.), and pink eye (*Pseudomonas* spp). But bacterial wilt caused by *P. solanacearum* is the most destructive disease in potato production, and is found all over the island. A survey conducted by Abeygunawardena and Siriwardena (1961) revealed that this common disease restricts the expansion of potato cultivation in Sri Lanka.

Potato tubers latently infected with race 3/biovar 2 have evidently served as the vehicle for the worldwide distribution of a major pathogen from the Andes of South America.

Seed tubers infected with bacterial wilt are the most effective means of dispersal of this disease (Keshwall et al., 1978; Sunaina et al., 1989). Cool-temperature biovar 2 found in the dry zone showed how effectively this disease could be dispersed through seeds (Velupillai and Seneviratne, 1983). Distribution, disease incidence, and biovar identification (Table 1) explain the spread of the pathogen (Kelaniyangoda, unpublished). Problems in producing quality seed material locally and the short supply of quality seed material cause farmers to use uncertified seeds, leading to the wide spread of the pathogen (Velupillai, 1985).

Continuous cultivation of solanaceous crops in certain parts of the country permitted the multiplication and survival of *P. solanacearum*. Seneviratne (1976) detected it at sites in the Horton plains after a 15-year fallow period.

Kelman (1953) reported that this disease affects more than 35 plant families. Gunawardena et al. (1980) recorded several hosts that were not reported earlier in Sri Lanka. These crops included *Anthurium andraeanum*, *Curcuma domestica* (turmeric), *Psophocarpus tetragonolobus*, *Momordica eharantia* (bitter gourd), and *Croton hirtus*. Other crops reported to be

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Table 1. Distribution of biovars (*Pseudomonas solanacearum*) and wilting incidence in various crops in some regions of Sri Lanka.

Region/agroclimatic zone	Average disease incidence of wilt (%)	Crop plants affected	Races/biovars of pathogens	Losses caused
Up-country wet zone	5-10	Potato	Race 3/biovar 2 Race 1/biovar 3	Wilting of plants Rotting of tubers/poor quality
Up-country intermediate zone	5-25	Potato, Tomato Eggplant	Race 1/biovars 3 & 4	Poor keeping quality
Low-country dry zone	>1	Potato, Tomato Eggplant, Chili	Race 1/biovar 3	Complete crop failure; deterioration of soil conditions, especially for future cultivation of solanaceous crops

alternate hosts are *Zingiber officinale* (ginger), *Phaseolus vulgaris* (common bean), and *Sesamum indicum* (gingelly).

Several hosts are known to be symptomless carriers: *Amarathus gucigans, Beta vulgaris, Calendula officinalis, Cyprus rotundus, Avena sativa, Echinochloa crusgalli,* and *Glycine max* (Keshwall, 1980).

There have been conflicting reports on soil survival of *P. solanacearum.* Seneviratne (1976) reported that wilt was not completely controlled even in paddy fields that had been flooded for several years. Survival of bacteria in soil depends mainly on soil temperature. The rise in soil temperature to above 40°C in Jaffna district explains why bacterial wilt is not a serious problem in the dry zone.

Several simple and advanced techniques were used to diagnose the disease:

- Ooze test in stems and tubers,
- Potassium hydroxide test (Gregersen, 1978),
- Urease test, and
- Triphenyl tetrazolium chloride salt (TTC) (Kelman, 1954).

All these tests generally identified the pathogen in crops. Timely identification of the pathogen, especially in a seed production program, will help eliminate the pathogen in fields.

Even though numerous attempts were made to find an effective control measure for the disease, we still lack a successful method. Many researchers have advocated adopting different cultural practices to reduce/minimize disease incidence. These methods include:

1. Crop rotation with nonhosts, which reduces soil inoculum.
2. Fallow.
3. Elimination of weedy hosts.
4. Sanitation: destruction of infected plants, disinfection of implements, and removal and destruction of crop residues after harvest.
5. Use of disease-free planting material.
6. Control of nematode populations.

Several research programs were undertaken to find economically feasible control measures. These programs include experiments on:

1. Chemical control with soil fumigants such as Ch_3Br, Cl_2, copper-based agrochemicals, etc.
2. Host plant resistance.

Even though immunity has not been identified in potato, several crosses involving *Solanum phureja* were evaluated under different climatic conditions and a few promising lines were identified as tolerant. However, we found that all lines showed a breakdown in resistance with time.

Almost all commercially cultivated varieties were found to be susceptible to bacterial wilt.

Several *Solanum* species were tested to find their effectiveness in controlling this pathogen. Although significant results were not obtained, certain species were found to give satisfactory results.

Some studies are geared to use biological control measures, which have immense potential to manage bacterial wilt. These experiments include the use of:

- Avirulent strains (Hayward, 1988),
- Soil amendments with organic matter, and
- Microbial antagonists such as *P. fluorescens, Bacillus polymixa,* and actinomycetes (Kelaniyangoda, 1992).

Although several methods were tried, none gave satisfactory control. Therefore, an integrated approach should be tried. This approach should include agronomic practices such as the use of potato seed from disease-free areas, timely earthing-up, the use of chemicals (e.g., bleaching powder and copper-based agrochemicals), and exposing soil to varying soil temperature and biological agents (e.g., avirulent strains and antagonistic organisms).

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CHAPTER 8

Techniques and Potential for Biological Control of *Pseudomonas solanacearum*

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Introduction

Prospects do not look good for a bactericidal chemical treatment to reduce the incidence of *Pseudomonas solanacearum* in the soil. There is also an increasing concern worldwide about the use of toxic chemicals in agriculture. We therefore need to focus on biological control of this organism as part of the integrated control of bacterial wilt in affected crops. We need the widest possible view of what constitutes biological control, a term that should include both internal and external intervention at the level of the plant.

The Nature of Biological Control

The history of control of soil-borne diseases by biological means is not encouraging. The reasons for this are not clear, but some contributing factors include:

- The inaccessibility of soil-borne pathogens, which are usually distributed through the volume of soil occupied by plant roots.
- Failure to understand the biological activity of the biocontrol agent.
- Undue emphasis on in vitro studies.
- Lack of understanding of the numbers and distribution of the pathogen and the incidence of disease.
- Inability to measure parameters of factors that influence pathogen numbers in the soil.
- Inherent difficulties in field-testing programs.

Organisms to be used in biological control procedures are usually selected by in vitro tests on the pathogen on agar media. This procedure

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usually results in the selection of organisms that act by one or more of three mechanisms—parasitism, antibiosis, or competition. The alternative is to select biocontrol agents by soil testing, a method that is subject to greater variation and is more time-consuming (Whipps, 1992).

To date, only a few commercial products are successful control agents. These include *Agrobacterium radiobacter* for control of *Agrobacterium tumefaciens*, which causes crown gall of fruit trees; *Pseudomonas fluorescens* to prevent blotch of mushrooms, caused by *Pseudomonas tolaasii;* and *Streptomyces griseoviridis* to prevent carnation wilt resulting from infection with *Fusarium oxysporum*.

It is clear from an examination of the species of bacteria that have been investigated as potential biocontrol agents that the genus *Pseudomonas* figures prominently (Table 1). Much of this work has been carried out with fungal root diseases, but there has been little work with bacterial diseases except for bacterial wilt (summarized in Hartman and Hayward, 1993), for which isolates of *Pseudomonas fluorescens* have been shown to reduce the incidence and severity of the disease.

Table 1. Role of Pseudomonads in biocontrol of plant diseases.

Biocontrol organism	Phytopathogen	Crop
Pseudomonas fluorescens	*Pythium ultimum*	Cotton
P. fluorescens	*Gaeumannomyces graminis* var. *tritici*	Wheat
P. fluorescens	*Thielaviopsis basicola*	Tobacco
P. putida	*Erwinia* spp.	Potato
P. syringae	*Ceratocystis ulmi*	Elm

It is clear from this and other work on the use of *Pseudomonas* as a biocontrol agent for bacterial wilt that efficient delivery of the antagonistic agent along the whole root system is crucial for effective protection. Thus, effective root-colonizing properties are a prerequisite for the Pseudomonads if they are to be used as crop protection agents (Lugtenberg and de Weger, 1992). Pseudomonads function as biocontrol agents usually by one or the other of two mechanisms. Plant beneficial *Pseudomonas* spp. produce large amounts of powerful siderophores, which react with the ferric ions in their surroundings with high affinity. The ferric-siderophore complexes or the ferric ions can be taken up by the fluorescent Pseudomonads themselves, but they are then not available for the pathogenic bacteria, which as a result decrease in number or activity.

In order to combat the deleterious effects of pathogenic microorganisms effectively, the fluorescent Pseudomonads must be able to

protect the plant's whole root system. Because of the continuous flow of nutrients supplied by the root exudate, the plant root is an excellent environment for the growth of microorganisms, including deleterious species. To protect the plant root effectively from these deleterious microorganisms, the beneficial Pseudomonads must deliver their siderophores along the whole root system. Thus, efficient colonization of the plant roots is essential for effective biocontrol by bacteria. Virtually nothing is known about bacterial colonization of the plant root at the molecular level. However, we know that Pseudomonads dominate the rhizosphere. We thus conclude that they are efficient root colonizers, but the genetic and molecular reasons for this remain unclear. Factors that may contribute to the success of Pseudomonads include the rate of growth of bacteria in the rhizosphere, their ability to adhere to the plant root, bacterial motility and movement, and the survival of bacteria in the soil, particularly under adverse conditions. It has been shown that the generation time of Pseudomonads in the rhizosphere is much lower than that of other soil bacteria, which may be due to their renowned ability to use a wide range of substrates as their source of carbon and energy. Molecules of plant origin that may play a role in the interaction of bacteria and the rhizosphere include glycoproteins, described as lectins or agglutinins. These molecules have been shown to be important in the symbiotic interaction between *Rhizobium* and leguminous hosts.

Strategies for Effective Biocontrol of Bacterial Wilt

We can propose three different strategies. The first uses the principle of infectious biocontrol. The ideal organism would have been developed without recombinant DNA techniques and would be retained in the soil for several years. It would be nontoxic for beneficial organisms in the rhizosphere but would itself establish a stable population there and would need to be toxic for *P. solanacearum*. This approach is based on the hope that a bacterium with the necessary antibiotic properties would act as a shield for the plant against infection with the causal organism of bacterial wilt.

The second possibility is to vaccinate the plant to prevent infection. Trigalet and Trigalet-Demery (1990) have proposed this strategy. It was shown that mutants of *P. solanacearum* for the *hrp* (hypersensitivity and pathogenicity) genes, which as a result had lost virulence, could still invade unwounded root systems and prevent subsequent colonization by wild-type virulent strains. The degree of protection depended upon the ratio of avirulent organisms, with the need for a tenfold ratio of the avirulent strain. While encouraging as a research approach, it is difficult to

envisage how this approach could be used for effective control of normal crops.

The third approach is to cause genetic modifications of the host plants so that they do not respond to invasion by virulent strains with the sequence of events that leads to the symptoms of bacterial wilt. This approach assumes that some plant gene products either prevent or enable infection. It also assumes that there is a gene product-signal relationship involved in the interaction between the bacterium and the plant. To date, knowledge of the infection process is insufficient to permit any significant advances with this approach.

However, the potato is well suited to this approach. It is easily transformed with DNA, the *Agrobacterium* vector is highly efficient with this plant, there is good regeneration of tissue cultures, and it has an excellent system of vegetative propagation. The potato is thus ideal for transgenic breeding procedures. We will need a greater acceptance of transgenic plants for food on an international basis, but, given all the inherent difficulties in the other strategies, the transgenic approach could be the most successful for effective biocontrol of bacterial wilt.

Summary

Biological control of bacterial wilt deserves more serious consideration. But we need more information on:

- The microbial ecology of the rhizosphere.
- Microbial interactions affecting the survival of *P. solanacearum.*
- The entry of *P. solanacearum* into the roots of susceptible plants.
- The multiplication of *P. solanacearum* in susceptible plants.
- The role of *hrp* and other bacterial virulence in initiating disease.
- The identification of plant genes affecting resistance and susceptibility to bacterial invasion.

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CHAPTER 9

Biocontrol of *Pseudomonas solanacearum*

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Introduction

Bacterial wilt of potato is a serious disease in many potato-growing countries, including India. Under Indian conditions, it continues to cause moderate to severe damage. Current management practices to control the disease are agronomic practices, crop rotations with nonhost crops, and the use of resistant varieties. Disease management through crop rotation is not always successful (Kelman, 1953; Graham and Lloyd, 1979; Granada and Sequeira, 1983) and rotation practices recommended for one region do not work well at other locations due to climatic limitations and differences in the bacterial strains involved. Management through the use of resistant varieties has met with little success because resistance is strain specific and liable to alterations under nematode-infested soils and changes in temperature (Thurston and Lozano, 1968; Sequeira and Rowe, 1969; French and De Lindo, 1982; Suatmadji, 1986).

Efforts are under way everywhere to evolve alternative methods based on bioagents. *Pseudomonas solanacearum* colonizes and infects plant roots in nature; therefore, bioagents chosen should be soil or rhizosphere inhabitants. The soil is rich in microbes like *P. fluorescens and Bacillus* spp. Both are fast multipliers and rhizosphere colonizers, and inhibit one or several microbes by producing antibiotics or siderophores. Recently, several studies were conducted on these organisms for use in controlling bacterial wilt of potato. This paper evaluates the benefits of these studies in management of the disease.

Experimental Evidence for the Successful Use of Bioagents

Kempe and Sequeira (1983) investigated biological control means involving microbial antagonists and avirulent and incompatible strains of *P.*

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solanacearum (race 2). The trials were conducted under glasshouse conditions using a precise method of stem inoculations. Potato seeds were treated with a test organism or injected in seed pieces by piercing the base of germinating buds. The test organisms, two *P. fluorescens* strains, *P. syringe* pv. *glycinea*, *P. syringe* pv. *lachrymans*, and incompatible *P. solanacearum*, were inhibitory to *P. solanacearum*, whereas avirulent and incompatible strains of *P. solanacearum* and *P. fluorescens* were effective in reducing severity of the disease, particularly when plants were challenged by stem inoculations at a relatively low level of inoculum or disease progress was slow in the control plants. When plants were challenged by root inoculations, there was a significant decrease in disease severity in treated plants. But the differences were lost when roots were injured. It was suggested that protection provided by avirulent *P. solanacearum* was probably due to induced physiological resistance. McLaughlin and Sequeira (1988) used avirulent strain B 82 of *P. solanacearum* extensively to investigate its potential under different climatic conditions and challenge inoculum was applied in different ways. There was a significant decrease in severity of the disease when challenge inoculum was applied by drenching the soil or when plantings were made in infested soils. The gain obtained by seed treatments was lost when temperatures were raised to 37°C or when sunlight was less. These researchers concluded that the avirulent strain was capable of colonizing the crown region inside the plants and the root surface. The protective effect obtained was due to competitive exclusion. Higher temperatures, however, favored the growth of the pathogen and inherent resistance in the plants was overcome. Aspiras and de la Cruz (1986) evaluated *Bacillus polymyxa* FU 6 (from roots of *Ficus ulmifolia*) and *P. fluorescens* (from water) against potato bacterial wilt using susceptible cultivar Red Pontiac. Rooted but slightly injured potato stem cuttings were dipped into the test culture suspension and planted in *P. solanacearum*-infested soil in bags. Plants were kept in a growth chamber with artificial light and maintained at 22°C. *P. fluorescens* was effective in reducing wilt incidence by 51%, and colonized the roots.

In India, work on bioagents for control of potato bacterial wilt began in the early 1980s. Evaluations included mild potato viruses (X and S), bacterial endophytes from potato tubers and stems (*Bacillus subtilis*, *B. polymyxa*, *B. spaericus*, and others), and actinomycetes isolated from diverse soils. It was concluded that *B. subtilis*, *B. polymyxa*, and actinomycete isolates delay the appearance of the disease and also reduce wilt incidence and infections in tubers (CPRI, 1983-86). Gadewar and Shekhawat (1988) conducted an exploratory experiment using *P. fluorescens* isolated from potato roots, which had a lethal effect on *P. solanacearum* when grown under mixed cultures in vitro. The experiments were conducted in natural uninfested soils in pots. Soil was infested with *P. solanacearum* isolate race 1

in two different ways. Potato seeds were treated with *P. fluorescens* by dipping for five minutes and were planted in potted soil. Challenge inoculum in the form of a suspension (20 ml, 10 cfu/ml) was poured around the seeds. In other experiments, soil was infested by inoculating tomato plants with *P. solanacearum* and mixing the plant material after wilting. Temperatures ranged from 25 to 30°C. A reduction in wilt and increase in incubation time were observed.

Field experiments conducted under Indian conditions in the mid hills showed encouraging results (Shekhawat et al., 1993). Replicated trials were laid out for three years using *Bacillus* spp. and strains of *P. fluorescens*. Before planting, potato seed tubers were treated with test cultures and planted in infested soils following normal cropping practices. Isolates of *Bacillus* spp. were promising for all three years, and reduced wilt incidence by 52-79%. *P. fluorescens* reduced wilt incidence by 43-51%. Additional benefits were a reduction in progeny tuber infections and an increase in yield of 19-90%. These isolates performed equally well in glasshouse studies.

Glasshouse and field trials conducted at different locations amply demonstrate the feasibility of biocontrol for reducing damage from *P. solanacearum*. Initial trials (Kempe and Sequeira, 1983; McLaughlin and Sequeira, 1988) involved the use of moderately resistant variety Ontario, but the rest of the trials used susceptible cultivars. Only a few trials involved an antagonist from the rhizosphere of potatoes (perhaps for obtaining sufficient root colonizations), which demonstrated the usefulness of biocontrol agents. Root injuries and temperature play an important role in the successful application of bioagents and neither can be avoided under field conditions. Injury favors the entry of the pathogen and higher temperatures overcome plant resistance, favoring the pathogen.

Bioagents operate in two ways: inducing active plant resistance mechanisms, and by exclusion of pathogen by colonizations. Plants remain accessible to infections for a long period and provide multiple entry points. Achieving full saturation by colonization of bioagents for the entire life of the plant and under fluctuating field conditions may be difficult. However, it may be appealing to use bioagents in conjunction with other presently available management practices and/or resistant/tolerant genotypes. The search for good bioagents (with affinity for potato roots, and able to colonize and saturate the entire root system before *P. solanacearum* reaches the threshold of infection) needs to be continued. Potato seeds have a good delivery system for introducing bioagents because their surface area can contain a larger amount of inoculum and colonization until the roots develop.

Pseudomonas solanacearum is basically a vascular pathogen. Once it colonizes the plant vascular system, it remains inaccessible to the bioagent colonizing the root surface and rhizosphere. Therefore, a bioagent able to colonize the vascular region without damaging the plant should be preferred. We need to consider the following points before using any bioagent on a large scale.

1. Most promising bioagents are *Pseudomonas* spp. and *Bacillus* spp. Some strains are pectolytic and can cause rots in tubers under warm and humid conditions that prevail during planting in the tropics and subtropics. These species also have harmful and beneficial attributes of increasing and decreasing yields. A residual population should not have an adverse effect on subsequent crops.
2. Avirulent strains of *P. solanacearum* fulfill the requirement of root colonization, are found in plant tissue, and generate defense mechanisms. However, recent findings reveal an ability of naturally produced avirulent types to revert to virulent types. This potential for change may undermine this control practice.
3. We need to know how bioagents work, that is, whether they work by forming antibiotics, siderophores, or bacteriocins, by competitive exclusion of the pathogen, or by inducing resistance.

Future Work

Bioagents belonging to *Pseudomonas* spp. and *Bacillus* spp. with promise in disease control without an adverse effect on germination, growth, and yield will now be extensively tested throughout India, in representative agroclimatic zones, under the umbrella of the All India Potato Improvement Project.

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CHAPTER 10

A Preliminary Study on Biocontrol of Bacterial Wilt in Potato and Tomato With Beneficial Bacteria

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Bacterial wilt (BW) caused by *Pseudomonas solanacearum* is a widespread and severe disease of many crops of economic importance in China and numerous other countries. It causes severe economic damage. To control this disease, researchers have done much work, explored many methods, and gained valuable experience. But it is difficult to control BW, and no method can control this disease completely.

Biocontrol is an important practice. Many kinds of beneficial antagonistic bacteria inhabit the soil and the rhizosphere of plants, and survive within plants. These antagonistic bacteria may reduce this disease's effects. To examine this control method, we must isolate strains that have strong antagonism and a control effect on BW in the greenhouse and field, and then examine their growth-promoting effect. This will provide the basis for biological control of BW.

Materials and Methods

Test bacterial strains and test media

Seven different *P. solanacearum* strains, coming from three different plants, were used in the experiments (Table 1). Five media were used, including NA, YDC, KB, TZC, and MS.

Test plants

Plants used in inoculation tests were potato (*Solanum tuberosum*) and tomato (*Lycopersicon esculentum*).

Culture conditions

All test strains were cultured at 28°C or 30°C. Test plants first grew at 20 ± 5°C, and after inoculation at 30 ± 5°C.

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Table 1. Test bacterial strains used in experiments.

Name of strain	Host plant
Po 41	Potato
Po 37	Potato
B4	Sweetpotato
B6	Sweetpotato
P8	Peanut
P4	Peanut
P16	Peanut

Inoculation methods

Most tests used inoculation after injuring the roots. The method that placed the bacterial suspension directly into the soil (the amount of bacteria reached 10^8 cfu/g soil) was also adopted. Inoculation procedures were similar to those described by He et al. (1983) and French and Sequeira (1970).

Treatment methods for antagonistic bacteria

Seed soaking or root soaking for 5 minutes with 10^9 cfu/ml suspensions of antagonistic bacteria were used as treatments in most tests.

Examining in vitro antagonism

The antagonistic activity of all strains of *P. solanacearum* was examined on agar plates. One hundred twenty strains that showed some inhibition to *P. solanacearum* were selected. Five strains showed strong inhibition, 28 had moderate inhibition, and the rest had weak inhibition.

Experiments on growth-promoting effect in the greenhouse

Greenhouse experiments showed that some antagonistic bacteria had obvious growth-promoting effects on potatoes and tomatoes with seed soaking and root soaking treatments. Fresh plant weight and height, root weight, and number of lateral roots of treated plants were obviously higher than those of the controls (Tables 2 and 3). The results of seed treatments in peanut, soybean, and green grain with suspensions of antagonistic bacteria indicated that some strains could promote germination and decrease seed rotting.

Experiments on control effect in the greenhouse

The disease-control experiments in the greenhouse showed that five antagonistic isolates had a good control effect on BW of potatoes: 35-100%, 30-40 days after inoculation (Table 4). Six antagonistic isolates also showed a

Table 2. Growth-promoting effect of biocontrol strains BD12, C2-2, GP7-13, GC2, IC5, and fss8 on tomato in the greenhouse.

Treatment	Number of plants	Total fresh weight (g)	Total root weight (mg)	Average height (cm)	Number of lateral roots
BD12	25	17.0	767	16.2	18.5
C2-2	25	15.5	580	15.0	16.7
GP7-13	25	18.0	850	14.9	20.8
GC2	25	17.5	620	16.6	19.9
IC5	25	18.2	737	15.9	18.3
fss8	25	17.2	626	15.4	20.8
Check	25	14.2	468	14.5	15.7

Table 3. Growth-promoting effect of biocontrol strains C2-2, BD12, IC5, and fss8 on tomato in the greenhouse.

Treatment	Average height (cm)	Average no. of leaves
C2-2	10.6 a	4.8 a
IC5	10.5 a	4.9 a
BD12	9.7 a	4.2 a
fss8	7.8 ab	3.3 ab
Check	6.0 b	2.5 b

good effect on tomatoes. The control effect 74 days after inoculation was 59.4-96.8% (Table 5).

