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Preliminary Studies for Tomato Bacterial Wilt (*Pseudomonas Solanacearum* E.F.Sm.) Resistance Mechanisms

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Abstract

The bacterial wilt resistant tomatoes Caraibo, Carmido (Caraibo + gene Mi), Hawaii 7996 and CRA 66 were contaminated with *P. solanacearum* and cultivated in the open field or in climatic room. Spread of the bacterium in the plants was investigated by ELISA at different stem level. Although the cultivars Hawaii, Caraibo and Carmido were tolerant in the field (respectively 5, 10 and 15% mortality), the analyses revealed that their vascular system was invaded by *P. solanacearum*. A topographical study of the colonization showed that progression of the pathogen in the stem was reduced at the collar level of Hawaii whereas it was found up to the apex of Caraibo and Carmido. In the collar of CRA 66 (no mortality) *P. solanacearum* was not detected. The consequences of the existence of such healthy carrier plants (80–100 p. cent in the field) are discussed.

Introduction

The widespread bacterial wilt (BW) due to *Pseudomonas solanacearum* has been observed in the majority of tropical and subtropical areas where it causes considerable damages especially in vegetable crops (Kelman, 1953). Attempts to control this soil-borne and vascular bacteriosis with chemicals have proved ineffective. The most widespread solution is to grow resistant cultivars, even though it is known, that resistance properties may fluctuate locally and with time (Denoyes, 1988). The tomato line Caraibo has been selected and cultivated in the French West Indies for its wilt

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resistant properties (Anaïs, 1986). If the literature does report the polygenic origin of this resistance (Messiaen, 1981; Singh, 1961), mechanisms have not yet been specified.

Recently, resistance overcome by the well-known synergical association between *P. solanacearum* and root-knots nematodes (Buddenhagen, 1986; Cadet et al., 1990). Studies of this association using strains of *P. solanacearum* with different levels of aggressiveness (Prior et al., 1989a) led us to the conclusion that nematodes facilitate bacterial invasiveness of plant roots. This is consistent with other reports (Napiere and Quimio, 1980; Routaray et al., 1986). In some cases, it seems that latent infections in tomato plants are present and that nematodes could then act as releasing wilt factors (Cadet et al., 1990). This research was undertaken to determine if such latent infections occur in resistant tomato cultivars.

Material and Methods

Bacterial strains

Two strains of *P. solanacearum* GT1 and GT4 isolated from tomato in Guadeloupe were used in this study. In an earlier report they proved highly pathogenic on tomato and were typed race 1, biovar I and III, respectively (Prior and Steva, 1989a). These strains were selected because they were also used for inoculation test in resistance breeding programmes (Prior et al., 1989b).

For inoculum preparation single colonies of the virulent, fluidal type grown at 28°C for 48 hr on selective tetrazolium chloride (TZC) medium (Kelman, 1954), were cultivated on TZC-free liquid medium. After incubation with shaking in a water-bath at 28°C, cells were rinsed twice in distilled water and harvested by centrifugation (15 mn, 5000 g, 14°C). Suspensions were adjusted to 10^7 CFU ml^{-1} which gave an optical density (OD) of 0.01 at 650 nm.

Seventeen different bacterial strains were isolated from healthy tomato stems, sampled at random, to test the specificity of *P. solanacearum* antiserum. Isolations were also made from ten symptomless plants carrying *P. solanacearum* and the pathogenicity of these strains was tested with the inoculation method described later.

ELISA procedure

Diagnosis of *P. solanacearum* in tomato stem cuttings was performed with an ELISA indirect test adapted from the method of Clark and Adams (1977). Specific antiserum (A-1110) was obtained from rabbit and was prepared against heat-killed entire cell antigens. Optimal concentrations of immunoreagents have been determined previously. The sensitivity of the test is $4.4\ 10^3$ CFU ml^{-1} of *P. solanacearum* in pure suspension and 5.10^4 CFU ml^{-1} in tomato sap extracts.

Tomato stem cuttings (2 cm) were extracted in adsorption carbonate buffer (15 hr, 5°C, pH 9.6) and 200 μl of absorbent gauze percolated sap extracts were deposited into

Nunc immunowells module (3 repetitions per cutting). Wells were incubated 1 hr at 37°C then washed with PBS (pH 7.2). Plates were saturated with 200 μl PBS-Bovine Serum Albumin (BSA) 1% blocking buffer for 30 mn at 37°C, then plates were washed twice with PBS-tween 20 (0.05%) and once with PBS. All other washings between the following steps were done identically. Rabbit antiserum (200 μl of a 25 $\mu g\,ml^{-1}$ globuline dilution in PBS-BSA 1%) was incubated 1 hr at 37°C, and then, goat alkaline phosphatase conjugate (1/1000 in PBS-BSA 1%) deposited. The final step consisted of 200 μl phosphatase substrate (0.6 $mg\,ml^{-1}$ in diethanolamine buffer, pH 9.6). Optical densities at 405 nm were recorded after 1 hr of enzymatic reaction. Sample wells were considered positive when OD_{405} was twice that of non-inoculated plant extracts.

