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SUSCEPTIBILITY OF TOMATO CULTIVARS TO BACTERIAL SPECK

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ABSTRACT

Bacterial speck caused by *Pseudomonas syringae* pv. *tomato* (PST) was previously reported in Portugal on tomato plants grown under greenhouse conditions. Recently, a similar disease affecting processing tomato cultivars was observed. The characteristics of isolates obtained from these infected plants were likely those produced by PST. Sixteen cultivars of tomato plants, including some adapted to processing industry, were inoculated with PST, in greenhouse conditions. Disease severity was evaluated in the upper, middle and lower third of the foliage in 10-wk-old plants. Although most cultivars tested were susceptible to bacterial speck, some of them presented significantly lower levels of susceptibility ($P \leq 0.05$).

INTRODUCTION

Bacterial speck of tomato (*Lycopersicon esculentum* Mill.), caused by *Pseudomonas syringae* pv. *tomato* (Okabe) Young *et al.* (hereafter designated PST), is a very important and widespread disease of processing tomato plants, affecting both quality and yield. The causal agent was first recorded in Portugal by OLIVEIRA & SANTA-MARTA (1983), but only on tomato plants grown under unheated polyethylene-covered greenhouse conditions. Since its appearance, bacterial speck has been recorded at many different locations of the country, mainly in autumn-winter protected tomato crops.

In the last two years, a similar disease has been observed in both transplant and production open fields, affecting the most currently tomato cultivars used for industry in Portugal (Rio Grande, Rio Fuego and Petopride), which are grown in spring-summer. Isolations made from these infected plants on King's medium B, KMB (KING *et al.*, 1954), consistently yielded a green fluorescent pseudomonad. The confirmation of the identity of this bacterium is important, since the symptoms induced by PST may be very similar to those produced either by *P. syringae* pv. *syringae* van Hall (PSS) or *P.*

viridiflava (Burkholder) Dowson, the causal agents of syringae leaf spot and bacterial leaf blight, respectively.

Under favourable conditions to bacterial speck development, control measures such as crop rotation, disease-free seeds and transplants and chemical treatments have been not completly satisfactory (LAWSON & SUMMERS, 1984; BAZZI *et al.*, 1989). An alternative measure is the use of tomato cultivars with a high level of resistance to PST. In our country, bacterial speck is important both under protected and open-field cultivation. However, attempts to use resistant cultivars frequently fail, because only a few cultivars, adapted for open-field production, are available.

This study was conducted to determine the accurate identification of pseudomonads isolated from processing tomato plants and the foliar reaction of 16 tomato cultivars to PST.

MATERIALS AND METHODS

Isolations and characterization of isolates

Suspected bacterial speck lesions of infected leaves and petioles of tomato transplants and plants of the cultivars Rio Grande, Rio Fuego and Petopride were macerated in sterile distilled water and streaked on KMB. All isolates were maintained on slants of nutrient yeast glucose agar at 5 °C (JONES *et al.*, 1981). For inoculum production, the isolates were grown either on nutrient agar (NA) or KMB for 24-48-hr at 25 °C, except where indicated otherwise.

The main criteria used for characterization of isolates were colony colour, morphology and the production of a fluorescent pigment on KMB when observed under ultra-violet light. Further identification was made by determining oxidase reaction (KOVÁCS, 1956), arginine dihydrolase activity (THORNLEY, 1960), levan production (LELLIOTT *et al.*, 1966), fermentative-oxidative acid production in glucose (HUGH & LEIFSON, 1953), potato soft-rot capacity (MISAGHI & GROGAN, 1969), ice-nucleating activity (LINDOW *et al.*,1978) and tobacco hipersensitivity (KLEMENT *et al.*, 1964). Nutritional tests were also made using the basal medium of MISAGHI & GROGAN (1969), without iron sources, to look for fluorescence and use of erythritol, DL-lactate and sucrose (JONES *et al.*, 1986). Gram reaction by the nonstaining (KOH) method, motility, catalase production and starch hydrolysis (plate tests) were performed by standard bacteriological methods (LELLIOTT & STEAD, 1987).

Pathogenicity tests were made by spraying the bacterial suspensions, containing approximately $2x10^8$ colony-forming units (cfu) per milliliter, on plants of the susceptible cultivar Rio Grande.

Greenhouse experiments

Tomato plants. Sixteen tomato cultivars, including a resistant cultivar to PST (Zenith), were used as host plants. The seeds were surface-sterilized with 1% sodium hypochlorite for 30 min and washed three times with sterile water to remove traces of hypochlorite. A pre-germination was made on watered filter paper inside Petri dishes, at 24 °C and darkness conditions. After 48-hr the seeds were sown in plastic trays (35x25x5 cm) containing a mixture of soil and peat (1:2 v/v) autoclaved three times for 45 min, at 121 °C. The seedbeds were maintained in a growth chamber set at 24 °C and 16-hr of photoperiod.

