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PHYTOBACTERIOLOGICAL RESEARCH PROGRAMS HELD FOR
BACTERIAL WILT CONTROL

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ABSTRACT

Bacterial wilt (BW) is due to Pseudomonas solanacearum (Smith) Smith, a soilborne and a xylem-invading bacteria. These properties and high variation in strains, aggressiveness, depending on environmental factors, make it difficult to bring this tropical world-wide disease under control. French research projects including co-workers from different institutes (CIRAD, INRA, ORSTOM) based in metropolitan France and in overseas departments are briefly reported. Recent results obtained by INRA-CRAAG's phyto-bacteriological laboratory include:

The behavior of P. solanacearum in receptive and suppressive soils;
Characteristics of local strains, especially host range and aggressiveness;

The existence of healthy carriers among tomato plants, i.e., plants bearing latent infections in vascular tissues.

Programs are being developed according to two complementary control strategies interdependent from results obtained by molecular biologists and breeders. They are:

Biological control of BW using genetically constructed avirulent mutants; and

BW resistance mechanisms, with particular attention to bacterial colonization (density, localization) and to incidence of environmental factors on resistance of cultivars.

RESUME

Pseudomonas solanacearum (Smith) Smith, l'agent du flétrissement bactérien, est un germe vasculaire d'origine tellurique. Ces propriétés de l'agent pathogène le rendent particulièrement apte à s'adapter aux contraintes de l'environnement. Il en résulte une forte variabilité de l'agressivité des souches qui rend difficile la lutte dans les zones tropicales concernées. Les programmes de recherche Français des équipes de différents Instituts (CIRAD, INRA, ORSTOM) basés en Métropole et dans les départements d'Outre Mer, sont brièvement cités. Les résultats récents du laboratoire de phyto-bactériologie du CRAAG concernent:

- L'étude du comportement de P. solanacearum dans des sols réceptifs et sup pressifs,
- Les caractéristiques des souches locales, principalement le spectre d'hôte et l'agressivité.
- La mise en évidence de plantes porteur sains, c'est-à-dire qui présentent des infections latentes dans les tissus vasculaires.

Les programmes développés concernent deux stratégies de lutte complémentaires, en interdépendance avec les résultats obtenus par les biologistes moléculaires et les améliorateurs:

- la lutte biologique par utilisation de mutants avirulents obtenus à INTRA-Tou louse par construction génétique.
- les mécanismes de résistance au flétrissement bactérien, en portant une attention particulière à la colonisation bactérienne (densité et localisation) et à l'incidence des facteurs de l'environnement sur la résistance des cultivars.

INTRODUCTION

Bacterial wilt (BW) is a vascular disease caused by Pseudomonas solanacearum (Smith) Smith, a soilborne pathogen. This tracheobacteriosis causes important losses in the tropics and subtropics on various crops, specially vegetables and bananas, but also on ornamentals (Kelman, 1985). As for other bacterial pathogens, one world-wide method of control consists of the cultivation of resistant varieties. Virulence (qualitative parameter) and aggressiveness (quantitative variation of virulence) plus variability of local strains compared to those of strains used for breeding BW resistance remain the limiting factors for long-term effectiveness of this strategy.

Host range classification of the strains was determined according to Buddenhagen et al. (1962):

- Race 1: These ubiquitous solanaceous strains affect many crops and a broad host range is reported. Those strains are active at high temperatures (25-30°C) in lowlands.
- Race 2: Musaceous strains which cause Moko disease of bananas.
- Race 3: These strains are pathogenic on potato and tomato. This race is active at lower temperatures (18-23°C) in elevated areas.
- Race 4: (described from The Philippines) and race 5 (described from China) have been reported pathogenic on ginger and mulberry, respectively.

This race classification systems used for P. solanacearum strains is peculiar because it does not retain a taxonomic value, but practical interest was considerable making it internationally admitted. Generally, strains are also complementary typed according to Hayward's biovar (Bv) classification

(Hayward, 1964), i.e., the ability of the strains to hydrolyze 3 hexose-alcohols and 3 disaccharides. Races and biovars do not correspond, except that strains classified race 3 are also typed Bv II.

BW resistance level of naturally (native bacterial population) of artificially (selected strain) bred varieties, manifestation of BW resistance properties, and development of pathogenic characteristics in the pathogen population are interdependent variables. This may explain the difficulties of developing a standard model to bring the disease under control.

Resistant cultivars and knowledge of population structures are first steps for an integrated control strategy (ICS). The purpose of this paper is to report rapid activity by French laboratories in BW research, and recent advances in the determination of some ICS parameters obtained in the French West Indies.

RAPID IDENTIFICATION BY THE FRENCH LABORATORIES CONCERNED IN BW STUDIES

Laboratories involved in BW research include co-workers from CIRAD, INRA, ORSTOM, and University (CIU). Approximately 20 researchers are working on different aspects of ICS like plant breeding, phyto bacteriology, agronomy, molecular biology, weed science, and soil science (Prior et al., 1990a). Those laboratories are located in metropolitan France, in the overseas departments (Guadeloupe, Martinique, and Réunion) and in experimental stations in Africa (Fig. 1). Molecular biology and biochemistry for investigation of *P. solanacearum* pathogenicity are advanced basic research developed in metropolitan France. Complementary programs from abroad perform in downstream and applied research.