Yield-increase experiments in the field

Field experiments demonstrated that isolates C2-2, GP7-13, and BD12 increased yield and control for potatoes and tomatoes. Seed tubers of potato cv. Zhongsu No. 2 were treated by soaking them in suspensions (10^9 cfu/ml) of strains C2-2 and GP7-13 for 5 minutes, and then planting them in BW-free field plots with three replications. This experiment was conducted in Mentougon village of the Beijing suburbs March 25-July 8, 1993. Results demonstrated that bacterial strains C2-2 and GP7-13 significantly increased tuber yield up to 40% compared with untreated potatoes (Table 6).

Control effect of field experiments

A field experiment on BW was conducted at Enshi, Hubei Province, in 1993. The biocontrol strains were converted into a powdery preparation, which contained 2.5 x 10^{10} cfu/g. A 40 g biocontrol preparation was added to 300 ml of water, and then seed tubers were soaked for 5 minutes and planted in BW field plots with three replications. The control was treated

Table 4. Control effect of antagonistic strains on bacterial wilt of potato.

Treatment	Days after inoculation							
	10		25		34		40	
	DI[a]	CE (%)	DI	CE (%)	DI	CE (%)	DI	CE (%)
C2-2	0	100	14	71	32	50	45	42
BD12	5	74	16	67	37	43	44	43
GPT-13	1	95	26	43	36	44	44	43
Check	20	—	47	—	64	—	78	—

a. DI = disease index, CE = control effect.

Table 5. Control effect of biocontrol strains on tomato in the greenhouse.

Treatment	Days after inoculation							
	7		20		45		74	
	DI[a]	CE (%)	DI	CE (%)	DI	CE (%)	DI	CE (%)
GC2	16.7	87.9	22.2	74.2	22.2	74.2	36.1	59.4
C2-2	0	100	2.8	96.7	2.8	96.7	1.8	96.8
fss8	2.8	96.6	5.6	93.5	8.3	90.4	11.1	87.5
IC5	0	100	2.8	96.7	8.3	90.4	13.9	84.4
GP7-13	2.8	96.6	16.6	80.7	16.6	80.7	16.6	81.2
BD12	0	100	2.8	96.7	5.6	93.5	5.6	93.7
Check	83.3		88.1		88.1		88.8	

a. DI = disease index, CE = control effect.

Table 6. Yield-increasing effect (YIE) of antagonistic bacterial strains on potatoes.

Treatment	Number of plants	Total yield (kg)	Average yield (g)	YIE (%)
C2-2	80	28.25	365 a	40.4
GP7-13	83	30.50	385 a	40.0
Check	81	20.95	260 b	—

with water. The disease index was investigated and yield measured. Results indicated that bacterial strains C2-2, GP7-13, and BD12 could significantly control bacterial wilt in potato (Table 7).

Tomato seedlings were treated by root soaking with the three bacterial suspensions and then planted in field plots with three replications and inoculated immediately with strain Po 41. Results demonstrated that isolates C2-2, GP7-13, and BD12 had obvious control effects on tomatoes (Table 8).

Identification of antagonistic bacteria

Morphological, cultural, and physio-chemical characteristics of 120 antagonistic strains of bacteria were studied according to Schaad's Laboratory Guide and Bergey's Manual of Systematic Bacteriology. Some bacillus strains were characterized and classified on the basis of R.E. Gorden et al. (*Genus bacillus*, 1993). Strain GP7-13 is tentatively identified as *Bacillus subtilis*, BD12 as *Bacillus licheniforzs*, and C2-2 as *Bacillus macerans*.

Discussion

Antagonism and control effect of antagonistic bacteria

Examinations for antagonism showed that 20% of the isolated strains had some inhibition to *P. solanacearum*. The strength and weakness of inhibition had no direct relationship to the control effect. Experiments showed that some strains that were antagonistic in vitro had no control effect in the greenhouse and field. On the other hand, some strains were not only antagonistic in vitro but also had a good control effect in the greenhouse and field. So the isolation of strains must therefore be examined through greenhouse and field experiments besides examining inhibition in vitro.

Table 7. Control effect of antagonistic bacterial strains on bacterial wilt of potato in the field at Enshi.

Antagonistic strain	BW index	Control effect (%)	Yield (kg/Mu)	Yield increase (%)
C2-2	0	100	1287	9
BD12	0	100	1467	24
GP7-13	17	75	1364	15
Check	67	—	1181	—

a. 1 Mu = 1/15 ha.

Table 8. Control effect of antagonistic bacterial strains on tomato in the field.

Treatment	Days after inoculations							
	10		32		43		56	
	DI	CE (%)	DI	CE	DI (%)	CE (%)	DI	CE (%)
GP7-13	0		32.5	34.0	38.0	44.0	53.0	29.0
C2-2	0		12.3	75.0	26.0	61.0	26.0	65.0
BD12	0		10.9	78.0	20.0	70.0	20	73.0
Check	0		48.3		67.0		75.0	

a. DI = disease index, CE = control effect.

Combination of several control methods

Experimental results showed that biocontrol of BW was a significant practice. It could control BW effectively in potato and tomato. But it could not control BW completely. Many methods (including physical, chemical, agronomic, and biological methods) may have some control effect. According to experience, the control effect of any one method was not ideal. But if we combine these methods, such as resistant cultivars and biocontrol, the control effect may be greater.

Current problems

First, a problem of biocontrol is its inconsistent performance. Different experiments produced different results. Many factors can cause these inconsistencies, such as the host, pathogen, and other conditions

(especially the temperature and humidity). We must seek appropriate conditions and preparations, so that the beneficial bacteria can control BW effectively.

Second, we must study the mechanisms of disease control and growth promotion. The more we know about them, the better we can use biocontrol. In the meantime, we need to further develop our technology of bacteria for biocontrol.

Acknowledgments

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CHAPTER 11

Effect of Changes in Seed Sources on Potato Bacterial Wilt

G.S. Shekhawat*

Potato seed is one of the main sources of inoculum for the spread and recurrence of bacterial wilt caused by *Pseudomonas solanacearum* (Kelman, 1953; Shekhawat et al., 1992a). Other sources of inoculum are alternate hosts, symptomless carrier plants, and root remnants of host plants in deep soil layers (Shekhawat et al., 1992b; Granada, 1988; Sunaina et al., 1989). Inoculum from the latter sources would require special conditions for infection to occur, while seed inoculum can cause disease under more varied environmental conditions. Therefore, disease epidemics can occur more through seed-borne inoculum than from inoculum from other sources. Potato seed tubers carry the bacterium in vascular tissue, lenticels, and on the surface (Kelman, 1953; Sunaina et al., 1989).

Bacterial wilt in India has changed dramatically with changes in seed sources, which have evolved as follows over the years (Singh and Grewal, 1986).

1. Imports from abroad (up to 1953).
2. Mid hills, low hills, eastern plains (1933-1945).
3. High hills, mid hills, plains, and central plateau (1940-1965).
4. Northwestern high hills (from 1965) and northwestern and central plains (from 1972).

Figures 1, 2, and 3 show the movement of seed to different areas from these sources.

Bacterial wilt was first reported in India in 1892 (Cappel, 1892) in Maharashtra (southern India) and subsequently in 1909 (Coleman, 1909) in Mysore State (now Kamataka in South India). In those days, seed was imported from Europe and multiplied for 3-5 years locally; thus, the endemic pathogen gradually built up in seed stocks and caused epidemics. In these areas, several weeds are now known to harbor the pathogen without showing symptoms.

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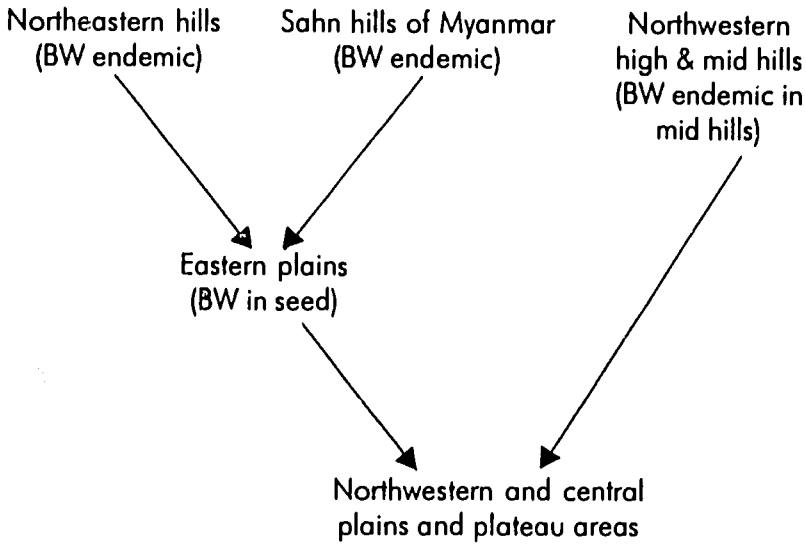

Figure1. Movement of potato seed in India during 1933-1945

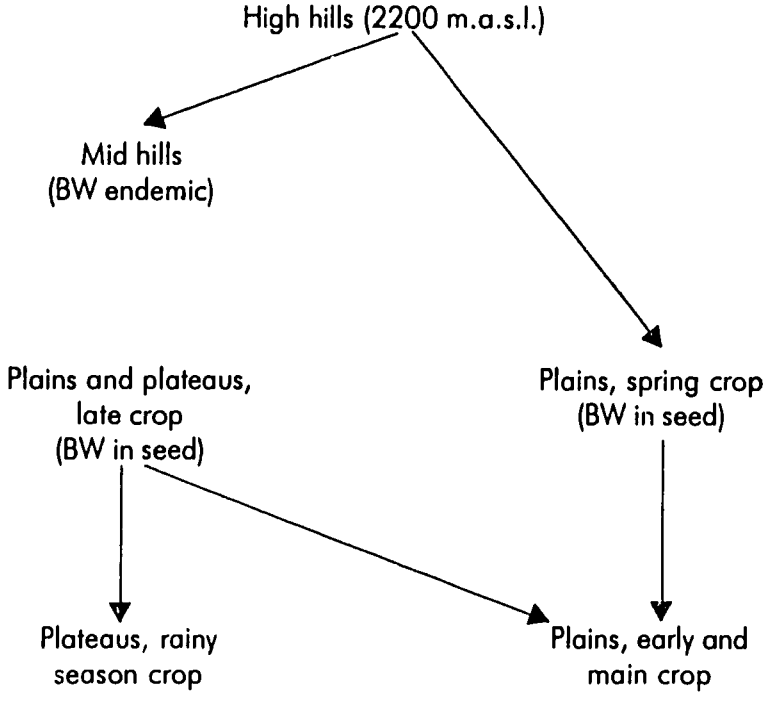

Figure 2. Movement of potato seed in India during the 1950s.

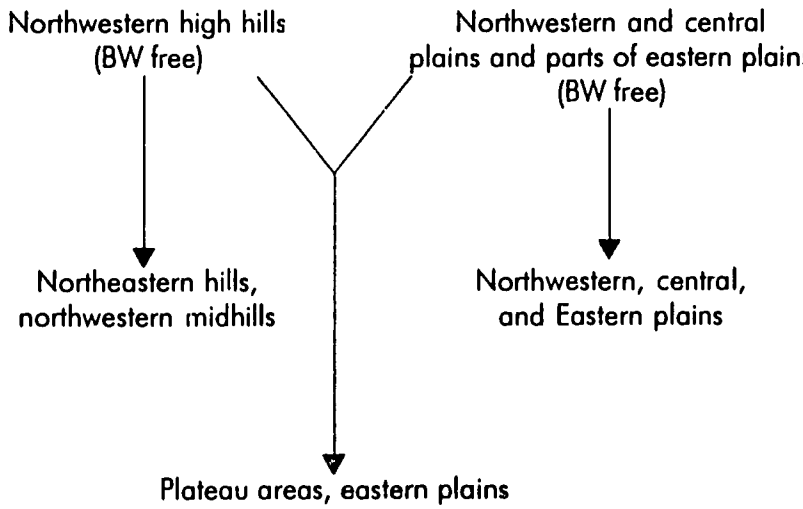

Figure 3. Movement of potato seed in India after 1972.

Seed imports were stopped in 1953 and seed became produced locally, mainly in the northeastern and northwestern high and mid hills; a limited quantity was imported from the Sahn hills of Myanmar (formerly Burma). Seed from the northeastern hills and Sahn hills used to be multiplied in the eastern plains of Bihar, Uttar Pradesh, and West Bengal. Seed from the eastern plains and from the northwestern hills used to move to the northwestern and central plains and plateau areas (Figure 1). In the northeastern hills and northwestern mid hills and parts of the eastern plains, bacterial wilt was, and even today is, endemic. The bacterium in these areas is known to survive in weeds, in tubers left in the field, and in seed tubers. Thus, inoculum constantly spread via seed tubers to all parts of the country. This pattern of seed movement continued from 1933 to 1955.

In the 1950s, we realized that potato seed grown in the hills was better due to low aphid incidence and, consequently, low virus incidence. As a result, a new seed channel developed wherein the main seed supply came from the high and mid hills (Figure 2). This seed was multiplied in the plains and central plateau late in the main cropping season and in the spring cropping season to tide over the dormancy of hill seed, and the produce was used as seed for the early and main crops in the plains. However, in this scheme, seed also moved through the mid hills and carried the bacterial inoculum. Seed produced during the autumn crop in the mid hills carried latent infection. In addition, the northwestern hill area was limited and could meet only about 10% of the requirement. Therefore, seed from earlier channels also continued to flow.

The maximum number of epidemics and maximum disease severity were recorded between 1933 and 1969, when the above two channels prevailed (Tables 1 and 2). Epidemics were recorded in Maharashtra (Bhide, 1948; Patel et al., 1952), Gujarat (Dutt, 1979), Kamataka (Rangaswami, 1962), Punjab (Dutt, 1958), Delhi (Hingorani, et al., 1956), the plains of Uttar Pradesh (Dey, 1947), the Bihar plains (Dutt, 1979), West Bengal (Mukherjee and Chattopadhyay, 1955), Andhra Pradesh (Vaheeduddin, 1955, Nath et al., 1956), and Assam (Nandi, 1944).

During the 1960s, researchers developed the concept of producing potato seed in the plains during zero or low aphid periods (Pushkarnath, 1959, 1967) and during the 1970s identified suitable areas and seasons for producing seed in the Indian plains. The technology of producing seed in the plains was called the "seed plot technique." Based on this technique, a potato seed production program was organized in 1966 for both the hills and plains. In the same year, the Government of India passed a seed law and formulated the Seed Act of 1966, which prescribed potato seed health standards. For bacterial wilt, the limit is zero in foundation I & II seed and three wilted plants/ha for certified seed (Tanwar and Singh, 1968).

Certain areas in the country were identified as primary and secondary seed-producing areas. The primary seed-producing areas included the northwestern high hills, northwestern and central plains, and parts of the eastern plains, where an aphid-free period was available from October to December. Bacterial wilt was not endemic in these areas. The remaining areas in the country were identified as secondary seed-producing areas because no aphid-free period was available. Bacterial wilt was endemic in these areas. As a result of these changes, a new pattern of seed movement developed (Figure 3). The northwestern high hills and northwestern and central plains, which are now free of bacterial wilt, emerged as the main sources of seed for the entire country. Seed from these areas is multiplied locally in different states to meet their domestic requirements. Thus, a constant flow of disease-free seed was ensured. As a result, bacterial wilt has ceased to be a devastating problem. In fact, many areas, such as the northwestern and central plains and the eastern plains of Bihar, are now totally free of bacterial wilt. Areas such as Pune (Maharashtra) and Bangalore (Kamataka) have almost become free of the disease. In other areas, the average disease incidence is in the range of 5-10% (Tables 1 and 2). Table 3 shows specific locations (CPRI/government farms) where continuous use of disease-free seed has either eradicated the disease or brought it down to economically insignificant levels.

Bacterial wilt of potato now remains a major problem in states such as Orissa, the northeastern frontier states, and in pockets of the Kumaon hills in Uttar Pradesh, Assam, Karnataka, and the Ranchi plateau where farmers

Table 1. Comparative incidence of bacterial wilt in plains and plateau areas of India before and after implementing the scientific seed production program.[a]

Area	Wilt incidence (%)	
	Before 1972	After 1988
Central plateau	20-50	0-10
Deccan plateau	10-50	0-10
Eastern plateau	15-20	5-15
Eastern plains		
Bihar	10-30	Nil
Orissa	20-50	10-20
Bengal	20-50	5-10
NE frontier states	10-20	5-7
Northwestern plains	0-30	Nil
Northcentral plains	10-50	Nil

a. Figures 1, 2, and 3 show the channels of seed movement before and after 1972.

SOURCES: CPRI and AICPIP annual reports; Dutt, 1958, 1979; Hingorani, 1956; Patel and Kulkarni, 1953; Shekhawat, 1978.

Table 2. Comparative incidence of bacterial wilt of potato in the Indian hills before and after implementing the scientific seed production program.[a]

Area	Wilt incidence (%)	
	Before 1972	After 1988
Northwestern high hills (2200 m.a.s.l.)	—[b]	Nil
Northwestern mid hills, Himachal Pradesh	10-50	5-7
Kumaon hills	20-50	5-10
Eastern hills	15-40	5-7
Nilgiris	20-30	5-10

a. Figures 1, 2, and 3 show the channels of seed movement before and after 1972.
b. Bacterial wilt was present, but percentage was not reported.

SOURCES: Shekhawat et al. (1978) and Dutt (1979).

cannot obtain enough healthy seed and are compelled to keep their own seed stocks for several generations.

The development of different channels of seed emerged from considerations of seed storage and the need to minimize virus incidence. They were not specifically targeted to control bacterial wilt. It was incidental that areas suitable for virus-free seed production were also free of bacterial wilt. The Indian experience, however, demonstrates that the bacterial wilt problem can be solved to a large extent by ensuring the supply of disease-free seed through a sound and scientific seed production program.

Experimental evidence (Patel and Kulkarni, 1953; Shekhawat et al., 1988, 1990; Verma and Shekhawat, 1990) also shows that continuous use of disease-free seed reduces bacterial wilt and brown rot infection by 50- 80% depending upon the area and availability of native vegetation that can harbor the pathogen. No other single control measure, except for the use of resistant varieties wherever available, is as effective as using disease-free seed to contain the disease, even in areas where symptomless carrier plants are prevalent. Also, no other control measure can be effective without healthy seed.

Table 3. Reduction in incidence of bacterial wilt of potato in some ICAR/state government farms as a result of using seed produced through the scientific seed production program.

Farm	Wilt incidence (%)	
	Before 1972	After 1988
ICAR farms Bhowali (Kumaon hills)	50	8
Mukteswar (Kumaon hills)	50	0.1
Shillong (eastern hills)	30	7
Rajgurunagar (Maharashtra)	35	Nil
State government farms		
Bagpashog (Himachal Pradesh, mid hills)	40	Nil
Rajgarh (Himachal Pradesh, mid hills)	40	10
Sufi (Uttar Pradesh, mid hills)	50	5

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CHAPTER 12

Integrated Management of Potato Bacterial Wilt

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The concept of integrated pest management (IPM) arose from a desperate need to stem the indiscriminate use of pesticides, which otherwise would have made our planet inhospitable. Today, it has become a general topic in scientific parlance and five of 11 papers presented on disease management at a recent international conference on bacterial wilt in Taiwan dealt with one or more aspects of integrated management (Hartman and Hayward, 1993).

Like many other soil-borne plant diseases, bacterial wilt (caused by *Pseudomonas solanacearum*) of potato is highly complex, and practical chemical control measures are still not available for this disease. Therefore, the integrated management approach for bacterial wilt is now being favored, not just to curtail environmental pollution but perhaps because no suitable alternative is available yet. Integrated management is information intensive and seeks experimental evidence on various aspects of the problem vis-à-vis its environment. We must realize that in nature nothing happens in isolation. Experiments on different aspects of bacterial wilt have already generated a large volume of information. We must now work out ecofriendly management practices that will achieve sustainability. The strategy for sustainable development advocates "improving the quality of human life while living within the carrying capacity of the supporting ecosystem" (Swaminathan, 1991). Integrated disease and pest management has a special significance in the sustainable development of complex, diverse, and risk-prone (CDR) areas where technologies will have to be tailored to suit specific socioeconomic and agroecological conditions (Chambers, 1991). A cursory glance at the distribution of bacterial wilt on different continents reveals that it is a constraint to potato production in these CDR areas.

Before attempting to develop integrated management practices for a particular area, we should first determine the priority of the problem in that area. The importance of a particular problem in an area can be

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objectively calculated by the formula

$$\left(\frac{A + B}{2} \times C\right) + D$$

where A = importance to farmers, B = importance according to scientists working in the area, C = economic impact (effect on yield), and D = geographical extent of the problem (Gandarillas and Devaux, 1992). Each component can be given a score from 1 (least important) to 5 (most important). Once bacterial wilt is identified as a constraint in a particular area, we should generate information on: (1) hosts affected by *P. solanacearum* in the region, (2) races and biovars prevalent, (3) sources of inoculum, (4) mode of perpetuation, (5) nematode population in the area, (6) management strategies advocated by technicians, and (7) techniques used by local farmers to control the disease. Based on this information, we have to prepare a reasonable strategy to contain bacterial wilt with minimum changes in existing local cultivation practices.

In India, the Central Potato Research Institute (CPRI) has been trying to evolve disease management practices that (1) can take care of most related and important diseases, (2) are based on minimum chemical inputs, and (3) involve host resistance/tolerance and agronomic practices as major components.

Much information is available on agronomic practices for management of bacterial wilt (Kelman, 1953; Shekhawat et al., 1990, 1992). In India, the practices recommended by the CPRI and All India Coordinated Potato Improvement Project based on multilocational trials are as follows (Shekhawat et al., 1988, 1990).

1. Use only healthy, disease-free whole tubers as seed. To do this, India has large areas in the northwestern high hills and northwestern and central plains free of bacterial wilt, where disease-free seed is produced.

2. Practice full earthing-up at planting time to reduce root injury and, consequently, infection.

3. Plough the soil after the potato harvest in April and leave it exposed to high temperature (35-40°C) in the summer in the plains. Plough the soil in October in the hills and leave it exposed to low temperatures (0-4°C) in the winter.

4. Follow a 2-3-year crop rotation with finger millet, maize, cereals, garlic, lupine, onion, sorghum, cabbage, cauliflower, knol-khol, carrot, or horse gram.

5. Apply stable bleaching powder at the rate of 12 kg/ha mixed with fertilizer in furrows at planting time.
6. Do early planting of the summer crop in February-March and harvesting by the first week of July in the hills.
7. Do late planting of the autumn crop in September in the hills.

In the mid hills of India, farmers have already adopted early planting of the summer crop and late planting of the autumn crop. In the eastern hills, farmers plant potato on raised beds and do not practice subsequent earthing-up. In the eastern hills of India, there is a practice called "Jhuming." Inhabitants clear the forest land and the cut vegetative mass is spread on the land and burned. Though "Jhum" cultivation is not ecofriendly and is being discouraged, we have observed that bacterial wilt incidence was negligible in "Jhum" lands. Experiments suggested that in situ burning of soil mulch can efficiently disinfect soil from *P. solanacearum*.

On the basis of a survey of information available on the disease, we identified the following four zones in India.

Zone I: Nonendemic areas (Gujarat, Maharashtra, the northwestern and northcentral plains, and the northwestern high hills at 2200 m.a.s.l.

Zone II: Northwestern mid hills (up to 2200 m.a.s.l.), northeastern hills, and the Nilgiri, Palni, and Mahableshwar hills in the south.

Zone III: Eastern plains and plateau and Deccan plateau.

Zone IV: Northwestern high hills at 2200 m.a.s.l.

The disease situation and management practices recommended in these zones follow.