Seedlings were transplanted at the stage of 3-4 true leaves into plastic pots (9x9x10 cm) containing the same mixture but, in this case, disinfected 15 days before transplantation with a 2% formaldehyde solution, applied at a rate of 10 l/m^2. The plants were transferred to the greenhouse and placed under favourable growth conditions until experimental inoculation.

Bacterial strain and inoculum production. To select the strain for using in this experiment, pathogenicity studies were made with several tomato isolates collected at different locations of Portugal, both from protected and open-field conditions. A strain isolated from cultivar Cobra, growing under greenhouse conditions, was selected because it proved to be the most virulent.

Inoculum was prepared by washing 24-hr NA slants with sterile distilled water. Final inoculum concentration was adjusted by dilution with sterile distilled water to approximately $2x10^8$ cfu/ml as determined by standard spectrophotometric (O.D. $_{650\,nm}$ 0.26) and dilution plate techniques (LELLIOTT & STEAD, 1987).

Inoculation technique and growth conditions. Tomato cultivars were inoculated 8 wk after sowing by spraying the bacterial suspension into the foliage (10 ml/plant), from a distance of 20-30 cm, until visible drops were apparent. This was achieved by using an electric vertical apparatus. After inoculation, plants were maintained at temperature and relative humidity ranges of 10-25 °C and 50-90%, respectively.

The experiments were carried out in a completely randomized design, with six replicates. Each replicate consisted in a group of six plants.

Two weeks after inoculation, disease severity was assessed by counting the number of speck lesions in two leaves selected at random from the lower, middle and upper third of foliage of all plants. Only the three apical leaflets of each leaf were used for countings. Isolations from diseased leaves were made and its key determinative characteristics studied. Data concerning countings were subjected to ANOVA and the means were compared according to Duncan´s multiple range test.

RESULTS

Isolations and characterization of isolates

All isolates studied were Gram-negative, motile, strictly aerobic, catalase positive and produced a green water soluble fluorescent pigment, but none hydrolyzed starch. When erythritol and DL-lactate were added to iron-deficient Misaghi & Grogan´s basal medium no growth occurred, whereas growth and fluorescence occurred on all plates containing sucrose. The isolated bacterium gave also negative reaction for ice-nucleating activity (Table 1).

Table 1 - Physiological, biochemical and pathogenicity tests of strains isolated from tomato plants grown under open-field conditions compared with some *Pseudomonas* spp.

Tests for	*P. viridiflava* [a]	*P. syringae* pv. *syringae* [a]	*P. syringae* pv. *tomato* [a]	Test strains [b]
Fluorescence on KMB	+	+	+	+
Oxidase reaction	–	–	–	–
Arginine dihydrolase	–	–	–	–
Potato soft-rot	+	–	–	–
Levan	–	+	+	+
Glucose (oxidatively)	+	+	+	+
DL-lactate	+	+	–	–
Sucrose	–	+	+	+
Erythritol	+	+	–	–
Ice nucleation [c]	+	+	–	–
Tobacco hipersensitivity	+	+	+	+
Tomato pathogenicity	+	+	+	+

[a] PALLERONI (1984)

[b] Strains isolated from tomato cultivars Rio Grande, Rio Fuego and Petopride

[c] JONES *et al.* (1984)

Greenhouse experiments

Typical small brown-black specks (1-4 mm diameter) surrounded by yellow chlorotic halo first appeared 4 days after inoculation in the foliage and the number of lesions continued to increase under favourable conditions. Although leaves were the most affected part of the plants, lesions were also visible on stems, petioles, pedicels and sepals.

Generally, bacterial speck was more severe on the lower third of foliage, where disease severity ranged from 1.07 lesions by leaflet in tomato cultivar Zenith to 25.06 in Rio Grande. The cultivar Zenith showed a high, but not absolute, level of resistance as demonstrated by the almost total absence of lesions in the lower, middle and upper leaflets (1.07, 0.73 and 0.27) (Table 2).

Table 2 - Bacterial speck severity of 16 tomato cultivars after experimental inoculation with *Pseudomonas syringae* pv. *tomato*

Tomato cultivars	No. of lesions/leaflet		
	Lower third of foliage	Middle third of foliage	Upper third of foliage
Zenith	1.07 a [a]	0.73 a	0.27 a
Presto	3.37 ab	4.90 abc	0.97 a
Fontana	4.66 ab	4.50 abc	1.31 a
Vision	5.36 abc	3.94 ab	0.60 a
Ophir	5.44 abc	6.37 bc	1.21 a
Estoril	5.62 abc	5.32 bc	1.40 a
Fiorin	5.90 abc	8.58 cde	1.56 a
Psx	6.38 abc	5.32 bc	1.48 a
Bermuda	6.61 abc	6.09 bc	1.67 a
Buffalo	8.82 bcd	6.77 bcd	0.96 a
Cobra	12.07 cde	7.87 bcde	1.27 a
Radius	14.82 de	11.29 e	2.36 ab
Lotus	16.36 ef	12.40 ef	2.45 ab
Petopride	21.55 fg	10.85 de	1.68 a
Rio Fuego	24.87 g	20.40 g	4.44 bc
Rio Grande	25.06 g	15.80 f	5.26 c

[a] Means followed by the same letter are not significantly different at $P \leq 0.05$ (Duncan's test).