PHYTOBACTERIOLOGICAL RESEARCH AT INRA-CRAAG

Results from the Past Five Years Concerning Soil-Plant-*P. solanacearum* Interactions.

The telluric phase of the bacteria has been studied in receptive and suppressive soils in Guadeloupe. In receptive ferritic soils, the disease may be controlled by fertilizing the soil with high nitrogen-content organic matter (Clairon, 1989; Prior and Bérarnis, 1990). In suppressive vertisols, a relation has been noted between the hydric behavior associated with microstruturated clays in this soil and the suppressive properties (Schmit et al., 1989).

Strains from the FWI are typed race 1/Bv I and III. Host range and aggressiveness of the strains isolated in Guadeloupe and Martinique were studied. A high variability of the strains was recorded according to the aggressiveness which was high compared to different international reference strains (Prior and Steva, 1990; Prior et al., 1990b). Knowledge of variations in strains has been considered in BW resistance breeding programs.

Studies for the BW resistance mechanisms were undertaken with particular attention to the bacterial colonization of three resistant tomato

Figure 1. Laboratories involved in bacterial wilt research and control

Location	Activity concerning BW
<u>INRA laboratories</u> <u>in metropolitan France:</u>	
Angers	Bacterial population dynamic: J. Luisetti, J.P. Paulin (collaboration with IRAT-Réunion)
Avignon	Breeding programs for BW resistance: H. Latterot
Paris	Molecular biology: A. Kotoujansky
Toulouse	(1) Genetic determinism of pathogenicity: C. Boucher (2) Biological control: A. Trigalet
Versailles	Behavior of bacteria in soil: J. Schmit (collaboration with CRAAG)
<u>CIO Laboratories</u> <u>in the overseas departments:</u>	
Guadeloupe (INRA-CRAAG)	Breeding: G. Anaïs Agronomy: M. Clairon Weed science: J. Fournet Phytobacteriology: Ph. Prior
Réunion (CIRAD-IRAT)	Bacterial population dynamic: J. F. Nicole Screening of variety for resistance: J. C. Girard
Martinique (CIRAD, ORSTOM)	Interaction between <u>P. solanacearum</u> and root-knot nematodes: P. Cadet, P. Quénéhervé
<u>Laboratories and experiment</u> <u>stations in Africa:</u>	
Congo (ORSTOM)	Phytotechny: C. Declerc
Burkina Faso (CIRAD)	Breeding: J. D'Arondel de Hayes

cultivars. For all those varieties, latent infections were put forward. The results showed a lack of resistance by the root to penetration by P. solanacearum and vascular tissues were reported tolerant to bacteria

for all those varieties (Prior et al., 1990c). The establishment and the characteristics of the latent infections appear to be new criteria readily accountable for inbreeding programs (Grimault and Prior, 1990). In addition, biological (nematodes) and/or physical (temperature) stress appeared to be sensitizer factors or releasing factors of the disease (Cadet et al., 1989).

Recent Developments in Biological Control of Pathogen and BW Resistance Mechanisms

The attempt to biologically control P. solanacearum is a program in collaboration with the group from INRA-Toulouse. The results obtained at the Toulouse laboratory show that avirulent strains obtained by transposon mutagenesis effectively control BW-susceptible plants from disease when infected by a virulent strain (Trigalet and Trigalet-Demery, 1990). The protective avirulent mutant colonizes the tomato stem. The protection efficiency and the effect of its incidence on the yield were first tested. Research was undertaken to determine mechanisms that control the protective effect. The biological control has been tested already in contained greenhouse under Guadeloupéen environmental conditions.

The program to study BW resistance mechanisms was carried out by focusing on spacial and temporal colonization of resistant cultivars compared to a susceptible one by P. solanacearum. Additional resistant varieties were collected to determine the relationship that may be involved between BW resistance level and characteristics of bacterial plants colonization. A histological study of the infections of different cultivars will be carried out in collaboration with the group from INRA-Versailles. After those parameters have been determined, this research project will include a study on the environmental effect on plant tolerance to P. solanacearum.

CONCLUSIONS

Results obtained on P. solanacearum survival and behavior in soil have to be integrated in a larger project on cultivation systems in irrigated areas. In addition, the tolerance of tomato vascular tissues to P. solanacearum and associated BW resistance was considered as useful information with important epidemiological consequences in various situations.

The first situation concerns the maintenance of high inoculum levels in diseased areas. Healthy carrier plants (80-100 per cent in the field) may be unsuspected bacterial sources, resulting in an increase of inoculum potential in fields where the disease is not prevalent (Prior et al., 1990).

The second situation concerns an original way of conducting research for BW sensitizer stress factors analysis. Stress will be considered as precipitating wilt factors. It is hopeful that under these conditions, environmental wilt factor incidences, which are poorly understood, could be analytically investigated. The principal and ambitious objective was a precise identification of complementary criteria for BW breeding programs.

Integrated controls for BW include modern biological molecular genetic studies. This branch of phytopathology provides considerable information on the basis of pathogenicity, resulting in a realistic biological control of virulent strains of P. solanacearum in planta. This strategy implies the need for important work for the selection of the best protective avirulent mutant in association with a study of genetic construction stability (recombination rate). Preliminary studies are to be performed on susceptible cultivars. A powerful control is expected which will probably be tested for pleiotropic effects against different vascular tomato pathogens.

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