Zone I. This zone is characterized by a hot and dry summer (April-June) with scanty vegetation; temperatures can go as high as 40-43°C. Farmers in this region helped make the Green Revolution a success during the 1960s and 1970s. Agriculture in this region is gradually being transformed into a big agribusiness. Bacterial wilt is no larger a major constraint to potato cultivation in the region.

Disease outbreaks have been mainly due to infected seed tubers obtained from endemic areas. Therefore, use of disease-free seed alone is adequate for control of the disease.

Zone II. This zone comprises typical, complex, diverse, and risk-prone areas where farmers are resource poor and productivity is also low. It is characterized by a mild summer, profuse vegetation, and maximum

temperatures in the range of 26-30°C. Winter temperatures may go as low as 3-6°C. Bacterial wilt is rampant in this area and a loss of up to 55% has been reported (Hari Kishore and Pushkamath, 1963). Race 3 is the most prevalent (Shekhawat et al., 1978), but race 1 has also been reported recently (Kishore et al., 1991). Many weed hosts, which can provide sheltered sites for survival of the bacterium during the off-seasons, have also been reported from this region. The region's soil is acidic and is infested with root-knot and other nematodes. Locally produced seed tubers or tubers left in the field also serve as sources of inoculum.

The use of disease-free seed plus application of stable bleaching powder at 12 kg/ha mixed with fertilizer at planting, ploughing the field in September-October, and leaving the soil exposed to winter temperatures are adequate for disease control. The application of bleaching powder can be substituted by a 2-year rotation with crops such as wheat, barley, finger millet, cabbage, cauliflower, knol-khol, carrot, onion, garlic, etc.

Early planting (at the latest by the first week of March) and early harvesting (by the third week of June) also reduce disease incidence by 70-80% (AICPIP, 1989; Shekhawat et al., 1990).

Zone III. Here potato is cultivated as a short-day crop during the winter months (October-March). Day temperature sometimes reaches 38°C. Heavy precipitation periodically occurs due to western disturbances. The area is relatively rich in vegetation. Farmers are either resource rich and can employ superior technologies, or practice low-input subsistence agriculture. Again, bacterial wilt is a constraint for subsistence farmers and losses of up to 75% have been reported (Gadewar et al., 1991).

The eastern plains and Deccan plateau have many symptomless hosts of the pathogen and therefore control of the disease is difficult. But it can be reduced to economic levels with agronomic practices such as the use of disease-free seed, the application of stable bleaching powder to soil, blind earthing and ploughing the land in March and leaving the soil exposed to summer temperatures during April-May, and crop rotations along with clean cultivation (AICPIP, 1989; Shekhawat et al., 1990).

Zone IV. This zone has a temperate climate with severe winters; daily temperatures range from -10 to 5°C during December-January. Snow is common during these months. Bacterial wilt is not endemic and the use of disease-free seed alone is adequate.

Host resistance. The presently available resistance in potato against *P. solanacearum* (Martin, 1979; CPRI, 1989) has two major problems: (1) it is strain specific and breaks down under warm climates, and (2) resistant clones are susceptible to latent infections that build up with successive vegetative propagation. To use this resistance/tolerance, we propose

adapting three approaches: (1) resistant varieties should be grown under cultural practices identified for control of the disease, (2) the rate of buildup of latent infection in resistant varieties should be determined, and (3) the seed stocks should be replaced before the latent infection reaches the threshold level for disease development.

Biocontrol. Certain bacteria such as *Pseudomonas flourescens, Bacillus polymyxa, Bacillus* spp., and actinomycetes have been found to delay development and reduce incidence of the disease and increase yield in glasshouse and field trials (Shekhawat et al., 1993). We thus propose developing biological control for the disease. These biocontrol agents are now being tested in multilocational field trials and the AICPIP. The biocontrol agents could prove useful alone or in combination with host tolerance and agronomic practices.

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CHAPTER 13

Production of Polyclonal Rabbit Antiserum and Protocol to Use Enzyme-linked Immunosorbent Assay (ELISA) to Detect *Pseudomonas solanacearum* from Latently Infected Potato Stems and Tubers

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I. Preparation of whole-cell antigen

1. Grow on CPG agar for 20 min.
2. Collect the growth with a minimum of 0.85% saline.
3. Centrifuge at 7,500 G for 20 min.
4. Wash pellet four times with saline.
5. Resuspend pellet from final washing in 0.3% formalin in saline solution.
6. Adjust bacterial density to ~2 x 10^9 cells/ml. Store at 4°C.

II. Immunization schedule

1. Collect normal serum of New Zealand white rabbit before immunization. Store with equal volume of glycerol at 4°C.
2. Inject 0.5 ml of antigen intravenously.
3. Inject 0.5, 1.0, 1.0, 2.0, and 2.0 ml of antigen at 3-day intervals.
4. Inject a booster dose of 5.0 ml 8 days after the last injection.
5. Collect blood after 7 days by ear bleeding.
6. Collect the serum and store with an equal volume of glycerol at 4°C.

III. Purification of immunoglobulin (IgG) fraction

1. Add 2 volumes of acetate buffer (pH 4.0) and adjust the pH to4.8.
2. Centrifuge at 7,500 G and remove the precipitate.
3. Dialyze the supernatant extensively against saline at 4°C.
4. Add an equal volume of saturated ammonium sulfate solution dropwise with constant stirring.
5. Place at 4°C for at least 6 hr to allow the IgG precipitate to flocculate.

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6. Centrifuge at 7,500 G for 20 min and dissolve the pellet in a minimum amount of saline.
7. Dialyze against several changes of saline until the solution is free of sulfate ions.
8. Estimate the protein concentration and store with 0.02% sodium azide at 4°C.

IV. Conjugation of IgG with alkaline phosphatase

1. Mix 1 mg of alkaline phosphatase with purified IgG containing 1 mg protein/ml.
2. Dialyze against 3-4 changes of half-strength phosphate buffer-saline solution (PBS) at 4°C.
3. Add 0.06% glutaraldehyde solution and keep at 20-22°C for 4 hr.
4. Dialyze against 3-4 changes of half-strength PBS at 4°C.
5. Store at 4°C after adding 10 mg/ml bovine serum albumin and a few drops of 0.02% sodium azide.

V. ELISA protocol

1. Deposit 100 μl of IgG solution (1:1,000 dilution) per well and incubate for 3 hr at 37°C.
2. Wash with 4-5 changes of PBS-Tween 20 (PBST) and deposit 100 μl analytes per well.
3. Incubate overnight at 4°C or at 37°C for 3 hr.
4. Wash as above and deposit 100 μl enzyme conjugate (1:1,000 dilution) per well and incubate at 37°C for 3 hr.
5. Wash and deposit 100 μl of p-nitrophenol phosphate (0.6 mg/ml in substrate buffer) per well and keep the plates covered with carbon paper for 30 min at room temperature.
6. Score the well visually or make a reading with an ELISA reader (410 nm absorbance).

VI. Sampling

Stem

1. Take a 1-inch length stem piece, preferably from the plant base.
2. Chop it into pieces and put them in a polythene bag.
3. Macerate in 1.0 ml PBST and keep at 4°C for at least 30 min before using the extracted liquid.

Tuber

1. Collect 5 g of tuber flesh from the heel end.
2. Macerate in 5 ml of PBST and keep at 4°C for at least 30 min before use.

CHAPTER 14

Polyclonal and Monoclonal Antibody-based Enzyme-linked Immunosorbent Assays for *Pseudomonas solanacearum*

Andrea Robinson-Smith*

Introduction

Bacterial diseases of plants are generally diagnosed on the basis of symptoms and subsequent isolation and characterization of the pathogen by classical, morphological, physiological, and nutritional tests. Because these tests are tedious and time-consuming, this has led to an interest in finding quicker methods of diagnosis.

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

Serological techniques have been used to detect and identify plant viruses for many years, but it was only in 1978 that the first serological assays were used for plant pathogenic bacteria (Cambra and Lopez, 1978; Vruggink, 1978). Several methods have now been proposed for the use of polyclonal antibodies (pAbs) to detect *P. solanacearum*, including latex agglutination (Nakashima and Nydegger, 1986), enzyme-linked immunosorbent assays (ELISA) (Bellstedt and van der Merwe, 1989), and immunofluoresence antibody staining (Janse, 1988). Because of nonspecific reactions of pAbs, the immunofluorescent technique has been the most widely used, even though it is laborious and relatively costly.

Few methods have yet been described for the use of monoclonal antibodies (mAbs) to detect *P. solanacearum*, but their use should eliminate many of the problems associated with pAbs. MAbs are secreted from hybridoma cells that result from the fusion of B-lymphocytes (antibody-producing cells) with myeloma cells (malignant cells that do not secrete antibody and are immortal in cell culture). They may therefore be produced indefinitely in vitro. Also, due to the nature of the hybridoma

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cells, those that secrete antibodies to a particular antigenic determinant may be selected.

This paper describes the production of both mAbs and pAbs for *P. solanacearum*, compares their sensitivity and specificity, and describes their subsequent usefulness for the rapid detection of *P. solanacearum*.

Materials and Methods

Bacterial isolates and antigen preparation

We cultured all bacterial isolates in 9-cm plastic petri dishes on a TZC medium, but without tetrazolium (Kelman, 1954), at 28°C for 24-48 hr, with the exception of *P. syzygii* and *P. celebensis*, which we cultured on a PW basal medium (after Davis et al., 1981). We harvested the cells in sterile distilled water (SDW), centrifuged them for 10 min at 10,000 G, and washed them three times in SDW. Finally, we resuspended them in 5 ml SDW and estimated their number by measuring the OD_{650} (an OD_{650} of 0.1 is equivalent to 1×10^8 cells/ml). For ELISA, we adjusted the cells to 2×10^9 cells/ml in SDW, fixed them with 0.1% formaldehyde (final concentration), and stored them at 4°C. For immunizations (with the exception of schedule C in mAb production, see the section on "Immunization schedule C"), we adjusted the cells to 1×10^9 cells/ml in 0.85% sterile saline and fixed them with glutaraldehyde, following the method of Allan and Kelman (1977). We then prepared 0.5 ml aliquots and stored them at -20°C until required.

For mAb production by schedule C, we used surface washings rather than whole bacterial cells as the immunogen. We obtained cell-free washings from a known number of cells as above and passed them 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 2×10^9 cells), which were redissolved in 1 ml sterile saline as required.

Polyclonal antibody production

We immunized female Dutch x Lop rabbits intramuscularly at two sites, with a total of 5×10^8 glutaraldehyde-fixed whole bacterial cells in 0.5 ml sterile saline and emulsified them in an equal volume of Freund's complete adjuvant (Difco Labs). Four weeks later, we again immunized the rabbits with the bacteria, but in Freund's incomplete adjuvant. We collected blood from the lateral ear vein at 2-3-week intervals thereafter. We allowed the blood to clot at room temperature, separated it by centrifugation (1500 G for 15 min), and collected the serum fraction. We determined antibody levels in the serum by ELISA.

Monoclonal antibody production

Immunization schedule A. We immunized female Balb/c mice 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, we bled the mice via the tail vein and measured antibody titers by ELISA. If titers were greater than 1:2,000, a final boost was given, as above. Otherwise, we continued immunizations at 4-week intervals until this titer was achieved.

Immunization schedule B. We immunized two female Balb/c mice 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, we immunized one mouse with 20 mg/kg cyclophosphamide (Sigma Chemical Co.) in sterile saline (0.4 ml). The second mouse was maintained as a control. We repeated this entire procedure three more times, at 2-week intervals. Test bleeds were taken during the intervening weeks and antibody titers compared. After a 3-week rest, we immunized the cyclophosphamide-treated mouse 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. We immunized female Balb/c mice intraperitoneally with low molecular weight washings from 5 x 10^8-1 x 10^9 cells in 0.5 ml sterile saline twice, at 4-week intervals. Two to four weeks later, we bled the mice via the tail vein and measured antibody titers by ELISA. If titers were greater than 1:2,000, a final boost was given, as above. Otherwise, we continued immunizations at 4-week intervals until this titer was achieved.

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

Hybridoma production. We placed fused cells in 96-well plates at 37°C with 8% CO_2. After 7-10 days, when growth of the hybridomas was almost confluent, we screened culture supernatants 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). We then bulked subclones in 25-ml tissue culture flasks and

supernatants were drawn for screening when growth was confluent and the medium was beginning to show signs of acid production (i.e., turning yellow). We froze all subclones of interest in a mixture of 90% fetal calf serum and 10% DMSO at -70°C in a Cryo freezing box (Nalge) for 24-48 hr and then transferred them to liquid nitrogen.

Antibody screening by ELISA

We coated 96-well microtiter plates (Nunc, polysorp) with a suspension of whole bacterial cells (100 ml/well), diluted from 1×10^2-1×10^8 cells/ml in 0.05M sodium carbonate coating buffer, pH 9.6. After coating for 1 hr at 37°C, we decanted the bacterial suspensions from the wells and washed the plates three times in phosphate-buffered saline plus 0.05% v/v Tween 20 (PBST). This washing step was done after each of the subsequent steps. Culture supernatants diluted 1:1 or polyclonal antisera diluted 1:1,000 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 (Nestlé) in PBS) were then added to the wells (100 ml) and incubated, again for 1 hr at 37°C. After washing, a horseradish peroxidase conjugated rabbit anti-mouse antibody diluted 1:2,000 (for culture supernatants) or goat anti-rabbit antibody diluted 1:5,000 (Sigma, for polyclonal antisera) in blocking buffer was added and incubated at 37°C for 1 hr. Finally, we added 100 ml of TMB substrate (1 mg/ml 3,3',5,5'-tetramethylbenzidine (Sigma), 0.1% v/v hydrogen peroxide, and 10% v/v sodium acetate, pH 5.8 in distilled water) to each well and incubated at room temperature until sufficient color developed. We stopped the reaction by adding 3M sulphuric acid (25 ml/well). We measured the absorbance at 450 nm on a Titretek Multiscan microtiter plate reader (ICN Flow). A positive reading was taken as being three times the mean of the negative controls.

Detection of *P. solanacearum* in plant samples

We screened artificially inoculated and naturally infected tomato and potato plants by ELISA for the presence of *P. solanacearum*. Leaves or sections of stem were taken from the plants and weighed, and we added an equal volume of 0.05M sodium carbonate coating buffer, pH 9.6, containing 0.2% sodium sulphite (as an antioxidant). We then macerated the sections of tissue mechanically and allowed them to settle. The resulting sap was removed, mixed 1:20 with the coating buffer, and used to coat the wells of a microtiter plate (100 ml per well). We then followed the ELISA described earlier (omitting the first stage of coating with whole bacterial cells).

Results

Polyclonal antibodies

In this study, we produced four pAbs for *P. solanacearum* (Rothamsted Culture Collection accessions R283, R303, R608, and R710) and one for *P. celebensis* (blood disease bacterium (BDB), R230) (Eden-Green and Robinson, unpublished). All *P. solanacearum* pAbs had high titers and reacted with all isolates of *P. solanacearum* at 1 x 10^8 cells/ml, but also cross-reacted with *P. syzygii, P. celebensis, P. picketti,* and *P. cepacia.* There was no detectable difference between the pAb developed for *P. celebensis* and those developed for *P. solanacearum* (Table 1). The smallest concentration of bacteria routinely detectable by the pAbs was 1 x 10^4 cells/ml, although several isolates were detectable at 1 x 10^2 cells/ml in pure culture.

Table 1. Detection of *Pseudomonas solanacearum* and related bacteria by polyclonal antibodies (+ = positive reaction, - = negative reaction).

Bacterial isolate			Polyclonal antibodies[a] (IACR-PS-)				
Code	Biovar	Race	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*[b]		—	—	—	—	—
R036	*X. campestris*		—	—	—	—	—
R111	*E. coli*		—	—	—	—	—
R137	*A. tumefaciens*		—	—	—	—	—
R226	*P. celebensis*		+	+	+	+	+
R228	*P. celebensis*		+	+	+	+	+
R707	*P. picketti*		+	+	+/—	+/—	+/—
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

a. 277 was raised to R283, 278 to R303, 291 to R608, 290 to R230, and 322 to R710.
b. *Bacillus subtilis, Xanthomonas campestris, Escherichia coli, Agrobacterium tumefaciens.*

Monoclonal antibodies

Schedule A. In general, serum with a titer in excess of 1:2,000 was obtained after two immunizations and so fusions were done after a third boost. A wide range of mAbs was obtained from the fusion, but none was able to detect all isolates of *P. solanacearum*, and they all cross-reacted with isolates of *P. syzygii*, *P. celebensis*, and *P. picketti*. There was no correlation between the reactions of these mAbs and the grouping systems used to classify *P. solanacearum* (i.e., biovars/races) (Table 2). The smallest concentration of bacteria detectable by the mAbs was 1 x 10^6 cells/ml in pure culture.

Schedule B. When we compared the serum titers of the cyclophosphamide-treated mouse and the control mouse, we 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 to one another, showing no cross-reactions to related bacteria (*P. syzygii*, *P. celebensis*, *P. cepacia*, *P. picketti*), but giving a spectrum of reactions to *P. solanacearum* isolates similar to those obtained by schedule A (Table 2). The smallest concentration of bacteria detectable by the mAbs was 1 x 10^6 cells/ml in pure culture.

Schedule C. Initially, mice immunized with washings died. We found that if the washings were obtained from bacteria that had been grown for longer than 48 hr, they were toxic. Preparations from bacteria younger than 48 hr were, however, tolerated by the mice.

As with schedule A, a wide range of mAbs was obtained from the fusion, but rather than showing greater specificity, as expected, most of the mAbs showed a greater range of activity than any of the mAbs produced by either schedule A or B. In fact, several reacted with almost all isolates of *P. solanacearum* (more than 260). There were also some cross-reactions with related bacteria, although one mAb produced (IACR-PS-144) only cross-reacted with a single isolate of *P. syzygii* (Table 2). The smallest concentration of bacteria detectable by these mAbs was, once again, 1 x 10^6 cells/ml in pure culture.

Detection of *P. solanacearum* in plant samples

Using the ELISA, *P. solanacearum* was detectable in 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 (+ = positive reaction, - = negative reaction).

Bacterial isolate			Monoclonal antibodies[a] (IACR-PS-)				
Code	Biovar	Race	054	089	117	124	144
R232	1	1	-	-	-	-	+
R296	1	1	-	+/-	-	-	+
R702	1	1	+	+	+	+	+
R281	1	1	+	+	+	+	+
R638	1	2, moko	-	-	-	-	+
R651	1	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	[illegible]	+/-	+	+	+	+
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*		+	+	-	-	+
R044	*P. cepacia*		-	-	-	-	-
R227	*P. celebensis*		+	+	-	-	-
R229	*P. celebensis*		+	+	-	-	-
R707	*P. picketti*		-	+	-	-	-
R036	*Xanthomonas campestris*		-	-	-	-	-

a. 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 ELISA, are able to detect all isolates of *P. solanacearum* routinely at concentrations as small as 1 x 10^4 cells/ml, but are unable to discriminate between *P. solanacearum* and *P. syzygii, P. celebensis, P. picketti,* or *P. cepacia*. While these cross-reactions would probably be of little practical significance when testing infected plant

Table 3. ELISA readings showing how the immune response of a cyclophosphamide-treated mouse is destroyed compared with that of a control mouse.

Bacterium	$OD_{450\,nm}$		
	Negative control	Mouse A[a] sera at 1:300	Mouse B sera at 1:300
Pseudomonas solanacearum biovar 1	0.018	0.034	0.254
P. solanacearum biovar 2	0.015	0.027	0.595
P. solanacearum biovar 3	0.017	0.018	0.306
P. solanacearum biovar 4	0.034	0.025	0.491
P. solanacearum biovar 5	0.043	0.025	0.451
P. syzygii R001	0.017	0.025	0.525
P. syzygii R002	0.022	0.022	0.687
	P. celebensis R002	0.022	0.030 0.568
	P. celebensis R002	0.020	0.028 0.511
	P. picketti	0.016	0.027 0.714
	P. cepacia	0.011	0.026 0.257

a. Mouse A was cyclophosphamide treated and mouse B was a control.

tissue (as the cross-reacting bacteria are unlikely to be present), they may cause a problem when testing soil samples in which *P. picketti* and *P. cepacia* can be present in large numbers. Also, in countries such 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.

MAbs were therefore developed to try to improve specificity, initially by immunizing mice with glutaraldehyde-fixed whole cells (schedule A). The use of these mAbs in the ELISA removed the cross-reactions with *P. cepacia* and reactions to several isolates of *P. solanacearum* (Table 2), and so replacement of pAbs with these mAbs in the ELISA would be of no advantage. There was no correlation between the spectrum of reaction of these mAbs and division into biovars or races. Similar results have been obtained in other laboratories (A. Alvarez, personal communication).

To improve the mAbs, we attempted to manipulate the response of the immune system of the mice. As a result, the production of antibodies to the shared epitopes of *P. solanacearum* and the cross-reacting bacteria was suppressed (schedule B). When an immune response is elicited, B and T lymphocytes proliferate. These dividing cells may be killed by the cytotoxic drug, cyclophosphamide, giving rise to "temporary tolerance" and permitting manipulation of the immune response so 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 titer of this mouse and that of a mouse that had only received the cross-reacting bacteria (control mouse) were compared. Titers of the mouse that had been given cyclophosphamide were far lower than that of the control mouse, showing that the cyclophosphamide was destroying the proliferating B and T cells. Subsequent immunizations were given until no antibody production was detected, with a total of four being required. Not only did the cyclophosphamide knock out antibodies for the cross-reacting bacteria, but also those for *P. solanacearum* (Table 3). Once the mouse had been re-immunized with a mixture of strains from *P. solanacearum*, antibodies that reacted with *P. solanacearum* were produced. MAbs resulting from this immunization schedule did not cross-react nor did they detect all isolates of *P. solanacearum*. Once again, the spectrum of reaction 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. As previously, these mAbs were too specific (reacting with only selected isolates of *P. solanacearum*) and would therefore be of no advantage if used to replace the pAbs in ELISA.

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

A final attempt at producing mAbs that could be used in the ELISA was made by immunizing mice with cell surface washings rather than cells (schedule C). The resulting mAbs differed tremendously, with some of those produced reacting with only a few isolates of *P. solanacearum*, while 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, with one of the mAbs, IACR-PS-144, only cross-reacting with a single isolate of *P. syzygii*. This mAb was found to belong to the group of mAbs that detected all isolates of *P. solanacearum*, making it ideal for use in the ELISA. Unfortunately, its limit of detection, like all of the mAbs, was not as great as the pAbs (i.e., only 1×10^6 cells/ml), meaning an ELISA using this mAb would not be as sensitive as one using any of the pAbs. 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 cross-reactors *P. syzygii*, *P. celebensis*, *P. picketti*, and *P. cepacia* in a matter of hours. For routine use in all other situations, however, the increased sensitivity of the pAbs suggests that we could use them in the ELISA until further improvements in the sensitivity of the mAbs can be made.

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CHAPTER 15

Methods for the Detection of *Pseudomonas solanacearum* in Potato Crops

J.G. Elphinstone*

Introduction

The ability of *Pseudomonas solanacearum* to infect and colonize the potato plant (as well as other crops and weed spp.), without causing symptoms, has resulted in its widespread dispersal and subsequent establishment in environments worldwide (Hayward, 1991). This is particularly true in the case of potato in temperate climates in which temperatures are usually below optimum for pathogen multiplication, resulting in latent infection rather than development of the disease.