Plant production, inoculations and observations

Five varieties of tomato (*Lycopersicon esculentum* Mill.) were selected for their resistance (R) or susceptibility (S) to *P. solanacearum* including Caraibo (R), Carmido (R) which is 'Caraibo' with root-knot resistance gene Mi, Hawaii 7996 (R), CRA 66(R), (the resistance source for Caraibo originating from Guadeloupe) and Floradel (S).

Seeds and seedlings were planted in vegetable mould in 10 cm pots and grown under greenhouse. Plants with 3–5 fully expanded leaves were inoculated by pouring 2 ml of mixed bacterial suspension (GT1 + GT4 adjusted to the final concentration of 2.10^7 CFU ml^{-1}) into each pot before planting out in open field or incubating in a climatic chamber (RH:75–100%; 30°C; 12 hr photoperiod).

Field trials consisted of 360 plants per cultivar (except CRA 66 which was not included) equally distributed in five randomized microplots. Each week, four symptomless plants per plot were sampled at random and tested by ELISA. Forty positive stem per resistant varieties were topographically tested at 1 cm above collar, in middle and in apical part of stem. There was no particular disposition for climatic chamber trial where 15 symptomless plants were sampled weekly at random for analysis. Plantations were regularly observed for BW mortality.

Results

None of the 17 strains isolated from tomato stems cross-reacted with serum A-1110. Ten strains of *P. solanacearum* were isolated from symptomless positive ELISA plants. All were typically fluidal on TZC medium and highly pathogenic when inoculated in Floradel, i.e. 100% mortality after 14 days.

Bacterial wilt mortality

All inoculated Floradel plants wilted within 4 and 2 weeks in field microplots and climatic chamber, respectively. In contrast, Hawaii 7996 and Caraibo behaved in the

field as resistant cultivars with only 5 and 10% mortality but losses of 20% were recorded for Carmido (Fig. 1A). Bacterial wilt increased in climatic chamber conditions (Fig. 1B) for all cultivars tested, holding on moderate for Hawaii 7996 (15% BW) and Caraibo (25% BW) but becoming elevate for Carmido (45% BW).