Rio Fuego and Rio Grande ranked as the most susceptible cultivars in all foliar positions evaluated. Cultivars such as Presto, Fontana, Vision, Ophir, Estoril, Fiorin, Psx and

Bermuda were not significantly different from the resistant cultivar Zenith, when the lower third was considered.

The pathogen was easily reisolated from leaf specks and its characteristics were in agreement with those concerning the inoculated strain.

DISCUSSION

Fluorescent pseudomonads isolated from foliar lesions of tomato plants, oxidase and arginine dihydrolase negative and tobacco hypersensitive positive, were usually assumed to be PST (JONES *et al.*, 1981). However, either PSS or *P. viridiflava* also exhibit these characteristics and may induce similar foliar lesions on tomato plants. Consequently, PST, PSS and *P. viridiflava* are frequently confused. The accurate identification of the pathogen is of concern, since only PST is economically important. JONES *et al.* (1986) showed that the ability of these fluorescent pseudomonads to grow and to fluoresce on media containing single-carbon sources, such as erythritol, DL-lactate and sucrose, are reliable tests for their differentiation. In addition, ice-nucleating activity appears to be another adequate test in separating these bacteria.

This study first reports PST as a foliar pathogen of open-field grown tomato in Portugal. Green fluorescent pseudomonads isolated both from tomato transplants and plants grown under open-field conditions were identified on the basis of morphological, cultural, biochemical, physiological and pathogenicity characteristics. Results of these determinative tests agreed closely with those obtained elsewere for PST, the causal agent of bacterial speck (JONES *et al.*, 1981; PALLERONI, 1984). The key tests referred by JONES *et al.* (1986) were also used and proved to be useful for the disease diagnosis.

Sources of inoculum involved in the first appearence of bacterial speck disease in tomato transplant fields were not studied in the present work. However, survival of PST in soil, host debris, seeds and weed hosts is currently reported (McCARTER *et al.*, 1983). In our environmental conditions, the overseasoning of PST in either soil or host debris during the warm season is unlikely, due to high temperatures (frequently $>30\ ^{\circ}C$). Studies made by McCARTER *et al.* (1983) support this opinion as they pointed out that PST had a short survival period in both soil and host tissue in southern Georgia under analogous conditions, but these ways of survival could be of importance in areas where temperatures are lower (GETZ *et al.*, 1981). Seeds and weed hosts could be assumed as responsible for the primary inoculum in tomato transplant fields in Portugal. According to SMITLEY & McCARTER (1982), even a low rate of contaminated

contaminated seeds can induce severe infections, since PST spreads rapidly. So, the infected transplants may have served as inoculum sources for spread in fruit-producing fields where transplants were shipped. Although the environmental conditions to disease development were not the most favourable in these fields, sprinkler-irrigation provided a high level of free moisture on foliage, necessary to subsequent infections. High temperatures observed during the fruit development probably prevented the extensive infection of fruits. Temperatures near 25 °C, free moisture and high relative humidity are optimum conditions for bacterial speck development (SMITLEY & McCARTER, 1982).

Our greenhouse experiments indicated that bacterial speck severity generally decreases from the lower to the upper third of foliage. Similar results were obtained by GITAITIS *et al.* (1982) and, more recently, by BAZZI *et al.* (1989). Symptoms on fruits were not observed, possibly due to unfavourable environmental conditions during the most susceptible growth stages of the fruit, specially the period following anthesis and before fruits have reached 3 cm diameter (GETZ *et al.*,1983).

When tomato plants adapted to grow under protected conditions were considered, cultivars such as Presto, Fontana and Vision were the less susceptible to PST (on the three thirds of foliage) and did not differ significantly from the resistant cultivar Zenith.

Results also suggest that the resistant tomato cultivar Zenith can be of value in replacing the cultivars Rio Grande and Rio Fuego, the most currently grown cultivars in the country for industry purposes. Furthermore, Zenith showed very good agronomic performances as observed by plant inspectors (E. Silva, '*personal communication*) in fields during the last growing season (1990/91). Further researches should be carried on under natural infection and field conditions to confirm our greenhouse studies.

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