One of the main constraints to developing strategies to efficiently control bacterial wilt has been the absence of rapid and accurate methods to detect the pathogen in large numbers of plant, soil, and water samples on a routine basis. There are several reasons for the need to develop more sensitive pathogen detection methods:

1. Current quarantine and seed-health tests rely mainly on visual inspection of the incidence of disease symptoms in the field or during storage. However, the absence of symptoms does not guarantee the absence of latent pathogen populations, which may often consist of too few bacteria to be detected by traditional methods.
2. Similarly, the selection of cultivars with resistance to bacterial wilt has been conducted by monitoring disease incidence rather than the ability of the pathogen to multiply and spread within the host plant. This has led to the selection of genotypes that remain symptomless but carry and protect virulent pathogen populations.
3. The effectiveness of integrated control strategies involving the use of resistant varieties, crop rotation, and other cultural measures has been monitored by measuring disease incidence in a subsequent

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crop. More accurate information could be derived by directly determining the effect of control treatments on pathogen populations.

There are several techniques available to detect *P. solanacearum*, ranging from simple but inaccurate conventional techniques to more recently developed methods that offer a high degree of sensitivity and specificity. A review of these techniques was recently compiled (Seal and Elphinstone, 1994). It is the intention of this paper to discuss the relative merits and disadvantages of various detection methods in the development of practical procedures for the monitoring of infection by *P. solanacearum* during the multiplication of seed potatoes.

Sampling Strategies

Little information is currently available on the effect of sampling strategies on the accuracy of methods to detect *P. solanacearum* in plant, soil, or water samples. Nevertheless, for both qualitative and quantitative detection, the sampling method is probably equally as important as the sensitivity of the technique employed.

Janse and Van Vaerenbergh (1987) and Janse (1989) recommend adapting the sampling methods adopted for the detection of bacterial ring rot (caused by *Clavibacter michiganensis* ssp. *sepedonicus*) in potato tubers. This involves the analysis of composite samples of 200 tubers each. Each tuber is washed, the periderm at the stolon end is removed, and a core of tissue containing xylem vessels (5-10 mm diameter x 5 mm length) is removed with a sterilized scalpel. The tissue cores from each 200-tuber sample are then homogenized together in phosphate buffer and the macerate supernatant is filtered (Whatman No. 1) and centrifuged at 10,000 G. Methods to detect the pathogen in the pellet are discussed below.

Sampling of tubers from the field just before harvest will allow collection of representative samples from all areas of the field. González (1977) used 5 samples of 90 visually healthy tubers each as a representative sample from a field in which 15% of the plants exhibited symptoms of bacterial wilt. Simultaneous monitoring of the incidence of bacterial wilt in affected plants will allow us to compare tuber samples from areas of the field with high and low wilt incidence. Outbreaks of bacterial wilt early in the season result in high incidences of tuber infection. However, late outbreaks may result in a greater proportion of the infection remaining latent (Ciampi et al., 1980). Monitoring of disease incidence at regular intervals throughout the season is therefore advisable.

Several methods have been proposed for the detection of *P. solanacearum* in soil but the optimum sampling strategies have yet to be determined. Sampling depths of 20-30 cm are usually recommended in order to avoid areas of high moisture and temperature fluctuation that influence bacterial populations. Composite samples from representative areas of the field should be thoroughly mixed before removing 10 g subsamples that can then be suspended in 90 ml of sterile phosphate buffer by shaking for 30 minutes. The presence or absence of the pathogen can then be determined in a series of dilutions prepared from this suspension.

Because the host plant acts as an efficient enrichment medium for the selective multiplication of the pathogen in the absence of saprophytic bacteria, it is much easier to detect *P. solanacearum* in the tissues of potato or other hosts than in soil or water, where they are more dilute in concentration and have to be distinguished from the range of other (often closely related) bacteria present. Hence, the analysis of roots or rhizosphere soil of rotation crops or weeds may give a better indication of the presence or absence of the pathogen in a particular field than sampling of the soil itself (Granada and Sequeira, 1983).

Depending on the detection technique employed, tissue, soil, or water samples may require additional preparation, concentration, or purification. This will be discussed further in the following descriptions of the various methods.

Detection Techniques

The choice of method will depend on several factors:

1. Degree of sensitivity needed (which, in turn, will depend on the level of pathogen population that results in significant economic losses).
2. Samples to be tested (soil, water, or tubers).
3. Experience of the user.
4. Laboratory facilities available.
5. Cost-benefit ratio.

Simple techniques

Symptom expression. The simplest way to determine the presence of *P. solanacearum* in a potato crop is to cut potato tubers or stems of plants exhibiting symptoms of wilt and observe the characteristic signs of the presence of the pathogen, a creamy bacterial ooze that exudes from the

(often discolored) vascular ring. Early stages of infection may be characterized by slight discoloration of the vascular tissues, especially near the point of attachment between stolon and tuber or between taproot and stem. Storage of tubers at temperatures above 25°C for 4 to 6 weeks can encourage symptom development in latently infected tubers and hence facilitate detection (González, 1977; Graham et al., 1979). Alternatively, samples of tubers may be grown in pots of sterilized soil at 25-30°C to stimulate the development of bacterial wilt from infected tubers (Graham et al., 1979).

A simple field test to confirm whether a diseased sample has bacterial wilt may be performed by suspending a piece cut from the base of a wilted stem or from the vascular ring of an infected tuber in clear water. Milky ooze drifts downward from the vascular tissues within a few minutes (Martin and French, 1985).

Use of indicator plants. Indicator plants have mainly been used to detect *P. solanacearum* in soils by either planting them directly in the field or in pots containing replicated soil samples in the greenhouse. The latter has the benefit that temperatures can be maintained at the optimum (25-30°C) for symptom expression in infected plants. However, if the pathogen population is low or localized in small areas of the field, then a large number of soil samples must be tested. Potato shoots cut from healthy tubers (Graham et al., 1979) or surface-sterilized tomato seeds (Felix and Ricaud, 1978) of susceptible cultivars have been used as planting materials for indicator plants. Tomato seedlings have also been recommended as indicators to detect the presence of *P. solanacearum* in the centrifuged pellet from homogenized tuber tissue (Janse, 1988) by injection of the resuspended pellet made from 200 tubers into the stem of 10 tomato (cv. Moneymaker) seedlings (at the 3-leaf stage) just above the cotyledons. The absence of symptoms after growing the seedlings under controlled conditions for 4 weeks (28/24°C day/night temperatures, 78% RH, and 10,000 lux light intensity for 16 hours per day) indicated a negative result.

If the variability of strains is known, their commonly occurring weed hosts can be used as indicators of their presence or distribution in a given area. If the weed hosts are latently infected only, different tests may be used to detect the pathogen in surface-sterilized roots.

The use of indicator plants can provide a relatively sensitive method for detecting *P. solanacearum* in nonsterile plant, soil, and water samples. However, there are several disadvantages:

1. The method is laborious and requires large areas of greenhouse space and controlled environmental conditions.

2. Pathogen strains that are weak or nonaggressive on a particular indicator species will not be detected. This may be overcome to some extent by using a second technique to detect latent infection in indicator plants that remain visually healthy.
3. Indicators may be susceptible to other pathogens that are commonly present in the sample (e.g., pectolytic *Erwinia* spp. or *Fusarium* spp.).
4. Results may not be known for several weeks after sample collection.

Verification of infection by *P. solanacearum*. The symptoms caused by *P. solanacearum* can be distinguished from symptoms of bacterial ring rot by conducting simple bacteriological tests. Accumulation of poly-ß-hydroxybutyrate (PHB) as intracellular inclusions is characteristic of *P. solanacearum* but not of *C. m. sepedonicus*. The inclusions can be detected by heat-fixing a smear of ooze from an infected tuber on a glass slide and staining with a 1% aqueous solution of Nile Blue A. PHB granules fluoresce bright orange under a fluorescent microscope (Lelliot and Stead, 1987). Alternatively, a Gram stain (Lelliot and Stead, 1987) can be performed on the heat-fixed smear. Cells of *P. solanacearum* are Gram-negative, whereas *C. m. sepedonicus* cells are Gram-positive. The Gram reaction can also be predicted by determining solubility of the bacterial ooze in 3% aqueous potassium hydroxide (KOH) solution (Suslow et al., 1982). If a loopful of bacterial ooze is mixed with a toothpick for 5-15 seconds with two drops of KOH solution on a glass slide, the formation of a milky thread upon lifting the toothpick indicates the presence of the Gram-negative rather than the Gram-positive pathogen (Martin and French, 1985).

Culturing techniques

Differential medium. Isolation on Kelman's (1954) tetrazolium medium allows us to identify colonies of *P. solanacearum* among those of other bacteria by their typical fluidal, smooth, white appearance with red internal whorling patterns. The medium contains 0.25-1.0% dextrose, 1.0% peptone (Difco), 0.1% casamino acids (Difco), and 1.5% agar in distilled water to which a filter-sterilized aqueous solution of 2,3,5 triphenyl tetrazolium chloride is added, after autoclaving, to give a final concentration of 0.005%.

Isolation by streaking ooze from diseased tubers or stems directly onto the medium usually results in growth of a pure culture of *P. solanacearum* after 48 hours of incubation at 30°C. Latent infection can sometimes be detected by suspending stem or tuber pieces, containing vascular tissues from near the base of the stem or stolon end of the tuber, in sterile distilled

water for 10 minutes and streaking loopfulls of the water onto the agar (French, 1984). Isolation from extensively rotted samples or from soil or water, in which large saprophytic populations are present, is more difficult because the presence of the pathogen may be obscured by bacteria that grow more rapidly on the nonselective medium or that are antagonistic.

Selective media. Several recipes for selective media have been published that rely on the specific resistance of *P. solanacearum* to different antibiotics (Karganilla and Buddenhagen, 1972; Nesmith and Jenkins, 1979; Granada and Sequeira, 1983). However, most are unsuitable for general use due to variation in resistance or susceptibility to the antibiotics used among different strains of the pathogen or among the saprophytic populations that prevail in different soil types. In general, a semiselective modification of the medium of Granada and Sequeira (1983) permits growth of most *P. solanacearum* strains within a 48-hour incubation period.

This medium is itself a modification of Kelman's (1954) tetrazolium medium to which a solution of crystal violet in 70% ethanol and a filter-sterilized aqueous solution of polymyxin-B sulphate are added after autoclaving to give final concentrations of 50 and 2.5 ppm, respectively. The presence of *P. solanacearum* in serial dilutions of soil suspensions can be detected by spreading 0.1 ml aliquots on replicated plates of this medium. Colonies of the bacterium have the same appearance as on Kelman's tetrazolium agar. However, as the medium is only semiselective, the pathogen can be difficult to detect in the lower dilutions due to extensive growth of saprophytes.

Serological techniques

Serological techniques now offer sensitive and easily used alternative methods for detecting *P. solanacearum* in plant samples. Furthermore, current research should soon provide the means for pathogen detection in soil and water samples and allow simultaneous identification of the race, biovar, or even pathovar of a particular strain, without the need for purification of the bacterium or highly developed laboratory facilities.

Antibodies. Polyclonal antibodies produced in rabbits in response to immunization with live or heat-killed whole cells of *P. solanacearum* are generally produced in high titers in the serum. Digat and Cambra (1976) described cell surface antigens in four categories: exopolysaccharide (EPS), cell wall structural (somatic) components ("O" antigens), extracellular glycoprotein, and flagella components ("H" antigens). Glycoproteins tend to be strain-specific whereas EPS and somatic antigens are common to all strains of *P. solanacearum* (Coleno et al., 1976; Digat and Cambra, 1976). In fact, some antigens are also common to other closely related *Pseudomonas* spp., such as *P. mallei* and *P. pseudomallei* (Coleno et al., 1976), *P. syzygii* (Eden-Green and Adhi, 1986), *P. pickettii*, and *P. cepacia* (Elphinstone,

unpublished). Hence, some degree of cross-reaction may be possible when polyclonal antisera are used, particularly when the closely related species are present in high numbers (10^8 cfu per ml or more). Although cross-reacting bacteria may be present in water or soil samples, they are unlikely to populate infected potato tissues from which relatively pure populations of *P. solanacearum* may be obtained, provided sufficient care is taken during the extraction process. Polyclonal antibodies may therefore be used for nonspecific detection of all *P. solanacearum* strains in infected plant material.

For more specific detection of particular strains of the pathogen, we need to use antibodies to particular antigens or their epitopes. Monospecific antibodies produced in response to purified glycoproteins have been used to differentiate different strains (serovars) of *P. solanacearum* in Brazil (Schaad et al., 1978). More recently, research on the production of monoclonal antibodies (McAbs) has begun in several places worldwide. Hybridoma cells, resulting from the fusion of mouse b-lymphocytes (antibody-producing cells) with myeloma cells (malignant cells that are immortal in cell culture), secrete McAbs during in vitro culture. Furthermore, clonal cell lines may be selected that secrete a homogeneous antibody population to a single antigen or epitope. Hence, it is possible to select highly specific McAbs for use in the recognition of intraspecific strains of *P. solanacearum*. Furthermore, McAb-secreting cell lines may be maintained indefinitely, ensuring a constant and uniform source of antibodies (unlike polyclonal antisera, for which batch variation exists) without the need for repeated immunization of animals.

Both *P. solanacearum*-specific and strain-specific McAbs have been selected in China (He, 1986; CIP, 1990), Hawaii (Alvarez et al., 1993), and the U.K. (Robinson, 1993). These have an advantage over polyclonal antibodies in that they do not cross-react with other *Pseudomonas* spp. So far, it has not been possible to select single strain-specific McAbs that differentiate all the strains of a given race of *P. solanacearum*. But it should be possible to select the desired specificity for practical applications by using a panel of different McAbs or mixing them to create a synthetic polyclonal reagent.

Latex agglutination. Nakashima and Nydegger (1986) used purified polyclonal antisera to a race 3 strain of *P. solanacearum* in an adaptation of the latex agglutination test of Slack et al. (1979), which was used to detect *C. m. sepedonicus*. In this case, latex beads (Sigma Chemical Company LB-11) were sensitized by binding with purified gammaglobulin extract (IgG) from the polyclonal antiserum. A simple slide agglutination test was then performed by mixing equal parts of sensitized latex and homogenate from diseased plant samples. Due to their large size, agglutination of the latex beads was easily observed even when bacterial concentrations were low.

Initial results confirmed that the method was highly sensitive, but problems due to long-term instability of the antibodies have prevented more extensive testing.

ELISA. Enzyme-linked immunosorbent assay (ELISA) has become a widely accepted method for detecting plant pathogens that combines high sensitivity, ease, and speed of use with the ability to quantify the amount of pathogen in extracts of plant tissues and other samples. Current research has shown that the indirect ELISA system can detect as few as 10^4 cfu per ml in pure or mixed bacterial suspensions or plant extracts (Robinson, 1993). This procedure involves the binding of soluble antigens from bacterial cells to polystyrene microtiter plates at pH 9.6, followed by reaction with pathogen-specific antibodies. The bound antigen-antibody complex is then reacted with a second antibody (produced in goat against rabbit IgG) that binds to the first. Excess unbound material is removed after each step by washing. As the second (antirabbit) antibody is conjugated to an enzyme (usually either horseradish peroxidase or alkaline phosphatase), the bound complex can be detected by adding an enzyme substrate that generates a colored product. If no antigen is present in the sample, then no color change occurs. If antigen is present, then the intensity of the color, which can be measured spectrophotometrically, is indicative of the amount of antigen present and the concentration of pathogen in the sample.

Current attempts to improve the method aim to increase the adhesion of bacterial cells and associated antigens to the plate and to improve methods of extraction of bacteria from soil and plant samples. To detect the pathogen in soil, improved separation of bacterial and soil fractions has been demonstrated recently using an extraction buffer containing sodium cholate and polyvinyl pyrrolidine (Robinson and Forde, n.d.).

An alternative ELISA method is the dot-blot system recently developed at the International Potato Center. This allows trapping of the bacteria on nitrocellulose membrane instead of adhesion to microtiter plates (El-Nashaar, personal communication). This is also a suitable method for detecting the pathogen in water samples because a large volume of water can be passed through a relatively small nitrocellulose filter, thus trapping the bacterial fraction on the filter, on which the ELISA can then be performed. If the concentration of bacteria in the water is too low to allow detection, the sample may be previously concentrated further by centrifugation or evaporation.

Both indirect and dot-blot ELISA systems are currently being tested for the detection of latent populations of *P. solanacearum* in potato tubers, but have yet to be widely adopted for use in seed certification or quarantine procedures.

Immunofluorescence. Probably the most sensitive of the serodiagnostic methods, and the method currently used by European quarantine services to detect latent populations of *P. solanacearum* in imported potato tubers, is indirect immunofluorescence antibody staining (IFAS) as described by Janse (1988). This involves direct microscopic counting of bacterial cells in pellets from centrifuged filtrate of the homogenate of tissue plugs from 200-tuber samples (as described in the section "Detection Techniques"). *P. solanacearum* cells are coated with polyclonal antiserum that is then detected by a second swine/antirabbit antibody that is conjugated with the fluorescent dye fluorescein isothiocyanate (FITC). Observation under a fluorescent microscope then allows us to quantify the total number of cells in the sample. The method, when used in conjunction with a tomato indicator plant test, was found to be particularly sensitive.

Immunocapture techniques. Methods using antibodies to trap or concentrate bacteria from plant homogenates, soil suspensions, or water samples, which can then be identified by other means, are currently under investigation. For example, the coating of small beads with specific antibodies, rather than a flat surface, provides a larger surface area where bonding with target bacterial cells or antigens can occur. Commercially available magnetic beads, sensitized and mixed with soil or tissue suspensions, can be selectively recovered with a magnetic field. Detection of the bound pathogen can then be completed with enzyme-linked antibodies or by culture or other methods. In this way, the advantages of several methods can be combined. Similarly, Seal and Elphinstone are currently looking at ways to trap bacterial cells from soil samples with antibodies coated onto the walls of plastic tubes. Once trapped, removal of the soil fraction by stringent washing may then allow us to detect pathogen-specific DNA, as described below.

Methods Involving Detection of Pathogen-specific DNA

Recent advances in the field of molecular genetics have led to new possibilities for pathogen detection that depend on the recognition of DNA sequences that are specific only to the pathogen genome or to that of a particular strain of the pathogen that is to be differentially detected. With the advent of nonradioactive methods to label and detect DNA, interest in the practical use of nucleic-acid-based techniques has dramatically increased. Methods already field tested for the detection of *P. solanacearum* in diseased and latently infected potatoes are briefly described below.

DNA probes. Seal et al. (1992) obtained a *P. solanacearum*-specific DNA probe (PS2096) by applying a process known as subtractive hybridization. The probe detected all strains of the pathogen tested except for three nonpathogenic strains. The method involves the fixing of single-stranded DNA sequences, either from purified DNA extracts or complex mixtures from squashes of infected plants, onto nitrocellulose or nylon membranes. The DNA probe, previously labeled with commercially available nonradioactive products such as horseradish peroxidase enzyme or digoxygenin, is then allowed to hybridize with the complementary specific sequence of the bound DNA strand. After washing to remove the unbound probe, the bound probe can be detected by reaction with a colorimetric or chemiluminescent substrate of the peroxidase enzyme or with an enzyme-linked antibody to digoxygenin, which is detected using the relevant enzyme substrate.

A probe for the specific detection of race 3 strains of *P. solanacearum* was produced by Cook and Sequeira (1991) using similar methods. However, this was later found to hybridize with 5 of 90 non-race 3 strains tested. Further work is under progress to obtain a probe that exclusively detects race 3 strains.

DNA amplification by the polymerase chain reaction (PCR). A highly sensitive and much less laborious method to detect pathogen-specific DNA is by repeated synthesis of labeled copies of the relevant sequence by means of the PCR. The procedure uses two oligonucleotide primers that are complementary to the regions flanking the specific DNA segment. By undergoing repeated cycles of heat denaturation, annealing of the primers, and copying of the complementary sequences as extensions from the primers using a thermostable DNA polymerase (Taq-polymerase), the target sequence is amplified up to one million times. This means that even if only one copy of the sequence is present in the original sample, it can still be detected after amplification, theoretically allowing detection of a single pathogen cell in the sample.

Seal et al. (1992) synthesized oligonucleotide primers (PS96-H and PS96-I) to the specific DNA probe PS2096. This enabled highly sensitive detection of all except the three avirulent strains of *P. solanacearum* prepared as purified DNA extracts, lysed cells, or extracts of wilt-infected potato or tomato plants. Following the PCR, a characteristic 148 base-pair product is generated, which is easily detected by electrophoresis on agarose gels following staining of the DNA with ethidium bromide. Even greater levels of sensitivity of this detection technique have since been reached using a new set of primers for a second probe, which hybridizes to a specific sequence on the 16S rRNA molecule. As this sequence is repeated several times in the molecule, greater quantities of the PCR product are

generated during the same number of cycles, thus increasing sensitivity of detection.

The PCR test has been fully automated using a heating block that strictly regulates the temperature cycles and ensures minimum time between successive temperature changes. Furthermore, the test can be completed within only 5 hours. This technology is already being used for research purposes to study epidemiological and etiological aspects of interactions between *P. solanacearum* and its hosts, which we hope will lead to the development of more efficient disease control measures.

Comparison of Detection Techniques

Immunodiagnostic or DNA-detection techniques have an obvious advantage over cultural methods in that they do not require bacteriological expertise to differentiate the pathogen from the saprophytic bacterial contaminants of the preparation. Furthermore, the serological and DNA-based methods can detect and identify the pathogen in a much shorter length of time than is required to culture and purify the organism and then perform large numbers of laborious physiological and biochemical tests to confirm its identification (a process that routinely takes 3-4 weeks to complete).

The main advantage of cultural detection methods, including those involving indicator plants, is that only the viable pathogen cells are detected, and information on their pathogenicity is obtained. Serological and DNA-based techniques, however, detect both live and dead pathogen cells. Further investigation of a positive sample is therefore required to determine whether or not it contains viable inoculum of pathological importance.

Table 1 shows the range in sensitivity of detection with different methods as determined experimentally. Nucleic acid probes and serological tests such as ELISA have limits to their sensitivity, which allow low levels of infection to pass undetected. PCR amplification allows both sensitive and specific detection of the pathogen and its strains. However, the high cost of application of these techniques, the need for highly developed laboratory facilities, high standards of aseptic technique, and specialized training mean that these methods have so far been useful at the scientific research level rather than as practical techniques for routine use in seed-health or quarantine testing. In contrast, serological methods such as ELISA and IFAS have already gained widespread acceptability for the routine testing of a wide range of viral and bacterial pathogens as a result of their relatively low cost, lack of need f

Table 1. Relative sensitivity of methods for the detection of *P. solanacearum*.

Detection method	Minimum bacterial concentration detected (cfu per ml)	Reference
Dilution plating	10^3-10^4	Janse, 1988
Indicator plant (tomato seedling)	10^2-10^4 (only infected plants counted)	Janse, 1988
	1-10 (latently infected plants counted)	
Indirect ELISA	10^4	Robinson, 1993
Immunofluorescent antibody stain	10^4	Janse, 1988
DNA probe	10^5-10^6	Seal et al., 1992
DNA amplification by the polymerase chain reaction	5-116	Seal et al., 1992

laboratory facilities, and ease of use (requiring virtually no specialist training).

Future Research

Until we obtain more precise estimates of losses caused by bacterial wilt in potato crops, it will not be possible to predict the cost-effectiveness of the application of pathogen monitoring systems during seed potato production. Similarly, the choice of detection method, based on the levels of sensitivity afforded by each, cannot be decided until the epidemiological relevance of different levels of infection in a potato crop to disease incidence in that and subsequent crops is known. There is, therefore, a clear need to monitor seed potato crops at all stages during their multiplication, using as wide a range of techniques as possible, to correlate results of pathogen detection and quantification in planting materials with the subsequent incidence of disease and associated losses.