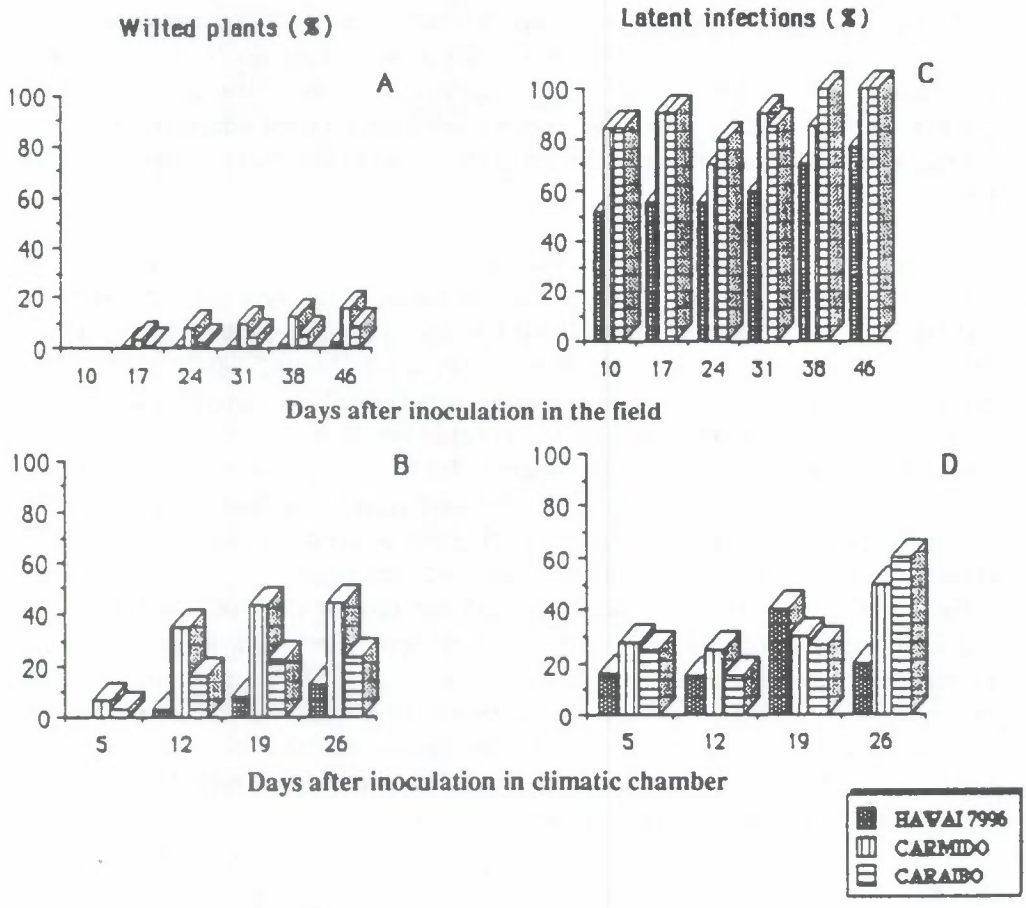

Figure 1. Bacterial wilt spread histograms observed (A) in field (360 plants per cultivar) and (B) climatic chamber (100 plants per cultivar). Cultural conditions 26 ± 2°C max.; 20 ± 4°C mini. and 30 ± 2°C, respectively. Latent infections were investigated weekly on 20 (C) and 15 (D) symptomless plants per cultivar.

Latent infections diagnosis

P. solanacearum diagnosis with ELISA demonstrated that latent stem infections were frequent (50–70% in Hawaii 7996, 80–100% in Caraibo and Carmido), early

established and balanced with time in the field (Fig. 1C). Plants growing in controlled conditions showed less latent infections as visible in Fig. 1D. In addition, it is worth noting that elevated temperature resulted in a better expression of *P. solanacearum.* Thus, latent infections with low BW mortality became general in the field whereas most of the infected plants wilted in controlled environment.

Seropositive resistant tomato cultivars differed in their ability to limit xylem colonization by *P. solanacearum.* As repoted in Fig. 2, Hawaii 7996 was more efficient with Caraïbo and Carmido in controlling bacterial spread in the stem. CRA 66 differed from other cultivars in that none of the inoculated plants neither wilted nor were carrying *P. solanacearum* in stem or collar.

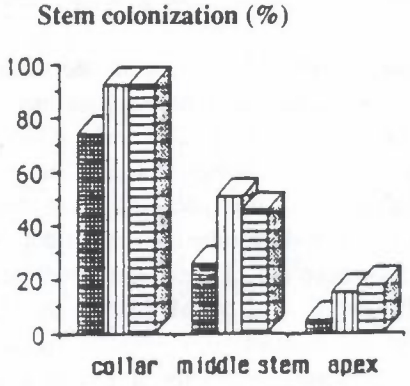

Figure 2. Topographical study of *P. solanacearum* stem colonization on healthy symptomless plants (40 per cultivar).

Discussion

The inoculation method used in these experiments was previously reported to be reliable and effective (Prior et al., 1989b), as proved by high mortality of susceptible cultivar Floradel. In addition, it is close to natural ways of entry of *P. solanacearum* (Kelman and Sequeira, 1965; Schmit, 1978). This is mainly why this test is used in breeding programmes for resistance to BW in the French West Indies. In the past, numerous selection programmes considered the absence of visible wilt symptoms as a sufficient resistance criterion, but none indicated plant resistance mechanisms involved. Presently, detailed resistance mechanisms are still unknown, but a lot of convergent reports have indicated factors like temperature, light, water stress or root-know nematode (Buddenhagen, 1986), as able to influence the susceptibility to BW and cause breakdown in field resistance of cultivars.

Strain isolations and ELISA diagnosis give clear evidence that latent infection with *P. solanacearum* are frequent in tolerant tomato cultivars tested. Similar observations were mentioned about a resistant potato clone (Ciampi and Sequeira, 1980), some tomato lines and resistant tobacco and peanut plants (Winstead and Kelman, 1952). Our results suggest that environmental conditions already mentioned often act towards plants as releasing wilt factors or modulate unknown combinations involving wilt factors.

Existence of latent infections orientate tomato breeding programmes for BW resistance toward new strategy. Several mechanisms may be put forward: resistance of root to *P. solanacearum* penetration, resistance to spread into stem vessels as observed with CRA 66 and/or tolerance to bacteria present in vessels as *P. solanacearum* recovered from latent infections maintains high pathogenicity. CRA 66 being the BW resistant parent for tomato line Caraibo, some genetic information may have been lost during selection.

Actually, knowledge about selectable criteria related to absence of root penetration by bacteria is still poor. In contrast, vascular colonization of the stem appears a very useful criterion, readily detectable with ELISA tests, which is now routinely applied in FWI breeding resistance programmes. As previously described (Digat, 1976) antigens from *P. solanacearum* strains are closely related and no A-1110 serum cross-reacting strains were found among tomato stem microflora, which ensure against mistaken diagnosis. Serological detection of *P. solanacearum* in plants has already been successfully investigated by Morton et al., (1965), but this is the first report on well established latent infection in resistant tomato plants.

Such latent infections of resistant cultivars may have epidemiologic consequences in various pedoclimatic situations. It could particularly concern the maintainance of high inoculum levels in diseased fields and also the unsuspected increase of inoculum potential in areas where the disease is not prevalent like suppressive soils.

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