At present, serological methods provide an efficient and widely accepted way to determine seed potato health. Improvement in specificity of detection has accompanied the development of monoclonal antibodies, but refinement of the techniques employed is still required in order to improve the sensitivity of detection. Of great potential for pathogen monitoring in both specialist research and routine plant-health indexing is the use of nucleic acid probes and PCR technology. Efforts are already under way to adapt these methodologies for routine use in more simplified forms. Current studies will also investigate the possible occurrence of inhibitory compounds in plant and soil extracts that may affect the outcome of these highly sensitive tests.

Several methods have been described for the detection of *P. solanacearum* in plant, soil, and water samples. As discussed above, each method has advantages and disadvantages. It is now the task of the scientist in the field to compare the reliability and cost-effectiveness of each alternative and to select the best method, or combination of methods, that can be used to accurately and efficiently predict the economic threshold of infection.

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CHAPTER 16

Role of Nonhost and Weed Plants in the Perpetuation of *Pseudomonas solanacearum*

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Bacterial wilt of potato caused by *Pseudomonas solanacearum* E.F. Smith is endemic in low, mid, and high hills (900-2300 m.a.s.l.) in the Kumaon region of Uttar Pradesh, India. Besides potato, host plants showing visible wilt symptoms such as tomato, brinjal, chili, and datura, and a diverse group of weed and nonhost plants carrying this pathogen symptomlessly, are attacked by *P. solanacearum* (Kishore et al., 1993; Rao and Sohi, 1976; Sunaina et al., 1989).

Potato seed tubers and leftover tubers are considered to be the main source of innoculum (Sunaina et al., 1989). Few studies have looked critically at the rhizosphere of weed and nonhost plants as a protected survival site for this bacterium (Dukes et al., 1965; Granada and Sequeira, 1983; Hayward, 1991; Moffet and Hayward, 1980). Moreover, plants becoming infected by strains of *P. solanacearum* in a symptomless way are becoming a major threat in potato-producing areas of this region (Kishore et al., 1993). In the absence of a susceptible host, the comprehensive understanding of the role played by weed and nonhost plants will enable us to manage the development and spread of this disease better in the future.

Locations

Three different locations in the northwestern hills representing different altitudes and temperature regimes were surveyed for two years (1988-1989) to determine the role played by weed and nonhost plants, along with other related factors responsible for maintaining and spreading *P. solanacearum* in the absence of a susceptible crop.

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Mukteshwar

Located at 2300 m.a.s.l., Mukteshwar is representative of high-hill potato farming. During the potato season, the values of mean minimum and maximum air temperatures ranged between 3.7 and 13.4°C and 14.8 and 23.5°C, with total rainfall of 1016.2 mm, and humidity of 27-87%. The soil is sandy loam with pH 5.7 (Table 1).

Table 1. Wilt incidence in potato in the high hills of the Kumaon region of Uttar Pradesh in relation to meteorological factors (March-July, 1988).

Location	Month	Average air temperature (°C)		Monthly rainfall	Humidity	pH	Average wilt
		Max.	Min.	(mm)	(%)		(%)
Mukteshwar (2300 m)	March	14.8	3.4	170.0	47.2	5.6	0
	April	19.7	5.2	58.4	31.0	—	0
	May	21.2	10.2	45.0	49.0	—	0
	June	23.5	13.4	351.0	76.8	—	2.0
	July	20.0	13.1	419.8	91.2	—	4.5
r-value		0.31	0.73	0.91	0.92	—	

Bhowali

Located at an altitude of 1400 m.a.s.l., it is representative of mid-hill potato farming. During the potato season, the mean minimum and maximum air temperatures ranged between 8.2 and 19.6°C and 23.4 and 28.8°C, with total rainfall of 873 mm, and humidity of 27-87%. The soil is sandy loam, with pH 5.7 (Table 2).

Table 2. Wilt incidence in potato in the mid hills of the Kumaon region of Uttar Pradesh in relation to meteorological factors (March-July, 1988).

Location	Month	Average air temperature (°C)		Monthly rainfall	Humidity	pH	Average wilt
		Max.	Min.	(mm)	(%)		(%)
Bhowali (1400 m)	March	23.4	8.2	142.7	41.5	5.7	0
	April	28.0	9.4	12.0	27.0	—	0
	May	28.8	10.8	29.3	39.4	—	2.5
	June	28.5	16.8	328.7	70.2	—	9.0
	July	27.2	19.6	347.1	87.0	—	17.5
r-value		0.24	0.94	0.85	0.96	—	

Nehla

Nehla is located at 900 m.a.s.l. During the potato season, the mean minimum and maximum air temperatures ranged between 10.5 and 22.0°C and 24.0 and 32.3°C, with total rainfall of 699 mm, and humidity of 24.0-79.2%. The soil is sandy loam, with pH 6.4 (Table 3).

Table 3. Wilt incidence in potato in the low hills of the Kumaon region of Uttar Pradesh in relation to meteorological factors (March-July, 1988).

Location	Month	Average air temperature (°C)		Monthly rainfall	Humidity	pH	Average wilt
		Max.	Min.	(mm)	(%)		(%)
Nehla (900 m)	March	24.0	10.5	87.0	39.2	6.4	0
	April	29.4	14.2	16.0	24.0	—	2
	May	31.6	19.4	30.0	32.4	—	4.5
	June	32.3	21.5	264.0	67.4	—	11.0
	July	31.2	22.0	302.0	79.2	—	24.5
r-value		0.55	0.78	0.86	0.89	—	

At each location, abandoned potato fields and fields in rotation with crops other than potato were surveyed at monthly intervals. An area of about 5.0 ha was covered at each place to obtain an idea of factors including weeds and nonhosts responsible for maintenance and spread of the bacterium.

Assessment of Wilt Inoculum

The nature of the occurrence of potato bacterial wilt in the high hills (Mukteshwar), mid hills (Bhowali), and low hills (Nehla) during the period of this testing (March-July, 1988) indicated the importance of temperature and moisture levels in the survival of *P. solanacearum* in soils at these locations. The average percentage of plants affected by bacterial wilt at these locations was variable, with average percentage wilt of 4.5 at Mukteshwar, 17.5 at Bhowali, and 24.5 at Nehla (Tables 1-3). These results revealed a positive correlation of wilt incidence with humidity, rainfall, and average minimum temperatures, but not with average maximum temperatures during this period.

Distribution of Races and Biovars of *P. solanacearum*

The isolates collected from different places in the Kumaon hills belonged to races 1 and 3 of *P. solanacearum*. Biovars 2 and 3 were present in all the locations, but biovar 4 was recorded only from isolates collected from the mid hills (Bhowali) (Table 4). The presence of race 1 strains of *P. solanacearum* in hosts other than potato in the high hills (2300 m), where it was not reported earlier, suggests that its presence, if not checked, will enhance its spread, which can have serious consequences because of the broad host range of race 1.

Table 4. Races and biovars of *Pseudomonas solanacearum* in the Kumaon hills.

Location	Elevation (m)	Biovar	Race
Mukteshwar	2300	2, 3	1, 3
Bhowali	1500	2, 3, 4	1, 3
Nehla	900	2, 3	1, 3

Incidence of Bacterial Wilt in Solanaceous Crops

Several crops were surveyed: potato *(Solanum tuberosum)*, tomato *(Lycopersicon esculentum)*, brinjal *(Solanum melogena)*, chili *(Capsicum annuum)*, and datura *(Datura stramonium)*. The average wilt incidence of two years (1988-1989) was recorded as 28.8% in tomato, 13.8% in potato, 21.0% in brinjal, 12.5% in chili, and 9.4% in datura (Tables 5 and 6). Results indicate that the pathogen persists in all three representative locations. The occurrence of high wilt incidence in the mid and low hills can be explained by conducive environmental conditions and the presence of two potato crops per year followed by the planting of other solanaceous crops throughout the year, compared with the planting of a single potato crop in the high hills.

Estimation of *P. solanacearum* Infection in Weeds and Nonhosts

The complete roots of weeds growing in abandoned potato fields and of nonhost plants grown in rotation with potato were assayed for presence or absence of *P. solanacearum*.

Table 5. Incidence of bacterial wilt of potato in the Kumaon hills.[a]

Location	Wilt incidence (%)
Mukteshwar	5.5
Bhowali	18.5
Nehla	27.5
Mean	13.8

a. Based on the average of data from two years (1988-1989).

Table 6. Incidence of bacterial wilt of solanaceous crops other than potato in the Kumaon hills.[a]

Crop	Locations			Average
	Mukteshwar[b]	Bhowali	Nehla	
Tomato	—	25.0	32.6	28.8
Brinjal	—	18.0	24.0	21.0
Chili	—	12.0	13.0	12.5
Datura	—	12.4	6.4	9.4

a. Based on the average of data from two years (1988-1989).
b. Solanaceous crops other than potato are not grown commercially.

Sampling

At regular monthly intervals, the complete root of each of the three plants per species was collected and the soil clinging to it was removed by shaking each root for 20 minutes in sterile distilled water (50-400 ml, depending on the size of the root). Samples of 0.1 ml from 10 fold dilutions were spread with a glass spreader on the selective medium of Granada and Sequeira (1981) and the bacterial population/g of rhizosphere soil for each root system was determined.

To determine the population of *P. solanacearum* in root tissues, thoroughly washed roots from each plant were surface sterilized with 70% alcohol for 5 minutes. The treated roots, after washing, were macerated in 50-200 ml of sterile distilled water and the bacterial population/ml for each root system was determined as described previously.

Population determination of *P. solanacearum* in weeds and nonhost plants

Results indicate that *P. solanacearum* can infect roots of many weeds and other plants that until now were considered as nonhosts (Tables 7 and 8). A quantitative estimation of the survival ability of this bacterium in

Table 7. Weeds found in the infested fields of the Kumaon hills, and presence of latent or visible infection.

Host	Annual[a]	Perennial	Infection type	
			Latent	Visible
Antirrhinum sp.	+	-	-	+
Artemissia sp.	+	-	+	-
Arabidopsis thaliana	+	-	+	-
Aritica dioca	+	-	+	-
Ageratum conyzoides	+	-	+	-
Blumea sp.	+	-	nil	nil
Chenopodium album	+	-	nil	nil
Cannabis sativa	-	+	+	-
Datura stramonium	+	-	-	+
Galium aparine	+	-	+	-
Geranium nepolense	+	-	+	-
Lathyrus apace	+	-	+	-
Nepata sp.	+	-	+	-
Oxalis sp.	+	-	+	-
Oenothera rosea	+	-	+	-
Polygala sp.	+	-	+	-
Polygonum sp.	+	-	+	-
Renunculus scleratus	-	-	+	-
Rumex sp.	+	-	+	-
Spergula arvensis	+	-	+	-
Solanum xanthocarpum	+	-	-	+
Scuttellaria scandens	+	-	+	-
Trifolium sp.	+	-	+	-
Thalictum javanicum	+	-	+	-
Thaliana sp.	+	-	+	-
Valeriana hardwickii	+	-	+	-
Vicia sp.	+	-	+	-

a. + = present, - = absent.

rhizosphere soil and in the roots on a selective medium indicated the presence of low populations of *P. solanacearum* in the rhizosphere and root tissues of symptomless hosts compared with bacterial populations in plant roots showing visible symptoms. A majority of the weeds and nonhost plants carrying the bacterium in roots showed no external symptoms. The weed hosts that showed wilt symptoms were *Antirrhinum* sp., *Solanum xanthocarpum,* and *Datura stramonium* (Table 9). Among nonhost plants, only one host, *Capsicum baccatum,* which represented most of the symptomless plants found, had *P. solanacearum* populations in root tissues of 10^2-10^3 cfu/ml/root tissue system compared with 10^5-10^7 cfu/ml/root tissue system in plants showing visible wilt symptoms. In essence, the

Table 8. Nonhost crops and one host crop grown in fields in rotation with potato, and incidence of latent or visible infection.

Host	Cultivar	Planting season		Infection type[a]	
		Summer	Autumn	Latent	Visible
Cabbage	Chaubatia (early)	+	+	+	-
Maize	P-66	+	-	+	-
Turmeric	Patna variety	+	-	+	-
Wheat	HB-208	-	+	+	-
Millet	P-171	+	-	+	-
Paddy	P-22	+	-	+	-
Bean	Contender	+	-	+	-
Arbi *(Colocasia sp.)*	S-11	+	-	+	-
Chili *(Capsicum baccatum)*	Bell pepper	+	-	-	+

a. + = present, - = absent.

weed plants tested carried a *P. solanacearum* population between 10^2 and 10^7 cfu/g rhizosphere soil and 0.0-10^6 cfu/ml/root tissue (Table 9). The nonhost plants tested were symptomless except for chili *(C. baccatum)*. The *P. solanacearum* population in nonhosts ranged between 0.0 and 10^5 cfu/ml/root tissue (Table 10).

From the results, it is clear that plants infected by strains of *P. solanacearum* in a symptomless way are becoming a real threat to potato-producing areas because symptomless plant species could play a major role in the longer survival of *P. solanacearum* in infested soil (Graham and Lloyd, 1973; Kishore et al., 1993; Olsson, 1976; Quimio and Chan, 1979; Rao and Sohi, 1976; Sunaina et al., 1989). The presence of race 1 in the high, mid, and low hills of the Kumaon region, in turn, makes the management of bacterial wilt even more difficult due to its wide host range, longer survival, and nutritional versatility.

Although strains of *P. solanacearum* can infect the roots of many weeds and nonhost plants, in symptomless plant species the release of *P. solanacearum* cells into soil is not as high as from susceptible hosts and thus the incidence of bacterial wilt is less with rotation with nonhost crops. But the pathogen is never eliminated. The presence of *P. solanacearum* in the rhizosphere of symptomless hosts removes doubts regarding the protracted survival of this bacterium in soil in the absence of a susceptible host (Granada and Sequeira, 1983; Jackson and González, 1981; Kishore et al., 1993; Moffett and Hayward, 1920), and also explains the failure of crop rotations.

Table 9. Population of *Pseudomonas solanacearum* in weed rhizosphere soil and root tissue.

Host	*P. solanacearum* population	
	Rhizosphere (cfu/g/soil)	Root tissue (cfu/ml)
Antirrhinum sp.	4.0×10^6	2.4×10^5
Artemissia sp.	2.8×10^3	1.3×10^3
Arabidopsis thaliana	2.6×10^2	3.0×10^3
Aritica dioca	4.2×10^4	4.7×10^3
Ageratum conyzoides	2.6×10^4	0.5×10^3
Blumea sp.	4.7×10^2	0.0
Chenopodium album	3.2×10^2	0.0
Cannabis sativa	4.0×10^4	0.7×10^3
Datura stramonium	3.4×10^7	7.2×10^6
Galium aparine	1.9×10^4	2.5×10^3
Geranium nepolense	2.0×10^3	1.7×10^2
Lathyrus apaca	1.7×10^4	6.0×10^3
Nepata sp.	2.6×10^4	2.5×10^3
Oxalis sp.	4.0×10^4	0.0
Oenothera rosea	1.3×10^3	1.5×10^2
Polygala sp.	2.6×10^3	0.0
Polygonum sp.	2.7×10^4	0.5×10^3
Renunculus scleratus	8.7×10^4	2.0×10^3
Rumex sp.	1.5×10^3	2.2×10^2
Spergula arvensis	2.8×10^4	1.2×10^2
Solanum xanthocarpum	2.7×10^6	3.2×10^6
Scuttellaria scandens	1.5×10^2	0.0
Trifolium sp.	3.2×10^3	2.5×10^2
Thalictum javanicum	1.9×10^3	2.3×10^2
Thaliana sp.	4.2×10^2	1.3×10^2
Valeriana hardwickii	2.7×10^4	3.5×10^3
Vicia sp.	2.2×10^2	0.0

Table 10. Population of *Pseudomonas solanacearum* in rhizosphere soil and root tissue of nonhost plants.

Host	Cultivar	*P. solanacearum* population	
		Rhizosphere cfu/g/soil)	Root tissue (cfu/ml)
Cabbage	Chaubatia (early)	4.7×10^3	1.2×10^2
Maize	P-66	1.2×10^3	4.1×10^2
Turmeric	Patna variety	2.8×10^4	3.2×10^3
Wheat	HB-208	2.9×10^3	5.0×10^2
Millet	P-171	1.2×10^2	0.0
Paddy	P-22	4.5×10^4	4.6×10^3
Bean	Contender	7.3×10^5	6.5×10^4
Arbi (*Colocasia* sp.)	S-11	6.2×10^4	4.2×10^3
Chili (*Capsicum baccatum*)	Bell pepper	1.4×10^4	5.7×10^5

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CHAPTER 17

Ecology and Epidemiology of *Pseudomonas solanacearum* Race 3

E.R. French*

Ecology

Race 3 (biovar 2-A) of *Pseudomonas solanacearum* is the causal agent of bacterial wilt of potato (BWP) nearly everywhere this disease occurs. But race 1, which is usually less aggressive at the same temperatures, is sometimes responsible in warmer climates where potato is not traditionally grown (French, 1994). Race 3 isolates have a lower optimum temperature for growth and cause latent infection at soil temperatures of 14°C or higher, whereas warmer temperatures of 16°C or higher are needed for race 1 isolates (French, 1986; French et al., 1993; Swanepoel, 1990). Although high temperatures favor BWP, if soil temperatures exceed 43°C for 6 hours daily in the absence of the crop, the bacterium is killed. On the other hand, cold temperatures below freezing have permitted overwintering in soil (Lopes, 1994).

BWP is most often spread by planting latently infected tubers that have their vascular system infected, or have infected lenticels resulting from being held in wet soils after maturity for delayed harvesting (French et al., 1972; Rodrigues-Neto et al., 1984; Shekhawat et al., 1992). It also spreads by root-to-root contact in soil, water flowing along rows or from infested fields to noninfested ones, and by transport of contaminated soil on equipment, tools, hooves, and shoes (French, 1994).

Epidemiology

Pseudomonas solanacearum persists in soils principally in crop residues and volunteer (self-sown) potato plants, but also on a few susceptible crops (such as tomato, which is seldom grown in rotation with potato) and weeds that may wilt, be symptomless carriers of latent infection, or provide a favorable rhizosphere (French, 1994).

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The bacterium can survive free in soil at depths of 1 m or more where microbial competition is low, or as slimy masses in more superficial layers. However, the disease threshold is usually between 10^4 and 10^7 bacteria per gram of soil (Lopes, 1994; Shekhawat et al., 1992). Soils vary in their suppressivity to this pathogen. Intercropping within the row with maize or beans reduces the spread and thus wilt incidence. High organic matter soil amendments and additions of nitrogen and calcium often reduce the incidence of wilt. Minimum till, nematode control, and planting during cooler weather reduce the amount of infection (French, 1994).

Several recent reviews cover this subject in great detail. For more information, see French, 1994; Hayward, 1991; Persley, 1986; and Shekhawat et al., 1992.

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CHAPTER 18

Epidemiology of Bacterial Wilt of Potato in the Tropics

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Damage from *Pseudomonas solanacearum* is widespread in potato-growing countries. In South Asia and Australia, damage has been recorded in India, the Philippines, Sri Lanka, Indonesia, Australia, Vietnam, Mauritius, China, Nepal, and other countries. Damage is caused by wide host range race 1 (biovars 3 and 4) and narrow host range race 3 (biovar 2). In India, potato is grown extensively in both hills and plains, and damage in hills comes from race 3/biovar 2 (Shekhawat et al., 1978). Race 1 has also been reported in plains, which is the major area under potato cultivation (Kishore et al., 1991 and unpublished). Extensive occurrence of wide host range race 1/biovar 3 is reported, along with scattered instances of biovar 4 (Shekhawat et al., 1978). This paper deals with the epidemiology of bacterial wilt in Karnataka State, representing the Deccan plateau region in India. Bacterial wilt of potato was recorded in 1909 by Coleman. *P. solanacearum* persistently occurs in crops such as tomato, chili, potato, and brinjal. This plateau is infested by race 1. Little is known about the epidemiology of bacterial wilt of potato extensively grown in different agroclimatic zones such as dry, transitional, and hilly zones of the state. Seed potatoes are obtained from wilt-free areas such as the northwestern plains of Uttar Pradesh, Punjab, and the high hills of Himachal Pradesh, besides local ware potaoes.

The state faces problems in obtaining high-quality seed, which often comes from long distances away (northern India). Therefore, attempts were made to find a local area free of bacterial wilt in which to multiply seed. The work reported here was done under this scheme.

In Karnataka, potato is grown extensively as both a rainfed and irrigated crop on more than 18,000 ha. Karnataka's soil has laterite sandy loam in the south and east, red clay loam and red laterite in the west, and black in the north. The rainfed crop is planted with the start of rains in May and is harvested in September, whereas the irrigated crop is planted in December and harvested in March. The cropping pattern of potato

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zones in the state varies considerably, but the major crops include sorghum, finger millets, sugarcane, and solanaceous and nonsolanaceous vegetables.

Materials and Methods

Long-term survival under field conditions

Government potato seed farms and farmers' fields with a known history of bacterial wilt were identified in the eastern dry zone, and northern and southern transitional climatic zones of the state. On these farms, potato and other solanaceous crops were abandoned because of bacterial wilt. Fields were either kept fallow or were under nonhost crops for a couple of years. *P. solanacearum* in the field was detected by planting a 2-ha field with disease-free breeders' seed of potato cultivar Kufri Jyoti. Observations on wilt were made twice during the cropping period, and 1,000 potato tubers per field were examined for *P. solanacearum.* Observations were confirmed by making isolations on a TZC agar medium (Kelman, 1954). Disease index was calculated using the method described by Winstead and Kelman (1952).

P. solanacearum populations in roots of common crops planted in the area

Common crop practices in the region were studied, and crops planted in rotations with potato were identified. To find a population of *P. solanacearum* in roots, a field trial was conducted in a wilt-infested plot at the Indian Institute of Horticultural Research in Bangalore. The plot was divided into 5 x 3 m blocks. Different crops were planted in different blocks.

Multiple root samples of each crop were analyzed periodically. Blocks were lightly irrigated before taking the samples. The plants were gently pulled, along with the soil, and brought to the laboratory, and then the soil and roots were separated.

To determine the infections in the roots, the roots were cleaned with running water, blotted, dipped in alcohol for 7 minutes, flamed, and chopped, and 1-g root samples were crushed in 100 ml of sterile water in order to count the *P. solanacearum* populations by both streaking and the dilution plate technique using a TZC agar medium.

Survival of *P. solanacearum* in seed tubers

A majority of the region's farmers obtain potato seed from wilt-free areas of Uttar Pradesh, Punjab, and the higher hills of Himachal Pradesh. However, some farmers use their own seeds. The seed is stored either in

cold stores or in country stores at room temperature. Bacterial-wilt-infested tubers are known to rot in transit or in storage at high temperature, but many still escape rot. On the other hand, low temperature is known to harm the pathogen. To find out whether or not *P. solanacearum* strains occurring in these areas survive under different storage conditions and become primary sources of inoculum, these studies were conducted. Four potato seed samples were collected from two fields having wilt incidence of 30-60%. Samples A and C belonged to one field and B and D to the other, but both belonged to the most popular cultivar, Kufri Jyoti. Visually healthy looking tubers without soil or ooze sticking to them were brought to the laboratory and surface dried, and had only closely adhering soil that could not be removed unless washed. Samples were stored in cold stores (4-6°C), a refrigerator (10°C), and at room temperature (25-30°C). Tubers at room temperature were kept spread on newspaper and were not allowed to touch each other. Potatoes that rotted during storage were removed by hand and the newspapers were replaced.

Every month, 10 tubers in every sample from each storage condition were taken out and 25 proliferated lenticels were removed from five tubers, using a sharp metallic blade, and crushed in 10 ml of water. The surface of the five tubers was swabbed in 10 ml of sterile water, decanted, and analyzed for the presence of *P. solanacearum*. The amount of inoculum estimated each month (dependent variable) and its relation to storage period (independent variable) under each condition were worked out by calculating the correlation coefficient (r). Survival in the vascular region was studied by taking pieces from the tuber's vascular region. Tuber wilt potential was studied every month by planting one or two eye buds scooped from the surface-sterilized tubers in sterilized soil. The wilting of one or both plants grown from these buds was recorded as one data entry.

Wilt incidence in farmers' fields in different agroclimatic zones

A total of 324 potato fields were observed twice at 30-40 and 60-70 days of plant age for bacterial wilt incidence in the eastern dry zone (Bangalore and Kolar), the southern transitional zone (Hassan), and the northern transitional zone (Belgaum and Dharwar). Five hundred plants in a 1-ha crop were observed at five places, and wilt incidence, seed source, and rotation crops were recorded.

Results and Discussion

It was difficult to detect bacterial wilt of potato in a standing crop in fields that were fallow or under a nonhost crop in a rotation for 3-6 years. Wilt incidence was low—from 1.20 to 3% (Table 1). But many tubers (30-96%)

were found to carry latent infection, as revealed by the presence of vascular browning and isolations of the pathogen. These studies indicate that the strain prevalent in the area can survive in fields for quite a long time, confirming observations made by Coleman (1909), Bhide (1969), Shekhawat et al. (1979), and Ram Kishun (1981). Some common weeds known to be affected in these areas are *Lagasca mollis, Solanum khasianum, Phyllanthus niruri, Cleome monophylla,* and *Accanthospermum hispidum* (Rao and Sohi, 1976; Ram Kishun et al., 1980). Infections were detected in roots of all crop plants except finger millets (Table 2). These studies indicate that long-term survival of *P. solanacearum* is possible by infecting plant roots that are the normal rotation crops in the area. Granada and Sequeira (1983) made similar observations.

Table 1. Incidence of bacterial wilt in fallow and cultivated fields.[a]

Location	Climatic zone	Soil pH	Soil type	Field history	Year of observation	Plant age (days)	Wilt incidence	Disease index	Tuber infection (%)
Nandi Cross	Dry	6.14	Red sandy loam	Fallow from 1978	1984	30-35 60-65	0.20 1.80	0.06 0.88	51
		6.20	Red sandy loam	Fallow from 1982	1985	30-35 60-65	0.60 1.20	0.12 0.72	30
Somanhalli Kaval	Transitional	6.46	Red sandy loam	Fallow from 1981	1984	30-35 60-65	0.10 1.80	0.02 0.66	54
		6.50	Red sandy loam	Fallow from 1980	1985	30-35 60-65	0.30 2.30	0.06 1.42	32
Dharwar	Transitional	8.38	Black clay	Kharif-1981 Potato Rabi-1981-82 Wheat Kharif-1982 Jowar Rabi-1982-83 Wheat Kharif-1983 Groundnut Rabi-1983-84 Wheat	1984	30-35 60-65	0.10 3.00	0.06 1.68	96

a. Data based on 300 tubers. Area planted was 60 m^2.

Table 2. Infection of *Pseudomonas solanacearum* in roots of nonhost crop plants.

Crop plant and cultivar	No. of roots infected/ no. of roots tested	Range of *P. solanacearum* (cfu x 10^3/g root)
Sorghum CSH-1	2/10	0.6-2.6
Maize Decan-103	5/9	1.3-2.6
Wheat HD 2189	6/8	0.6-32.0
Finger millet Indaf-7	0/9	Nil
Knol khol White Vienna	6/8	4.6-48.0
Cabbage	7/11	10.6-24.0

Seed storage practices in cold or country stores do not eliminate *P. solanacearum* and tuber latent infection can still subsequently result in wilting plants (Table 3). More tubers resulted in wilting plants when they came from cold storage than when stored at room temperature (25-30°C), perhaps because of elimination of infected tubers (because they rotted when kept in room storage).

Tubers from infested soil carried inoculum in lenticels and/or vascular tissues for varying periods. But surface inoculum was not detected after 4 months of cold storage (4-6°C), although it could be detected up to 9 months in tubers stored at room temperature (Table 3).

Bacterial wilt occurred in all the climatic zones, under irrigated or nonirrigated conditions, to a varying extent (Table 4). There were 38 types of crop rotations involving short duration (onion, garlic, cabbage, cauliflower, etc.), medium duration (sorghum, maize, paddy, etc.), or long duration (sugarcane). Bacterial wilt occurred in infested soil even when seed was disease free, indicating that bacterial wilt of potato would remain a problem in the state of Karnataka even when following crop rotations and using healthy seeds.

Table 3. Monthly average inoculum levels (cfu) on each tuber surface (S) and lenticel (L), survival in vascular tissues, and wilt potential of potato tubers stored at different temperatures.[a]

Storage period (month)	*P. solanacearum* population (cfu x 10^3) and within plants								
	4-5°C			10°C			25-30°C		
	S	L	% wilt	S	L	% wilt	S	L	% wilt
0	7.60	0.58	—	7.31	3.18	—	7.60	2.35	—
1	0.23	0.04	—	5.21	2.09	—	11.12	0.35	—
2	0.57	0.07	—	2.31	1.40	—	2.28	0.28	—
3	0.84	0.09	—	2.74	4.45	—	0.12	0.10	—
4	0.18	0.08	25	17.38	0.55	25	0.72	0.11	25
5	0	0.06	45	17.55	1.09	15	8.16	0.48	15
6	0	0.04	40	1.25	0.38	30	0.31	0.04	10
7	0	0.03	40	8.77	1.58	30	0.40	0.10	15
8	0	0.04	30	9.29	2.10	35	0.14	0.05	10
9	0	0.04	20	8.47	1.34	55	0.18	0.04	—
10	0	0	20	13.57	2.43	15	0[b]	0[b]	—
11	0	0	20	1.24	0.12	30	—	—	—
12	0	0	20	1.11	0.22	20	—	—	—
Correlation coefficient r values	-0.534	-0.596*		-0.072	-0.463		-0.635*	-0.614	

a. Based on two samples (five tubers of each) at each storage condition.
b. Compound scraping of skin of five tubers in each sample. Percentage of wilt in plants raised from ten tubers of each sample and based on sprouts established from every tuber.
* Significant at 5% level.

Table 4. Potato bacterial wilt incidence in different agroclimatic zones.

Agroclimatic zone / Place name	Average disease incidence (%)		Number of fields observed	Range of disease incidence (%)
	Rainfed	Irrigated		
North transitional zone				
Belgaum	13.3[c]	18.8[b]	71	0-98.0
Dharwar	0.7[c]	—	40	0-2.4
South transitional zone				
Hassan	3.0[c]	17.7[b]	52	0-97.2
Eastern dry zone				
Kolar	0.3[b]	0.8[c]	50	0-25.0
Chickballapur	31.0[a]	5.3[b]	41	0-58.0
Hoskote	—	4.1[c]	34	0-27.0
Devenhalli	6.0[a]	4.6[c]	36	0-34.8

a. Data based on one crop season. b. Data based on two crop seasons. c. Data based on three crop seasons.

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CHAPTER 19

Epidemiology of Bacterial Wilt in the Northeastern Hills of India

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Introduction

The ability of *Pseudomonas solanacearum* to cause bacterial wilt is linked to its ability to survive its aggressiveness as a pathogen and predisposing environmental factors. Possible sources of *P. solanacearum* include infested soil, infected tubers, and the host plant, among others. Hence, study of these aspects began in 1985 at the Central Potato Research Institute's research station at Shillong, located in the East Khasi Hills of Meghalaya at 25.35° N and 91.54° E, at 1600 m.a.s.l., in northeastern India. This report aims to present findings made.

Field Incidence and Occurrence of Races and Biovars

Bacterial wilt of potato is a serious problem throughout the northeastern hills and is widespread in several mid-hill elevations of Arunachal Pradesh, Mizoram, Meghalaya, the plains of Assam, and valleys of Manipur, where it survives in a virulent form on cultivated crops such as potato, tomato, chili, capsicum, brinjal, ginger, and peanut. In the Khasi Hills, wilt incidence in potato raised under low-lying but well-drained paddy fields in the spring season did not exceed 0.5%. In the summer crop grown under raised beds, wilt incidence did not exceed 10%, but tuber rot was proportionately higher. Under "Jhum" cultivation, wilt incidence was below 1%, but tuber rot was proportionately higher. In autumn, wilt incidence exceeded 11% and in some cases was as high as 35-45%. Isolates of *P. solanacearum* from different hosts collected from the northeastern region belonged to race 1 and biovar 3. All the isolates were pathogenic to potato, chili, tomato, and brinjal, and used lactose, maltose, and oxidized mannitol and sorbitol. Hence, it appears that in this region only the solanaceous race is prevalent, and is well distributed.

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Population of *R solanacearum* in Soil

Population levels of *P. solanacearum* in two infested plots, one with potato raised from disease-free seed of variety Kufri Jyoti and the other under fallow for two years, were determined during 1987. Studies revealed that the pathogen survives in the soil throughout the year without the soil population reaching zero even after keeping the plots without a potato crop for two years. Studies further indicated that the soil population of *P. solanacearum* fluctuated between 1.6×10^2 and 8.0×10^5 cfu/g of soil. Field trials conducted in India have also indicated that the bacterium persisted in soil for at least two years even in the absence of host plants, but its population gradually declined (Shekhawat et al., 1979, 1982; Indrasenan et al., 1981; Addy et al., 1980). Supporting evidence (Granada, 1987) indicates that root remnants of host plants or the roots of presumed nonhost plants and leftover tubers, or survival deep in soil of the organism (Sunaina et al., 1989b) allows it to survive almost indefinitely.

In the northeastern region, soils are rich in organic matter that supports weed growth and high soil moisture year-round. These conditions favor the survival of *P. solanacearum* in soil.

Pseudomonas solanacearum is sensitive to high temperature and cannot withstand exposure to soil temperature above 43°C (Seneviratne, 1987). Meteorological data at CPRI indicated that mean monthly temperature ranged from 5.2-17.7°C to 17.0-23.7°C for minimum and maximum, respectively. These temperatures imposed no stress on the survival of *P. solanacearum*. Because of high soil moisture and favorable temperature during most of the year, it is able to survive in the soil.

Latent Infection in Tubers and Lenticels

Infected seed tubers are the most efficient means of survial of the pathogen (Patel and Kulkarni, 1953; Shekhawat et al., 1982a). Several researchers have reported the role of vascular, lenticular, and surface contamination as a source of inoculum (Sunaina et al., 1989a). Our studies also revealed that 9.36% of tubers had vascular infection and 6.1% of tubers had lenticular infection. Analysis of tubers harvested on different dates also showed that the bacterial population survived in lenticels for 90-100 days. After that, it dropped to a nondetectable level.

Latent Infection in Symptomless Weed Host Plants

Weed hosts and crop plants have been reported to carry latent infection with or without symptom expression (Sunaina et al., 1989a; Shekhawat et al., 1979). These hosts include potato, tomato, chili, capsicum, brinjal, ginger, groundnut, and many weeds. Studies on seven common weed host plants as possible latent carriers of *P. solanacearum* have revealed that *Polygonum hydropiper* was a successful and potential source of latent survival of *P. solanacearum* in the soil and was found to carry populations of *P. solanacearum* up to January.

Studies made at this institute have revealed that bacterial wilt of potato is a serious problem throughout the northeastern hills and is widespread in mid-hill elevations of Arunachal Pradesh, Mizoram, Mechalaya, the plains of Assam, and valleys of Manipur, where it survives in a virulent form. Studies have also revealed that *P. solanacearum* survives in the soil aided by latently infected tubers and weed hosts. Weeds such as *Polygonum hydropiper* and cultivated crops such as tomato, chili, capsicum, brinjal, peanut, and ginger help *P. solanacearum* survive in the soil. Intensity of wilt incidence in different parts of this region is mainly governed by soil infection, compounded by latently infected tubers and weed hosts. Survival and increase or decrease of the pathogen are governed by the changing temperature and soil moisture regime, which remains favorable during most of the crop growth stage.

We could conclude that growing potatoes under conditions of *P. solanacearum* infestation in this region requires successful coexistence with the disease. Coexistence is aided by practices such as cultivation on raised beds, crop rotation with finger millets, early planting and early harvesting, and a specific practice—jhuming (a local practice of soil burning).

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CHAPTER 20

The Detection of Variation in *Pseudomonas solanacearum*

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Introduction

Pseudomonas solanacearum is a highly diverse species, with field isolates demonstrating variation in host range, geographical distribution, pathogenicity, epidemiological relationships, and physiological properties (Hartman and Hayward, 1993). Some of these characteristics have been associated with changes at the DNA level, with the identification of restriction fragment length polymorphisms (Cook et al., 1989). Needs to identify isolates have led to a classification into biovars and races (Hayward, 1991), which, although consistent, useful, and practical, does not clearly establish a genetic basis for naturally occurring variation. A way to classify isolates based on specific genetic differences may also be more relevant for establishing rational strategies for control of bacterial wilt and breeding of resistance for this disease.

Over the past ten years, new molecular genetics techniques have become available that enable more precise genetic differentiation of isolates of *P. solanacearum*. These techniques include ones that enable the whole genome structure of this bacterium to be characterized.

These techniques need to address questions such as:

- How much variation occurs in genes that affect host range and virulence?
- Is genetic variation located in both the chromosome and plasmid components of the genome?
- Is such variation restricted to specific regions of the genome components?

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The Aims of Genetic Analysis of Bacteria

The combined use of molecular and traditional means of genetic analysis for bacteria now makes it possible to define genome size, the number and size of individual replicons, the location of chromosomal and plasmid-borne genes, and gene arrangements that have significance for biological properties. The ability to characterize whole genomes of bacteria provides additional precision for DNA fingerprinting techniques.

Techniques of Bacterial Genetic Analysis

Whereas the classical techniques of conjugation, transduction, and transformation have been essential for the characterization of bacterial genomes, the new molecular techniques have permitted the genetic characterization of newly isolated strains of bacteria. This replaced the situation in which genetic analysis was restricted to a few highly characterized laboratory strains. This feature of the new techniques is important for agriculturally significant organisms such as *P. solanacearum*. These techniques include pulsed field gel electrophoresis (PFGE), polymerase chain reaction (PCR), restriction endonuclease site mapping, synthesis of oligonucleotides of known sequence, DNA probing, developing a cosmid clone library of whole genomes, complementation of gene function, DNA and protein sequencing, and the construction and availability of data banks of DNA and protein sequences.

Physical and Genetic Analysis of *P. solanacearum*

There are no effective classical methods for mapping the genome of *P. solanacearum*. The aims of the genetic analysis of this organism can be defined as follows:

- To discriminate between genes on the chromosome and genes on megaplasmids.
- To identify genes contributing to plant disease.
- To develop a DNA fingerprinting system for identifying field isolates for virulence properties and host range.
- To compare the chromosome in *P. solanacearum* with that in other Pseudomonads.

We have prepared cosmid genome libraries using the vector pLA2917 (Allen and Hanson, 1985). This is a derivative of PK2 and pBR322, which retains the wide host range of PK2 and includes the *cos* site of lambda. Libraries have been constructed in this way with *P. aeruginosa, P. putida,* and *P. solanacearum* as well as other Gram-negative and Gram-positive bacteria.

Two strains of *P. solanacearum* have been examined, both isolated in Australia and both race 1/biovar 3. A preliminary *Spe*I map has been constructed and 30 fragments identified (Table 1), with both strains showing the same *Spe*I fragment pattern. To determine the linkage relationships of these fragments, a library of linking clones was constructed, each clone containing a *Spe*I site, a method used successfully by Ratnaningsih et al. (1990) to construct a physical map of *P. aeruginosa.* A total of 16 *Spe*I junctions involving 22 *Spe*I fragments were identified, and these were arranged into six unlinked groups (Figure 1) (Holloway et al., 1993).

Table 1. Sizes of *Spe*I fragments of *P. solanacearum* strains PSO1 and PS1000 obtained using the CHEF mode of PFGE.

*Spe*I band[a]	Size (kb)	*Spe*I band[a]	Size (kb)
A	467	P	173
B	448	Q	162
C	431	R	162
D	431	S	140
E	410	T	118
F	347	U	84
G	269	V	66
H	243	W	60
I	229	X	52
J	208	Y	29
K	200	Z	21
L	200	AA	18
M	190	AB	14
N	183	AC	11
O	173	AD	8
Genome size			5548

a. The fragments have been designated by letters of the English alphabet based on the proposed nomenclature of Tummler et al. (1992). Bands with identical size are inferred double bands based on appearance and intensity of fluorescence of the ethidium bromide stained gel.

AD—H—AA—U—[B C/D]—Y

M—G—J—V—Z

W—A—Q/R—F

E—AB—S

K/L—R

O—P

Figure 1. Linkage arrangements of *Spe*I fragments in *P. solanacearum* PS1000. The letters refer to the fragments identified in Table 1. Fragments B, C, and D have not yet been distinguished as to their linkage to the flanking fragments U and Y.

A number of individual genes have been located on individual *Spe*I fragments by probing using individual cosmids known to carry specific genes or cloned gene fragments.

DNA Fingerprinting Techniques for Identification and Classification of Field Isolates of *P. solanacearum*

The genomic data obtained by the approaches outlined above are of considerable value in the development of DNA fingerprinting techniques that will allow precise identification of isolates of this bacterium from diseased plants in the field. We can compare the specific DNA sequences in different biovars to determine their genetic similarity.

Selected individual cosmids from the cosmid banks of the biovar 3 strains referred to above were probed against whole genomic DNA of both a biovar 3 strain and a biovar 2 strain. A search was made for cosmids that hybridized with high efficiency to biovar 3 but poorly to biovar 2, and a number of such cosmids were identified. The DNA of these cosmids was digested with selected restriction enzymes and the fragments of *P. solanacearum* chromosomal DNA so isolated were used as probes against genomic DNA isolated from a range of different biovar 2, 3, and 4 isolates.

The results were encouraging, indicating that these probes could distinguish the different biovars. In addition, variation was observed with different isolates within each biovar. The DNA of these probes can now be mapped to individual *Spe*I fragments, thus providing the first data as to whether the variable regions of the genome are clustered or distributed over the whole genome. These data will be important for the construction

of further probes to distinguish field isolates of *P. solanacearum*.

Summary

An understanding of the genetic basis of variability in *P. solanacearum* can be achieved using modern techniques of molecular genetics. The knowledge so obtained will be highly effective for the development of rational strategies for control of this important crop disease.

Acknowledgments

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CHAPTER 21

Diversity of *Pseudomonas solanacearum*

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Diversity of a particular species reflects its evolutionary success and ecological plasticity. Current diversity of the wilt-causing bacterium *Pseudomonas solanacearum*, therefore, shows its ecological competency. Though the origin of the bacterium cannot be traced back through paleontological evidence, modern techniques of genetic analysis suggest that it probably originated from a common ancestor, possibly at a single location near the equator (Cook et al., 1991; Martin et al., 1982; Sequeira, 1993). Further evolution of the bacterium then occurred with several wild hosts, possibly in a forest ecosystem in geographically isolated areas (Buddenhagen and Kelman, 1964; Sequeira and Averre, 1961; Lomer et al., 1992). Criteria used to study diversity of *P. solanacearum* mostly rely on either host range or phenotypic differences such as ability to oxidize different carbohydrates, nitrate metabolism, variabilities in optimum growth temperature, serotyping, phage typing, etc. Much circumstantial evidence now suggests that present-day crop plants are not the natural host with which *P. solanacearum* has evolved. If this is true, then the current variability in host range of *P. solanacearum* is probably only the tip of the iceberg and many surprises await us if we study the bacterium in its natural habitat.

Phenotype

The bacterium is known to segregate into virulent and avirulent forms, which can be distinguished on a medium containing 2,3,5 triphenyl tetrazolium chloride (Kelman, 1954) on the basis of colony morphology. Virulent types form white and round or irregular fluidal colonies with or without pink centers. The pink center may be homogeneous or may show a spiral or concentric ring pattern. Colonies are rarely truly round and have abundant polysaccharide. The virulent colonies of the potato race are often irregularly lobed and each lobe sometimes has a pinhead-size clear

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plaque. Avirulent types produce truly round dark red colonies that are deficient in polysaccharide.

The virulent bacterial cells lack flagella, whereas the avirulent cells possess polar flagella. The strains of the bacterium also differ in pigment (melanin) production. Some strains produce black pigment while others produce no pigment. Intermediate types are also observed. The strain's ability to produce pigment also determines the degree of vascular discoloration in the host.

Symptoms

Pseudomonas solanacearum is a unique bacterium that can produce almost all types of symptoms known to be caused by other phytopathogenic bacteria. It produces wilt in plants and vascular rot in underground stems. It can infect potato tubers through lenticels and cause localized decay lesions in tuber flesh (Shekhawat at al., 1992a). It causes leaf spot in tomato and chilies in Australia (Hayward and Moffett, 1978). Depending on the strain's virulence, the wilt can be rapid or slow. Avirulent strains often produce swelling around the point of inoculation. In tomato, avirulent strains produce excessive adventitious roots. Epinasty and yellowing of leaves are more prominent when the pathogen's infecting strain is less virulent.

Races and Biovars

The extreme diversity within the species is reflected in the virtual dichotomy in its infraspecific classification. Below the formal species level, the pathogen is informally grouped into races on the basis of host range, and into biovars (formerly biotypes) according to differential use of carbohydrates. Classification of *P. solanacearum* into races and biovars is not governed by the International Code of Nomenclature. Groupings of *P. solanacearum* on the basis of host range are practical and useful for plant pathologists who work for good crop health, but seem highly artificial to systematists, who seek information on phylogeny and evolution. Broad host range and nonavailability of a suitable differential set compelled Buddenhagen and Kelman (1964) to modify the conventional race classification scheme for infraspecific grouping of *P. solanacearum*. Conventionally, a set of cultivars/genotypes of a single species is used for race classification. On the contrary, plants representing different species and even genera were used for race identification of *P. solanacearum*. Three races were originally identified by Buddenhagen and Kelman in 1964. Later,

two more races were added (Aragaki and Quinon, 1965; He et al., 1993; Hayward, 1991).

Hayward (1964) used different biochemical tests for infraspecific classification of *P. solanacearum*. Originally, four biovars were identified and later one more biovar had to be created (He et al., 1993; Hayward, 1991). In general, race 3 belongs to biovar 2 and race 5 to biovar 5. Race 1, consisting of biovars 1, 3, and 4, contributes significantly to diversity of the pathogen. The virtual overlap between race 3 and biovar 2 has recently been found untenable. Martin et al. (1982) reported few biovar 2 strains affecting tobacco, potato, and tomato. Biovar 2 strains are even sometimes isolated from brinjal (eggplant) and the weed host *Potulaca* sp. (Hayward et al., 1990). The potato-specific highland strains are now termed biovar-2A (Andean) and the lowland strains biovar-2T (tropical), which have a slightly broader host range (Hayward et al., 1992). Race 2, which infects species of Musaceae (banana, abaca, *Heliconia*, plantain, etc.), was also regrouped into different strains based on their colony appearance on semiselective media and epidemiological behavior (French, 1986). Race 5 infects mulberry and is so far synonymous with biovar 5. Extreme diversity is encountered within race 1 strains and host-specificity to a different extent is observed among them.

Analysis by molecular techniques such as membrane protein pattern (Dristig and Dianese, 1990), fatty acid composition (Janse, 1991; Stead, 1993), DNA homology (Cook et al., 1989), and others also revealed much diversity within the species. Conventionally, homologies at the DNA level are used to compare similarities between strains within a species. Cook et al. (1989) first studied the DNA homology pattern of nearly 200 different strains collected from diverse geographical areas representing all five races and biovars. They used seven different probes encoding information essential for virulence and induction of hypersensitive response. Thirty-five different homology groups were constructed based on restriction fragment length polymorphism (RFLP) analysis. Construction of dendograms (trees) based on similarity coefficients separated all the strains into two divisions that had only a 13.5% similarity between them. Division I contained all members of race 1/biovars 3, 4, and 5, and division II contained all members of race 1/biovar 1, and races 2 and 3. Further work by Cook et al. (1991) demonstrated that probably early in its evolution *P. solanacearum* divided into two groups and strains representing RFLP division I evolved in Australasia and division II in America. Race 3 strains that are isolated from Asia, Africa, and Australia are probably introduced with plant materials. Investigation into the fatty acid composition, membrane protein pattern, restriction analysis of a polygalacturonase gene fragment by polymerase chain reaction (PCR) amplification (Gillings et al., 1993), and the sequence analysis of 16S rRNA genes (Li et al., 1933)

supports the broad classification of *P. solanacearum* strains in two divisions. Nutritionally, biovars 1 and 2 are less versatile than biovars 3 and 4. Li et al. (1993) proposed that the two RFLP divisions of Cook et al. (1989) should now be given the formal status of subspecies, which would then be governed by the International Code.

Host Range

Variability in host range of Indian strains of *P. solanacearum* has long been recognized. Isolates collected from potato were not found to cause disease in chilies (Patel et al., 1952; Hingorani et al., 1956). Khan et al. (1979) showed that chili isolates did not cause disease in potato. In India, *P. solanacearum* affects crops such as potato, brinjal, chili, tomato, and nearly 114 other plants belonging to 37 families (Shekhawat et al., 1992a). Recently, nutmeg (*Myristica fragrans* L.) and mulberry (*Morus alba* L.) have been identified as natural hosts of the bacterium (Mathew at al., 1993a, 1993b). Rapeseed (*Brassica napus* L.), cabbage (*Brassica oleracea* L. conver. *capitata* (L.), Alef. var. *capitata* L.), and groundnut (*Arachis hypogaea* L.) were also found to be natural hosts of the pathogen in the northeastern hills of India (Singh and Husain, 1991; Singh, 1992, 1993). Shekhawat et al. (1978) studied 150 *P. solanacearum* isolates collected from all over India, representing diverse agroclimates. They concluded that race 3/biovar 2 is prevalent in hills and race 1/biovars 3 and 4 is predominant in the plains of India. Recent reports indicate the occurrence of race 1 in the higher hills also. Gnanamanickam et al. (1979) first reported the occurrence of race 2/ biovar 1 in Madras (south India). Their isolate, however, was pathogenic to tomato in addition to banana. Molecular diagnostic techniques revealed similarities between the banana strain from India and a similar strain from Honduras (Seal et al., 1993). If this is true, then probably a different strain of race 2 is prevalent in parts of southern India, causing moko disease of Robusta and Poovan bananas (Sivamani and Gnanamanickam, 1987). Recently, the SFV strain of race 2 was intercepted in a consignment of *Heliconia* sp. received from Hawaii (USA) in the Bombay airport (Reddy and Nikale, 1992). It is still uncertain whether race 2 strains have already been introduced in India. *P. solanacearum* infecting mulberry (race 5) has also been reported in southern India (Mathew et al., 1993b). Though the authors have not indicated the biovar of the isolate, we presume that it belongs to biovar 5. So far, all the mulberry isolates studied belong to biovar 5. Until recently, race 1/biovar 1 has not been encountered in India. However, Singh (1993) reported the existence of biovar 1 in the northeastern hills, where a forest ecosystem with precipitation year-round exists. It will be interesting to investigate whether nutritionally less

versatile biovar 1 has been introduced with planting materials or it has evolved locally from other nutritionally versatile progenitors. It is thus evident that all races and biovars of the pathogen exist in India. The Indian isolates studied so far are nonpathogenic to tobacco, and wilt of peanut occurs only in pockets. It is fortunate that major groundnut-growing parts of India are free from *P. solanacearum* strains that affect the crop. Appropriate quarantine regulations should now be formulated to curtail the domestic spread of groundnut isolates from northeastern India to other states.

One peculiarity of some Indian isolates is that the spontaneously formed afluidal mutants reverted to their respective wild-type, fluidal state with high frequency (Shekhawat et al., 1992b). Antibiotics such as oxytetracycline and tetracycline increased the frequency of reversion (Gadewar et al., 1993; Chakrabarti et al., 1993). Spontaneous reversion to a wild type has not yet been reported in any strain of *P. solanacearum* from other Asian countries or from America, Africa, and Australia. A question immediately arises: Is reversion a unique property of Indian isolates or does it also occur in strains of other countries? However, we presume that a similar reversion of afluidal types to fluidal wild types can be detected in strains from Nepal, Pakistan, Bhutan, Bangladesh, Sri Lanka, and other Asian countries. If this is so, then we have to reevaluate many of our earlier hypotheses about ecology of the pathogen.

Host Genome-Specific Variability

The natural populations of the bacterium are quite heterogeneous particularly in the tropics and subtropics. Variants/strains that exist or develop can selectively infect a specific host. This phenomenon has become apparent with the introduction of wilt-resistant hybrids/varieties derived from *Solanum phureja* in potato. Almost all the resistant clones are prone to latent infection (Ciampi et al., 1980; Granada, 1988). This means that populations of the bacterium capable of infecting the particular genome remain supressed in nature and when the host with that particular genome is planted, it is colonized by that population but does not show wilt because the inoculum is below the threshold level. Because potato is a vegetatively propagated crop, the latent infection builds up in subsequent generations and after some years when the inoculum crosses the threshold level for wilt development, the resistance of the clone breaks down. A similar phenomenon might be operating in wilt-resistant genotypes of other crops. However, because most of the other crops are propagated by true seed, chances of selection and buildup of the bacterial population virulent to the genome are rare.

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CHAPTER 22

Shift in Virulence of *Pseudomonas solanacearum*

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The bacterial wilt pathogen *Pseudomonas solanacearum* infects plants belonging to more than 35 families (Kelman, 1953) and causes wilt in many of them. It is widespread in tropical, subtropical, and warm climates. The pathogen's ecology, epidemiology, control, and genetics are actively studied because it causes devastating losses to crops such as potato, tomato, tobacco, groundnut, and others.

A unique feature of *P. solanacearum* is its ability to shift virulence spontaneously at a high rate (more than classical mutations, 10^{-7}). This change in virulence is routinely identified in vitro by the colony type (red, round, butyrous to rough) it forms on an agar medium containing 2,3,5 triphenyltetrazolium chloride (Kelman, 1954). Multiple changes in traits associated with virulence occur during this shift. Well-characterized changes during the shift to avirulence are in compositions of lipopolysaccharide (Lozano and Sequeira, 1970; Whatley et al., 1980) and exopolysaccharide (EPS) (Orgambide et al., 1991). Production of EPS (Husain and Kelman, 1958; Brumbley and Denny, 1990), endoglucanase, and pectin methyl esterase is reduced, while that of endopolygalacturonase is enhanced (Schell et al., 1993). Bacterial cells become motile (Kelman and Hruschka, 1973). Some avirulent strains cause stunting and swelling in plants (Husain and Kelman, 1958; Sequeira and Kelman, 1962; our unpublished observations). These multiple changes associated with the organism later came to be known as phenotypic conversions (PC) (Brumbley and Denny, 1990), and the resultant mutants as PC types.

A majority of the spontaneously formed mutants can grow in plants (Denny and Baek, 1991). The apparent and easily identifiable and detectable change is in the colony type associated with the change in EPS. Kelman (1954) studied the role played by EPS in wilting of plants. Recent studies (Denny and Baek, 1991; Kao and Sequeira, 1992) reveal EPS as a major virulence factor and endoglucanase and endopolygalacturonase as relatively minor factors (Denny et al., 1990). Several clusters of genes

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involved in biosynthesis of EPS have been identified: eps I and eps II (Denny and Baek, 1991) and the eps gene cluster (Cook and Sequeira, 1991). Four regions in the bacterial genome were identified as being associated with EPS production in two Australian strains using speI (Holloway et al., 1993). However, whether or not genes in these clusters are affected in spontaneously formed avirulent or weakly virulent forms is not known. Brumbley and Denny (1990) identified a regulatory gene phcA that upon losing its function affects multiple traits associated with virulence. In many of the spontaneous mutants, EPS was restored by this gene. Another regulator, epsR, has been identified in strain K 60, whose overexpression demonstrated a shift toward avirulence (Huang and Sequeira, 1990). Recent evidence from Japan indicates formation of a miniplasmid in nonpathogenic forms (Negishi et al., 1993).

We observed spontaneous or induced reversion of avirulent forms to virulent forms (Shekhawat et al., 1992; Gadewar et al., 1993). The revertant fluidal types are exactly like wild types in colony formation, appearance, and virulence. The phenomenon observed might occur in nature and is therefore important in ecology and management of the pathogen. The mechanism operating in these isolates appears to be different from those reported elsewhere. The present paper deals with the effect of temperature on shifts in virulence in one of the isolates.

Background information

Wild-type *P. solanacearum* isolates GSC 26 and CSG 39 form deep red, round, small afluidal colonies spontaneously, which, in stem inoculations in tomato, produce mild or no wilt. Seventy-two-hour-old fluidal colonies of isolate GSC 26 consist of 0-7.7% of wild-type cells that form afluidal colonies on a TZC agar medium. These afluidal colonies have 1.1-8.6% of cells that form fluidal colonies on a TZC agar medium, indicating that afluidal cells of this isolate are unstable (Shekhawat et al., 1992). Attempts to isolate stable afluidal variants failed. However, for isolate CSG 39, formation of stable afluidal forms was found to be very low (0.066%) (unpublished data). In our previous trials, a suspension obtained from single afluidal colonies of GSC 26 produced an average of 3.3-3.8 fluidal colonies on TZC agar medium but it was increased 5-16 times with antibiotic oxytetracycline or tetracycline (Gadewar et al., 1993). The trend was similar for isolate GSC 39 (Chakrabarti et al., 1994). However, a spontaneous stable variant of CSG 39 did not produce a fluidal revertant spontaneously in plants or by antibiotic amendment (unpublished data).

The shift in virulence was not influenced by other antibiotics, namely, chloromycetin, vancomycin, and streptomycin (Gadewar et al., 1993).

However, this indicated that shift in virulence, i.e., formation of fluidal revertants, can be influenced by specific external factors. Antibiotics are not used extensively to control bacterial wilt.

Temperature is known to influence the outcome of host (potato) and pathogen (*P. solanacearum*) interaction (Tung et al., 1990). Therefore, the effect of temperature on formation of the revertant fluidal type was studied in isolate GSC 26, which formed only unstable afluidal variants.

Materials and Methods

Optimum temperature for the formation of fluidal and afluidal colonies was found to be 30°C for isolate GSC 26. Preliminary trials were carried out using temperatures higher than the optimum. At a temperature of 40°C, colony formation took longer and was erratic. Although growth was slower and erratic at 37°C, it was better than at 40°C. Therefore, 37°C was chosen as a maximum temperature for the experiment. An intermediate temperature of 35°C was included in the studies as a standing crop often experiences this temperature in the field.

The effect of temperature on the reversion phenomenon was studied in a total of 23 afluidal 72-hour-old colonies obtained from a TZC agar medium by following the method described by Gadewar et al. (1993). The experiment consisted of three temperatures (30, 35, and 37°C) and the medium used was TZC agar. The diluted (10^{-5}) suspension (100 µl) of a single colony was spread on each plate of TZC and six plates were incubated at each temperature.

Observations on total colonies and number of fluidal and afluidal colonies at 30°C were made at 72 hours, whereas plates at 35 and 37°C were shifted to 30°C after 84 hours and 168 hours, respectively, and further incubated at 30°C for 84 hours. Fluidal and afluidal colonies were counted. Pathogenicity of fluidal types obtained from treatments of 35 and 37°C was tested on 1-month-old tomato seedlings by the stem stab method of inoculation and final data for wilt index were recorded using the Winstead and Kelman (1952) scale.

Results and Discussion

The total number of colonies growing on plates incubated at 37°C was always lower than the total of colonies growing at 30°C. However, colony formation at 37°C generally took 7 days. The colonies growing at 37°C were red, compact, and afluidal. Development of small but otherwise

typical fluidal colonies rarely occurred. However, an atypical phenomenon was observed after shifting to 30°C in the following 84 hours:

1. Plates had no growth while shifting, but fluidal and afluidal colonies appeared in the next 84 hours.
2. Colonies looking afluidal, red, and compact turned out to be fluidal with white margins.
3. Colonies looking afluidal, red, and compact did not change except for increasing slightly in size.
4. Additional fluidal colonies appeared.
5. Additional afluidal colonies appeared.

At 30°C, numbers of fluidal and afluidal colonies averaged 2.4 and 66.9, respectively. Plates first incubated at 35 and 37°C and later shifted to 30°C showed a 4-fold and 6.5-fold increase in fluidal colonies (Table 1). Fluidal colonies from 35 and 37°C were pathogenic to tomato plants, like those of normal fluidal revertants.

Table 1. Mean number of colonies in incubation at different temperatures.[a]

Temperature (°C)	Mean no. of colonies		Total	% decrease in total	Fold increase in fluidal types
	Fluidal	Afluidal			
30	2.4	66.9	69.3	0	0
35	9.7	39.9	49.6	28.4	4.0
37	15.5	24.7	40.2	42.0	6.5

a. Based on colony forming units (cfu) from 23 afluidal colonies.

Fluidal colonies did not appear during incubation at 35 and 37°C, but appeared only after the plates were shifted to 30°C. This indicates that formation of EPS was inhibited at 35 and 37°C, but was triggered in some cells and expressed only at 30°C.

We can be draw from these findings the following conclusions:

1. All the cells in the inoculum cannot be made to convert to fluidal forms by raising the temperature, although the conversion can be triggered.
2. We do not yet know how and why such forward and backward conversions occur so frequently.

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CHAPTER 23

Possibilities for Manipulating *Pseudomonas solanacearum* for Its Control

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Plant diseases have been managed successfully through modification of the host by means of traditional resistance breeding between compatible parents. Control is now being expanded by creating transgenic plants. Manipulation of the pathogen for its control has been attempted in only a few cases due to more difficulties in manipulating the pathogen than the host. This has been successful in a stubborn disease such as chestnut blight (Van Alfen, 1982).

Resistance and immunity in the host plant are expressed by restricting the entry of the pathogen, restricting its growth in plants, and harboring the pathogen but tolerating its presence. Management of *Pseudomonas solanacearum* through resistance breeding in potato has met with little success, although it has been successful in tobacco and groundnut. Resistance in *Solanum phureja* and other species has been identified and an inhibitory factor to *P. solanacearum* has been identified in them (Zalewski and Sequeira, 1975), but all the bred varieties become susceptible to one strain or another. Resistance is also influenced by environmental factors that produce different responses to the same strain. Indian cultivars like Kufri Lalima delay the multiplication of the pathogen, but subsequently allow it to multiply as fast as other susceptible varieties do (Chakrabarti and Shekhawat, 1991).

Other widely practiced methods are management through crop rotation and use of biocontrol agents. Crop rotations may or may not be successful in all situations and small farmers may not like to rotate a crop due to the economic importance of potato. Although the use of biocontrol agents is promising, it will still be difficult to find a biocontrol agent that stops the entry and progress of the pathogen.

Our field trials indicate that damage from *P. solanacearum* can be mitigated by using avirulent strains of it (unpublished data). Similarly, recent studies have shown that hrp mutants created using interposon protect tomato plants to a large extent. However, the virulent cell

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population in tomato plants was quite large, indicating that the pathogen does gain entry into plants and multiplies, but the resistance shown is due to a host defense mechanism activated because of avirulent cell inoculation (Frey et al., 1993). In our studies with unstable isolates consisting of a mixture of 3% virulent cells and a 97% avirulent population, tomato plants show no wilt or slight wilt, indicating that virulent cells are not favored in the plants (Gadewar et al., 1993). Earlier studies by Averre and Kelman (1964) support this observation. We have observed that sometimes a few susceptible potato and tomato plants inoculated with virulent forms of *P. solanacearum* wilt under favorable conditions although other plants inoculated at the same time and under the same conditions do not wilt. The isolates from these plants produce wilt in subsequent inoculations to other plants, and this phenomenon indicates that even susceptible plants have a mechanism to induce tolerance, or a resistance mechanism that may need to be activated. The phenomenon of activation of a defense mechanism can be used to advantage. Earlier studies indicated that plant wilting depends upon the ratio of virulent and avirulent cells (Averre and Kelman, 1964).

Pseudomonas solanacearum spontaneously changes from highly virulent types to avirulent or weakly virulent types. Whether or not the conversions are due to classical incidental mutations (reported in a few cases as a frame shift, Denny et al., 1993) or programmed regular happenings, their frequency is high. These conversions are highly influenced by host factors (which generally appear late in plants), cultural conditions (Kelman and Hruschka, 1973; Denny et al., 1993), higher temperature, and other environmental factors (Shekhawat and Perombelon, 1991). Similarly, reverse conversions in unstable isolates are also affected by other external factors such as antibiotics (Gadewar et al., 1993) and temperature (Gadewar et al., Chapter 22, this volume). These observations indicate that conversions in *P. solanacearum* may be accessible to manipulations.

The chemical nature of compounds causing the avirulent conversion has not been identified. Observations indicate that compounds in the cells set the conversion in motion. If such compounds could be identified and plants made to form them, they may convert entering *P. solanacearum* cells into the avirulent form instantly. These converted cells could invoke the plant defense mechanism and thus avert wilting. Until now, emphasis was on studying the character that eliminates the pathogen or restricts its entry.

Endothia parasitica has been manipulated successfully (Van Alfen, 1982). In nature, strains of the pathogen existed that were hypovirulent, and it has been known to carry double-stranded RNA (Elliston, 1978). The factor known to cause hypovirulence has been found to be cytoplasmically

transmissible (Grente and Sauret, 1969; Van Alfen et al., 1975). In *P. solanacearum*, recent studies have shown that many sites make the pathogen avirulent on transposition of transposing elements. There are reports that many Malaysian isolates of *P. solanacearum* are lysogenic (Abdullah, 1993), but it is not known whether lysogenic isolates are less virulent than others. Taking these factors into consideration and with modern techniques available, it seems quite likely that we can manipulate *P. solanacearum* for its control.

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CHAPTER 24

Breeding Potato for Resistance to Bacterial Wilt (*Pseudomonas Solanacearum*): Looking for Stable Resistance?

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There can be no question about the usefulness of resistance for integrated control of bacterial wilt (BW, *Pseudomonas solanacearum*) of potato in the tropics and subtropics. During the past 25 years, much effort has been made to search for and to breed for this resistance. Quick progress was made in identifying resistance sources and using these specific sources to create potato breeding populations (Schmiediche, 1983, 1985; French and Sequeira, 1988). Resistance to wilt, however, appears to be unstable. The use of resistant clones has been successful in some cases, but not in others. The complex nature of resistance itself and the strong host x pathogen x environment interaction are probably major obstacles to achieving stable resistance. In this paper, we shall restrict our discussion to these aspects of breeding for BW resistance.

Resistance: Inheritance and Expression

Our recent observations indicate that resistance to BW in potato is a partially dominant character and is more of a polygenic type (Tung et al., 1993). The number of genes involved is uncertain, but there is evidence that genes with both major and minor effects are involved in the expression of resistance to wilt (Tung et al., 1992a, 1992b). Results from our investigations are thus in general agreement with the hypothesis of Rowe and co-workers (Rowe and Sequeira, 1970; Rowe et al., 1972). But the inheritance of resistance and its expression are complex. Both additive and nonadditive gene actions are of significance in inheritance of resistance to wilt (Tung et al., 1992b, 1993). Their relative magnitudes, however, indicate that the latter component is more important. As a result, significant general and specific combining abilities for resistance are observed (Tung et al., 1990a, 1993).

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There is also evidence of a high interaction between genes for resistance and genes for adaptation (Tung et al., 1993), which further confirms the hypothesis that genes for adaptation are involved in conditioning the resistance expression (Tung et al., 1990a; Schmiediche, 1983, 1988; Kloos and Fernández, 1986). This also highlights the importance of the adaptive potential of a resistant genotype for the expression of the resistance. It also indicates that epistatic gene effects are probably large in the inheritance of resistance to BW. These epistatic gene effects are certainly conserved by artificial clonal selection for resistance and vegetative propagation of the selected resistant clones in breeding programs. A breakdown of the intact parental genotypes upon cross-breeding will result in a partial loss of favorable epistatic associations for resistance. The consequence is often a large reduction in the average level of resistance in selfed and F1 progenies of resistant parental clones. Progeny testing is thus a prelude to identifying the best general combiners for breeding for true potato seed progenies resistant to BW.

Resistance genes

Research at CIP showed that resistant accessions of various wild *Solanum* species have been collected from areas where *P. solanacearum* has never been found (CIP, 1987). It is likely that true resistance genes have never been molded by natural selection. A gene-for-gene relationship (Flor, 1971) thus does not seem to be applicable to bacterial wilt. This raises the question: Why are there genes that exert major effects on resistance expression and what is their nature? Certain genes other than those "for resistance alone" might have turned out to have the novel (pleiotropic) effects in conferring the resistance once the potato plant has come into contact with the pathogen under a certain set of environmental conditions. Those genes are then eventually called "genes for resistance" once a certain level of resistance is detected. It is conceivable that some of those genes are genes for adaptation because resistance tends to break down whenever the carrier host genotype faces new ecological conditions. The major or minor status of those genes may depend on the particular genotype of the pathogen, the genetic background of the carrier host genotype, and the particular environmental conditions that influence their expression. This is probably why Zalewski (cit. Sequeira, 1979) found that four major genes instead of three (Rowe and Sequeira, 1970; Rowe et al., 1972) govern the resistance and why contradictory results were obtained from their later studies (Sequeira, 1979).

As BW resistance is associated with fitness, breeding for resistance, which has taken place only during the past two decades, would seem a difficult and challenging task. Attempts to transfer resistance from wild *Solanum* species into the common potato would result in excessive recombinations, which are the most vulnerable to breakdown upon

intercrossing. Furthermore, as Mather (1960) mentioned, over the short term, the unit of transmission and adjustment is not so much the genes as the chromosome segments carrying genes that affect more than one character. From an evolutionary point of view, adjustments and adaptations imposed on polygenic systems in wild species by natural selection in the past will obviously channel and limit response to selection, whether natural or artificial (Mather, 1960). Breeding for resistance to BW in warm environments of the lower elevations in the tropics will indeed demand readjustment and reorganization of the genic systems conditioning both resistance and adaptation. As a consequence, progress during the first few cycles of selection would be slow and inconsistent.

Specificity of resistance

Strain specificity, and not race-cultivar specificity, is a common feature in many tests of potato cultivars for resistance to BW that further supports the fact that the gene-for-gene concept does not apply for bacterial wilt of potato. Host x pathogen interaction, and hence strain specificity, seems to be more of the polygenic type described by Parlevliet and Zadoks (1977). In tests (French and De Lindo, 1982; Thurston and Lozano, 1968; Tung et al., 1990b), host x pathogen interaction effects, though they may be highly significant, often comprise only a small fraction of the total variation, which is often at the magnitude of experimental errors. Main effects due to host genotypes and pathogen isolates are the major source of variation. Many genes seem involved in controlling the resistance expression, and this resistance is quantitative.

Like the resistance itself, strain specificity also varies. The ranking order of host genotypes in terms of resistance to a particular pathogen isolate tends to change when tests are carried out over environments (Tung et al., 1990b). Thus, the host genotype x pathogen genotype interaction in the potato-*P. solanacearum* system often seems to be artifactual. Both the host and pathogen are sensitive to environmental changes. Therefore, host genotype x pathogen genotype interaction may also be a result of host genotype x environment and/or pathogen genotype x environment interactions as discussed by Kulkarni and Chopra (1982). Apparently, strain specificity in the potato-*P. solanacearum* pathosystem seems to be a consequence of differential adaptation of particular host and pathogen genotypes to environments as discussed by Crute (1985).

Breeding for Resistance

Practical evidence during the past two decades indicates that breeding for resistance, though successful to some extent, has made slow progress. Some fundamental problems were discussed in the previous sections.

Whereas the tremendous variability in pathotypes of *P. solanacearum* requires a broad-based type of resistance, we need to put more emphasis on adaptation aspects of the host genotype. Wide adaptation is necessary for stable expression of resistance.

Selection

Breeding procedures will largely depend on the concrete situations with which a breeding program is faced: sources of resistance available, prevalence of a particular pathotype of the bacterium, ecological conditions, agronomic requirements, etc. For any case, the presence of significant nonadditive gene action suggests that clonal selection will be successful in developing clonal cultivars for specific growing conditions. However, such cultivars would not offer stable resistance if selection were not based on a broad genetic background for resistance and adaptation. For population improvement, breeding procedures should be designed to make use of both additive and nonadditive gene actions. Open options should be available for continuous introduction of resistance from new sources. In a broad-based population, recurrent selection of individual resistant plants should be successful.

Screening

The success of any method of breeding for resistance is a function of the efficiency of the screening method applied for selection of superior genotypes. With the most effective inoculation method, the choice of an appropriate isolate/strain of *P. solanacearum* is probably the most important factor affecting the effectiveness of selection for high and stable resistance. Variability in pathogenicity according to environmental changes, especially temperature, has been shown to be great in most strains of *P. solanacearum*. A highly virulent strain that maintains virulence and aggressiveness over a wide range of environmental conditions would be the most suitable for testing and selecting for stable resistance. The test may seem very rigorous, but could assure a high level of stable resistance. This hypothesis derives from the concept that host x pathogen x environment interaction in the potato-*P. solanacearum* pathosystem is a reflection of differential adaptation of host genotype and pathogen genotype to environments discussed previously. Although further proof may be desirable, it seems evident that a strain that behaves differently in different environments is not suitable for testing and selecting for stable resistance.

Latent infection

Almost nothing is known about the mechanism and genetics of resistance to latent infection. In addition, latent infection seems to be a neglected objective in potato breeding, and was touched upon only recently, perhaps because latent infection is not associated with above-

ground symptoms (Ciampi and Sequeira, 1980). As more and more potatoes are grown in the tropics, latent infection appears to be a serious problem because of the danger of spreading bacterial wilt by latently infected tubers. Perhaps we need to do more research on genetics and breeding for resistance to latent infection in the near future.

Conclusions

It appears that until genetic engineering can provide a general type of resistance, many things can still be done through conventional breeding in order to achieve a high level of stable resistance to BW. In the short and medium terms, the use of the type of resistance that we currently have will still be an important component in the integrated system of controlling the disease. Interaction of *P. solanacearum* with root-knot nematodes in inducing more wilt requires good ways to control the latter in combination with other agronomic and sanitary measures.

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CHAPTER 25

Stability of Resistance to Bacterial Wilt in Potato: The Indian Experience

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Shortly after scientists became aware of Mendel's law of inheritance, rust resistance in cereals was demonstrated to be inherited in a Mendelian fashion (Biffen, 1904). This stimulated research to identify and incorporate disease resistance in crops through breeding. The search for bacterial wilt (BW) resistance and its use in potato is a fascinating story, with much frustration and little success (Thurston, 1976; Martin, 1980; Schmiediche, 1986). In India, a systematic search for resistance was initiated by Mahesh D. Upadhya and coworkers during 1968-1972 (Upadhya et al., 1976). For five years, a large collection of genetic stock (Table 1) was evaluated in the glasshouse and in fields. Two groups of cultures (BR/A and BR/AM) were raised from true potato seeds provided by Robert V. Akeley of USDA-ARS, Beltsville, Maryland (USA), in 1968. Performance of these cultures was evaluated by root injury and stem stab methods as well as by natural

Table 1. Germplasm screened during 1968-1972.

Source	Accession code	Number screened
CPRI	CP	1,474
tuberosum	CP	213
andigena	EX	313
Tuber-bearing *Solanum* spp.	SS	274 (52 spp.)
Polyhaploids	PH	326
Irradiated materials	-	144
Dr. Robert V. Akeley, USDA-ARS, Beltsville, Maryland, USA	BR/AM	681
	BR/A	578
Dr. Rowe, Plant Introduction Station, Sturgeon Bay, Wisconsin, USA	BR/69	647
Total		4,650

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infection under field conditions. Although none of the cultures escaped wilt, wilt appearance was delayed in certain cultures. But this was also inconsistent during different years. In 1969, 647 cultures of 41 families were received from the Plant Introduction Station in Sturgeon Bay, Wisconsin, and they were numbered serially from BR/69 to BR/69-647. These cultures derived BW resistance from the cultivated diploid species *Solanum phureja* and late blight resistance from the hexaploid *S. demissum*. During the first year, 110 BR/69 cultures were selected, which were free of tuber rot after storage. Further screening showed that none of the cultures escaped wilt. Beside these, thousands of clones from the CPRI collection (both *S. tuberosum* ssp. *tuberosum* and ssp. *andigena*), polyhaploids, hybrids, and irradiated materials were also tested. No useful source of resistance with persistent results could be detected. Seedlings raised from seeds of BR/A, BR/AM, and BR/69 collected from self- and open-pollinated berries were also tested. Survival percentage was poor, and it was observed that the resistant seedlings mostly fell under late-maturity groups. Therefore, they were not used for additional breeding. From this experiment, it was concluded that:

1. Most varieties had an erratic response to wilting from season to season; cultures that escaped the disease in the first year showed susceptibility to brown rot in later years, and the stage at which wilting developed also varied.
2. There was no correlation between the time of foliage expression of the disease and tuber loss at harvest.
3. Comparatively late-maturing cultures developed wilting late, but tuber damage varied.
4. Percentage of tuber loss was comparatively low in the varieties that produced numerous tubers per plant.
5. It was not possible to isolate any mutant resistant to brown rot among the mutagenized population screened (Upadhya et al., 1976).

G.S. Shekhawat and coworkers made another attempt during 1973-1984 to locate sources of resistance (Table 2). Among 7,183 different cultures tested, useful resistance was detected in only one accession (SS 529-1) of the diploid wild species *S. microdontum* (Shekhawat et al., 1980). We therefore tried to begin transferring BW resistance from *S. microdontum* to commercial cultivars (Tyagi et al., 1980). A diploid clone of *S. tuberosum* (PH 54-30) was used for this purpose. Nearly 500 viable seeds were obtained from the cross PH 54-30 x *S. microdontum*, out of which three diploid hybrid (BRB/A-21, 23, and 24) were promising. In the following years, the diploid resistant hybrid BRB/A-24 was crossed with tetraploid

Table 2. Germplasm screened during 1973-1984.

Source	Accession code	Number screened
CPRI and CIP	BR/A, BRB/A, BRB/B, BH, MT	2,813
andigena	EX	860
Solanum spp.	SS	310
Polyhaploids	PH	200
Mutagenized materials	-	3,000
Total		7,183

lines (EB/B-413/740 and VB/A-92) to produce meiotic tetraploids. Two tetraploid hybrids (BRB/MT-2 and BRB/MT-5) were promising. However, we obtained inconsistent results when these lines were tested in different agroecological zones. Meanwhile, we demonstrated that the use of clean seed along with bleaching powder at 12 kg/ha could efficiently manage bacterial wilt in wilt-endemic areas (Shekhawat et al., 1988). Therefore, attempts to breed BW-resistant potato cultivars were abandoned. During the 1980s, it was demonstrated elsewhere that BW resistance in potato is strain and temperature specific (French and de Lindo, 1982), and the so-called resistant cultivars are in fact wilt tolerant, as they harbored a large population of *P. solanacearum* in vivo. Screening efforts were therefore made to detect clones with a high degree of tolerance instead of resistance (Chakrabarti et al., 1992, 1993). We presumed that if a tolerant cultivar is grown under an umbrella of preventive cultural practices, a good crop of ware potato can be harvested in wilt-endemic areas. A few clones that can delay wilt appearance even in the presence of *Meloidogyne incognita* (Nagesh et al., 1993) have been identified. In the future, we will evaluate the performance of these clones on a large scale and explore the possibility of transferring BW tolerance to commercial cultivars.

The above discussion reveals that like that of other countries, the Indian experience in breeding BW-resistant potato cultivars is indeed frustrating. Why is it so difficult to obtain a stable BW-resistant cultivar in potato? The answer is evident if we analyze critically the nature of presently available host resistance and variability in the pathogen. The classical concept of the gene-for-gene interaction states that plant resistance is governed by dominant genes, whereas avirulence is characteristic of pathogens. This concept fits well in a host-pathogen system that coevolved for eons (like the potato-*Phytophthora infestans* system in the Toluca valley of Mexico). Genetic and biochemical evidence suggests that *P. solanacearum* is a highly heterogeneous species that has probably evolved with several wild hosts in geographically isolated areas. Present-day crop plants probably became chance victims of this bacterium

when they were introduced in infested areas (Sequeira and Averre, 1961; Buddenhagen and Kelman, 1964; Buddenhagen, 1986; Hayward, 1991; Lomer et al., 1992; Sequeira, 1993). Detection of a remarkably high level of resistance in clone STANMSL-24 983 originally collected from Austria, where *P. solanacearum* is absent, also suggests an independent evolution of resistance in potato (CIP, 1988). Therefore, it will likely be futile to search for a major gene-like resistance source in potato. Resistance in *S. phureja*, which has been extensively used in breeding programs, is quite complex. At first, it was proposed that three independent, dominant major genes govern specific resistance to race 1 strains of *P. solanacearum* in *S. phureja* (Rowe and Sequeira, 1970; Rowe et al., 1972). However, further work by the same group showed that resistance could be due to as many as 10 major genes and its nature is highly complex (Rowe and Sequeira, 1970). Virtually no information is available about the nature of resistance in other wild species such as *S. chacoense*, *S. sparsipilum*, *S. microdontum*, etc. Moreover, the resistance from *S. phureja* depends on many factors, such as temperature (Ciampi and Sequeira, 1980), strains of the pathogen (French and de Lindo, 1982; French, 1986), nematode population in soil (Jatala et al., 1975), and others. More recent evidence shows that resistance is apparently polygenic and quantitative in nature, involving genes with major and minor effects, and even nonadditive gene action such as epistasis probably plays a role in determining resistance (Tung, 1992). Moreover, it has been demonstrated that a genetic background for adaptation and heat tolerance is of crucial importance for expression of resistance (Kloos and Fernández, 1986; Tung et al., 1992a,b). Higher ploidy level and heterozygosity of present-day potato cultivars also pose a formidable problem for a successful breeding program. This discussion suggests that no specific resistance to *P. solanacearum* has been acquired by potato or its wild relatives during evolution and whatever resistance we see in our experiments is the result of genes that did not result from coevolution.

The complex, nonspecific nature of resistance in potato is further aggravated by the fact that the pathogen is a highly heterogeneous species and is probably capable of creating variability in a population with exceptionally high frequency. It has been repeatedly demonstrated that race 3/biovar 2 has a surprising ability to adapt and survive under extremely low temperature conditions (Olsson, 1976; French, 1986). It is now believed that the prototype race 3/biovar-2A has probably evolved from lowland biovar-2T, which has a relatively broad host range (French et al., 1993). The higher frequency of variability in *P. solanacearum* was also appreciated by Robinson (1968), who postulated that the frequent breakdown of vertical resistance in potato was due to the appearance of vertical pathotypes in *P. solanacearum*. Investigation at the DNA level revealed the complex heterogeneous nature of *P. solanacearum*. Cook et al.

(1989) had to construct no less than 35 homology groups to classify only 200 strains by restriction fragment length polymorphism (RFLP). More and more distinct groups ought to be created in the future if we extend RFLP analysis to more strains. If we consider the biovar concept of Hayward (1964), we realize that potato can be infected by biovars 1, 2, 3, and 4. Therefore, in any breeding program, we are in fact dealing with a group of related pathogens, but not with a single homogeneous species.

Breeding for resistance is also hindered because we still cannot pinpoint a single crucial host-pathogen interaction that irreversibly commits the host to wilt development. Molecular genetic techniques revealed several interesting facets of virulence in the pathogen (Boucher et al., 1992; Denny and Schell, 1992), but the key step in pathogenesis is still not clear. A technical difficulty in elucidating the host-pathogen interaction arises because *P. solanacearum* prefers to colonize in xylem vessels that cannot be investigated in a nondestructive manner. It has been convincingly demonstrated that *P. solanacearum* can multiply in xylem vessels of resistant potato cultivars without symptom expression (Ciampi and Sequeira, 1980). Recent studies in tomato also revealed that resistance is not directly related to exclusion of root penetration by bacteria, but it is related to an active/passive tolerance mechanism of conducting vessels (Grimault and Prior, 1992). Therefore, we have to establish some nondestructive techniques (Shaw and Kado, 1986) to critically investigate the behavior of *P. solanacearum* inside the xylem vessels of susceptible and resistant cultivars.

At present, it would be difficult to obtain a major gene-like resistance in potato. We should therefore explore ways to strengthen the basic constitution of the host plant by either pooling resistance sources from different partially resistant *Solanum* spp. into a single population (Schmiediche, 1986) or by using nonconventional methods. Incorporation of lytic peptide genes from *Hyalophora cecropia* into well-adapted, bacterial-wilt-tolerant potato cultivars through genetic engineering will play an important role in the future development of resistant/tolerant cultivars (Jaynes and Bol, 1993). Integration of these genes with a suitable promoter like PR-la (Rhee and Bol, 1993) will facilitate induction of transient expression of such genes when required by external application of inducers such as salicylic acid (Gaffney et al., 1993). Selective induction of these bactericidal genes may eliminate the bacteria from xylem vessels of plants carrying latent infection.

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CHAPTER 26

Manipulation of Host Resistance for Bacterial Wilt in Potato

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Introduction

Potato (*Solanum tuberosum* L.) occupies a significant position among solanaceous crops in Karnataka, with an area of 35,000 hectares, second only to hot pepper (160,000 ha). Other members of the family—tomato, eggplant, and sweet pepper—cover an area of about 33,000 ha, 23,000 ha, and 3,000 ha, respectively. The production of these crops suffers from an important disease, bacterial wilt (BW), caused by *Pseudomonas solanacearum* E.F. Smith. The potato crop alone suffered a 25% loss in 1993 as a result of BW in Kharif, in the transitional belt of Karnataka. Potato was affected much more than other solanaceous crops. The incidence of this disease is likely to increase further because the same land is being used for cultivation of solanaceous crops, which host the same causal organism. Our experience from interaction with farmers who inquire about control of this problem has increased over the years. Recommendations such as the use of bleaching powder around affected plants hardly have any effect in disease management. Hence, the most effective method of BW management should be the use of resistant cultivars. Dividends have been found without a doubt in tomato and eggplant, in which Megha (L-15) and Composite 2 bred for BW resistance found immediate acceptance in farmers' fields (Madalageri and Dharmatti, 1991; Madalageri and Ramanjini Gowda, 1988). This experience should be useful in attempting to develop resistant cultivars in potato also. As new, heat-adapted clones are produced, allowing potato to be grown in lowland tropical conditions, BW control has a high priority.

Methodology

Strain reaction

The destructiveness of the BW pathogen, *P. solanacearum*, is attributed

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to its widespread occurrence, different strains, and exceptional ability to survive in the soil and roots of nonhost plants. Before we attempt any work on host manipulation, we need firsthand information on the existing strains of *P. solanacearum* in the important potato-growing regions, particularly Dharwar, Belgaum, Hassan, Bangalore, and Kolar districts, where potatoes are grown as both a rainfed and irrigated crop. Considerable variation in strain reactions for virulence has been observed (Table 1).

Table 1. Pathogenicity of *P. solanacearum* strains on tomato, eggplant, and hot peppers.[a]

Strains	Tomato		Eggplant		Hot pepper	
	A[b]	B[c]	A	B	A	B
K60	—	—	—	—	—	—
K60-A	X	XX	—	—	—	—
K60-B	—	—				
295	X	XX	XX			
295 B	—	—	—	X	—	—
295 C	—	X	—	—		
295 X	—	X				
W 80	X	XX				
W 82	—	—				
W 82A	—	—	—	—		
W 82B	—	—	—	—		
FF	X	XX	—	—	—	—
FFSc	X	XX	X	XX	X	XX
FP12	—	—				
PF	—	—				
80-1	X	XX	X	XX	X	XX
TBF 8-1	—	—				
TFP 12	—	—				
TFP 13	—	—				
A 21	—	—	—	—	—	—
C-19-4	—	—				
301	—	—				
301 SYR	—	—				
PS 301	—	—				
K60 AO 12	—	—				
FFSc + 80-1 + 295	X	XX	X	XX	X	XX
Control	—	—	—	—	—	—

a. X = virulent, XX = virulent and early symptoms.
b. Regular environment.
c. At 100% humidity.

It is our experience that, out of 25 strains inoculated on tomato, eggplant, and hot pepper for identifying virulence capacity, only FFSc, 80-1, and 295 were virulent. A combination of these virulent strains in injection has shown greater symptoms than injection of an individual strain. Similar work is necessary in potato. Maintenance of virulence upon subculturing is a problem; therefore, repeated culturing by inoculation of a susceptible potato line is necessary.

Inoculation method

For quick and efficient screening of seedlings, the hypodermal injection method in the stem is the most accurate (Table 2). A second method, in which field conditions are simulated, is soil inoculation in the presence of nematodes (*Meloidogyne incognita*). This method delays the production of symptoms. However, for both methods it is essential to maintain high humidity (>95%) and temperature (30°C).

Table 2. Species reaction and inoculation method.

Method	Days to expression of symptoms		
	Eggplant	Tomato	Pepper
Hypodermal injection	3	3	10
Soil inoculation + nematode	12	12	30
Root dip	12	11	35

In our experiments, each 25-day-old seedling was stem injected with 0.2 ml of bacterial suspension with a 106 cfu/ml concentration, and resulted in symptoms in just 3 days, compared with soil-inoculated seedlings with 1.0 ml of bacterial suspension and 3.0 ml of nematodes (500 larvae/ml), which produced symptoms 12 days after inoculation.

Host reaction to *P. solanacearum*

Differential host symptoms caused by the same pathogen are exhibited in the solanaceous family. Capsicums, although susceptible, do not show sudden wilting upon injection, but instead remain stunted for a long period without reaching the reproductive phase, unlike susceptible eggplants and tomatoes, which succumb rapidly to infection (sudden wilt).

Non-tuber-bearing *Solanums* such as *S. sisymbrifolium* Lam. and *S. ciliatum* Lam., which were found to be immune to the pathogen, restricted the multiplication of pathogen at the point of infection, leaving only a

black scar. The intermediates in the beginning oozed bacteria at the point of injection and started wilting after a few days.

In tuber-bearing *Solanums*, Dodds (1989) reported that wilting of leaves and collapse of stems may be severe in young, succulent plants of highly susceptible varieties. Initially, only one branch in a hill may show wilting. If disease development is rapid, all the leaves on the plants in a hill may wilt quickly, without much change in color. Tubers are also affected by general decay and soft rot.

Our experience with potato in farmers' fields indicated that there were huge patches of sudden wilting at the half-grown stage (flowering) during years with normal rains, whereas during years with heavy rainfall at the time of planting, cut tubers rotted because of brown rot.

Sources of Resistance

For potato, breeders and plant pathologists for a long time have been concerned about reliance on a small gene pool that is limited to two Colombian clones of *S. phureja*, which impart dominant resistance. However, Hermsen (1987) found valuable BW resistance, associated or not with resistance to root-knot nematode (*M. incognita*), in *S. sparsipilum*, *S. multidissectum*, *S. stenotomum*, *S. chacoense*, *S. raphanifolium*, *S. pinnatisectum*, and *S. jamesii*, besides *S. phureja*, among the tuber-bearing *Solanums*.

Our 15 years of experience with resistance breeding for BW in non-tuber-bearing *Solanums* implies that resistance genes can be acquired even from species like *S. sisymbrifolium* (PI 337597) of Argentina, *S. ciliatum* (PI 196300) of Nicaragua, *S. torvum* and *S. macrocarpon* in eggplant, and *Lycopersicon pimpinellifolium* (CL 1131-0-0-38-40) in tomato.

It might not be easy to incorporate genes from these species (Patil, 1989) into cultivated potatoes by conventional breeding techniques, but they open an avenue for gene donation through novel methods such as genetic engineering. With current transformation technology, we might be able to transfer the resistance genes into cultivated potato. Here, RFLP might help locate the genes. Once this is done, the rest is taken care of by genetic engineering. Such efforts are worth attempting.

We should make a serious effort to explore the gene pool from within the *tuberosum* group in its centers of origin to find an accession resistant to BW. This kind of work has had success with eggplant in isolating WCGR 112-8 from the west coast belt (India) for BW resistance in the *S. melongena* group (Belkhindi, 1985).

The monogenic dominant gene of this accession was easily transferred to the superior cultivar Pusa Kranti (Gopinath, 1983) and selection pressure against BW resulted in the commercially acceptable and resistant type Composite 2. Likewise, in tomato, BW-resistant variety Megha has been released for commercial cultivation by crossing NTDR-1 (nematode resistant) with small-fruited CL 1131-0-0-38-40 (AVRDC accession) known for its BW resistance. We need to do this kind of work in potato.

Dodds (1989) reported innovative methods such as the incorporation of genes coding for antibacterial proteins: the chicken egg white lysozyme, cecropin, and attacin.

A genetically engineered plant would possess a rather potent arsenal composed of three different antibacterial proteins that appear to work in different ways to actively destroy the bacterial pathogen. This multilevel defense system would present a formidable challenge to invading bacteria.

Prospects

Widespread solanaceous crops throughout the world and the common pathogen that attacks them make BW a worldwide problem that has spread from the tropics to temperate regions. A serious effort is therefore needed to develop sustainable integrated disease management, which is possible only through host-resistance breeding.

Collaborative work at the international level could be an effective solution for developing an acceptable technology. We need a central testing site to develop a broad genetic base through genetic engineering in conjunction with conventional breeding. A broad-spectrum resistance base population should be tested rigorously. Selections should be taken to potato-growing regions around the world to field test them and measure acceptability. We need an international network of scientists and adequate support. AVRDC has begun this kind of collaborative work in tomato in a project spearheaded by Professor Bruce Holloway, of Monash University (Australia).

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Acronyms

ACIAR	Australian Centre for International Agricultural Research
AICPIP	All India Coordinated Potato Improvement Project
BADC	Bangladesh Agricultural Development Corporation
BARI	Bangladesh Agricultural Research Institute
BSAI	Bashang Agricultural Institute, China
BW	bacterial wilt
CAAS	Chinese Academy of Agricultural Sciences
CDR	complex, diverse, and risk-prone
CIP	International Potato Center, Peru
CPRI	Central Potato Research Institute, India
CRI	Crop Research Institute, China
EG	endoglucase
ELISA	enzyme-linked immunosorbent assay
EPS	exopolysaccharide
EPS	extracellular polysaccharide
IACR	Institute of Arable Crops Research, U.K.
IFAS	immunofluorescent antibody staining
IPM	integrated pest management
IPP	Institute of Plant Protection, China
LRARC	Lumle Regional Agricultural Research Center, Nepal
PC	phenotypic conversions
PCR	polymerase chain reaction
PCSC	Potato Center of South China
PFGE	pulsed field gel electrophoresis
PMG	polymethylgalacturonase
RFLP	restriction fragment length polymorphism
SDW	sterile distilled water
TCRC	Tuber Crops Research Centre, Bangladesh
TZC	tetrazolium chloride
VRI	Vegetable Research Institute, China

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