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Inheritance and sources of resistance to bacterial speck of tomato caused by *Pseudomonas syringae* pv. *tomato*

BY E. FALLIK*, Y. BASHAN**, Y. OKON**†, A. CAHANER* AND N. KEDAR*

**Department of Field Crops and Vegetables, **Department of Plant Pathology and Microbiology, Faculty of Agriculture, The Hebrew University of Jerusalem, P. O. Box 12, Rehovot 76100, Israel*

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SUMMARY

Inheritance of resistance to bacterial speck of tomato was determined by analysing F_1, F_2 and backcross progenies of crosses involving a susceptible (VF-198) and a resistant cultivar (Rehovot-13). The results fit the hypothesis that resistance is controlled by a single dominant gene in interaction with minor genes. Cultivar susceptibility to *Pseudomonas syringae* pv. *tomato* was tested under greenhouse conditions under high inoculum pressure using infested tomato seeds together with infested soils and spray-inoculated wounded plants. Of 21 species, cultivars and lines, Rehovot-13, Ontario 7710 and *Lycopersicon pimpinellifolium* P.I. 126927 were found to be resistant to the pathogen. VF-198 and Tropic-VF were the most susceptible. Extra Marmande, Saladette, Acc.339944-3 and the wild type *Lycopersicon esculentum* var. *cerasiforme* were moderately resistant.

INTRODUCTION

Bacterial speck of tomato caused by *Pseudomonas syringae* pv. *tomato* (Okabe 1933) Young, Dye & Wilkie, 1978 (Dye *et al.*, 1980), causes severe damage to tomato crops in Israel, mainly in winter and early spring crops and it is also an important world-wide tomato disease (Bashan, Okon & Henis, 1978; Goode & Sasser, 1980; Yunis, Bashan, Okon & Henis, 1980*b*). Genetic resistance may be a better alternative for disease control than expensive chemical treatments and five sources of resistance have recently been found. Resistance of two accessions is controlled by a single dominant gene (Pilowsky & Zutra, 1982; Pitblado & Kerr, 1979), but the modes of inheritance of the resistance factors from the other sources have not yet been demonstrated (Cinar, 1978; Yunis, Bashan, Okon & Henis, 1980*a*). However, the acquisition of additional genetic factors for resistance and an understanding of the mode of action of their resistance genes are desirable and even essential for the success of future breeding programmes.

The purpose of this study was to investigate the inheritance of resistance to bacterial speck in cv. Rehovot 13 (an old commercial variety of the Marmande type) which was found to possess a high degree of field resistance to the disease (Yunis *et al.*, 1980*a*). The purpose was also to test the susceptibility of tomato cultivars, species and lines to bacterial speck of tomato, and to explore new sources of resistance to the disease.

MATERIALS AND METHODS

Plant materials and growing conditions

Tomato cultivars tested included the processing variety VF-198 (susceptible; P_2) and the fresh market cultivar Rehovot 13 (resistant; P_1). Seeds of the two cultivars were obtained from

† To whom all correspondence should be addressed.

Hazera Seed Co., Haifa, Israel. Seeds of F_1, F_2, BC_1 ($F_1 \times P_1$) and BC_1 ($F_1 \times P_2$) were obtained from plants growing in sandy loam soil in the greenhouse. Seeds were sown in 'Speedling' trays with peat and vermiculite (1 : 1, v/v) as growth medium. Each tray contained 52 plants and was irrigated with 1% N:P:K commercial fertiliser containing microelements (Haifa Chemicals). Experiments were conducted six times in a completely randomised fashion in six replicates, using 10 plants as a replicate.

The resistance tests were conducted with 20 tomato cultivars, species and lines (see Table 1).

Disease index – (Yunis, Bashan, Okon & Henis, 1980*a*)

Disease severity was estimated using an index of: 0 = no symptoms; 1 = 1–5 specks, either clustered together or spread all over the leaf; 2 = 6–10 specks; 3 = more than 11 specks. Index was determined on the third, fourth and fifth upper leaves of each plant. Number of specks per leaf were counted separately and the mean of the three leaves was considered as the disease index of the plant. All plants in each experiment were examined for their disease index.

Methods of inoculation

Two local isolates of *P. syringae* pv. *tomato* WT-1 and Bet Dagan 134.1, were used in separate experiments. Inoculum was prepared by incubating *P. syringae* pv. *tomato* in yeast-peptone broth at 30 °C (Bashan, Okon & Henis, 1978). Seeds were infested by soaking samples in a *P. syringae* pv. *tomato* suspension containing 10^{10} colony forming units (CFU)/ml under vacuum. The vacuum was released abruptly to favour entry of the pathogens into the seed cavities. Seeds were then sown and 10 ml of the same suspension was dripped into each pot.

Seedling leaves (three true leaves) were princubated under periodic mist (5 s mist every 30 min, 22 ± 2 °C) (Devash, Okon & Henis, 1980) for 24 h and then inoculated daily for 4 days by spraying to run off with a suspension containing *P. syringae* pv. *tomato* (10^9 CFU/ml) and carborundum (300 grid, 1 g/litre). Later, the plants were incubated for 8 days under periodic mist. Screening of cultivar susceptibility and inheritance tests was done by a single inoculation of the same suspension at the sixth true leaf stage and plants were then incubated as above. Control plants were sprayed with sterile water and incubated under the same conditions. Disease index was assessed after 9 days.

The newly discovered resistant line *L. pimpinellifolium* P.I. 126927 was subjected simultaneously to inoculation by several methods. This involved growing 52 plants from artificially infected seeds in artificially infested soil. Plants of the susceptible line VF-198 were treated in the same way and placed alternately with P.I. 126927 plants in 'Speedling' trays. Foliage was inoculated four times using carborundum and the plants were incubated at 25 ± 2 °C with periodic mist for 5 s every 30 min for 8 days. The experiment was repeated twice.

Detached leaves (15 leaves per replicate, five replicates) were surface-disinfected in 0·5% NaOCl for 3 min, washed five times with sterile water, placed on 0·5% water agar and inoculated with 2 ml suspension of 10^7 CFU/ml of *P. syringae* pv. *tomato* or sterile water. After 2 or 8 days of incubation under fluorescent light (100 W/m^2) at 24 °C, the leaves were again surface-disinfested and washed in sterile water. The leaves were then homogenised in sterile-phosphate buffer (0·06 M) and aliquots of dilutions of the homogenate were placed on King-B medium supplemented with 9 mg/litre fuchsin and 14 μg/litre triphenyl-tetrazolium-chloride. After 48 h incubation at 30 °C, typical fluorescent *P. syringae* pv. *tomato* were counted according to the recently developed enrichment method of Sharon, Okon, Bashan & Henis (1981).

RESULTS AND DISCUSSION

The inheritance of resistance of cv. Rehovot-13 to bacterial speck of tomato was investigated using cv. Rehovot-13 as resistant parent, VF-198 as susceptible parent, F_1 and F_2 of crosses between the parents and backcrosses to either the resistant or the susceptible parents.

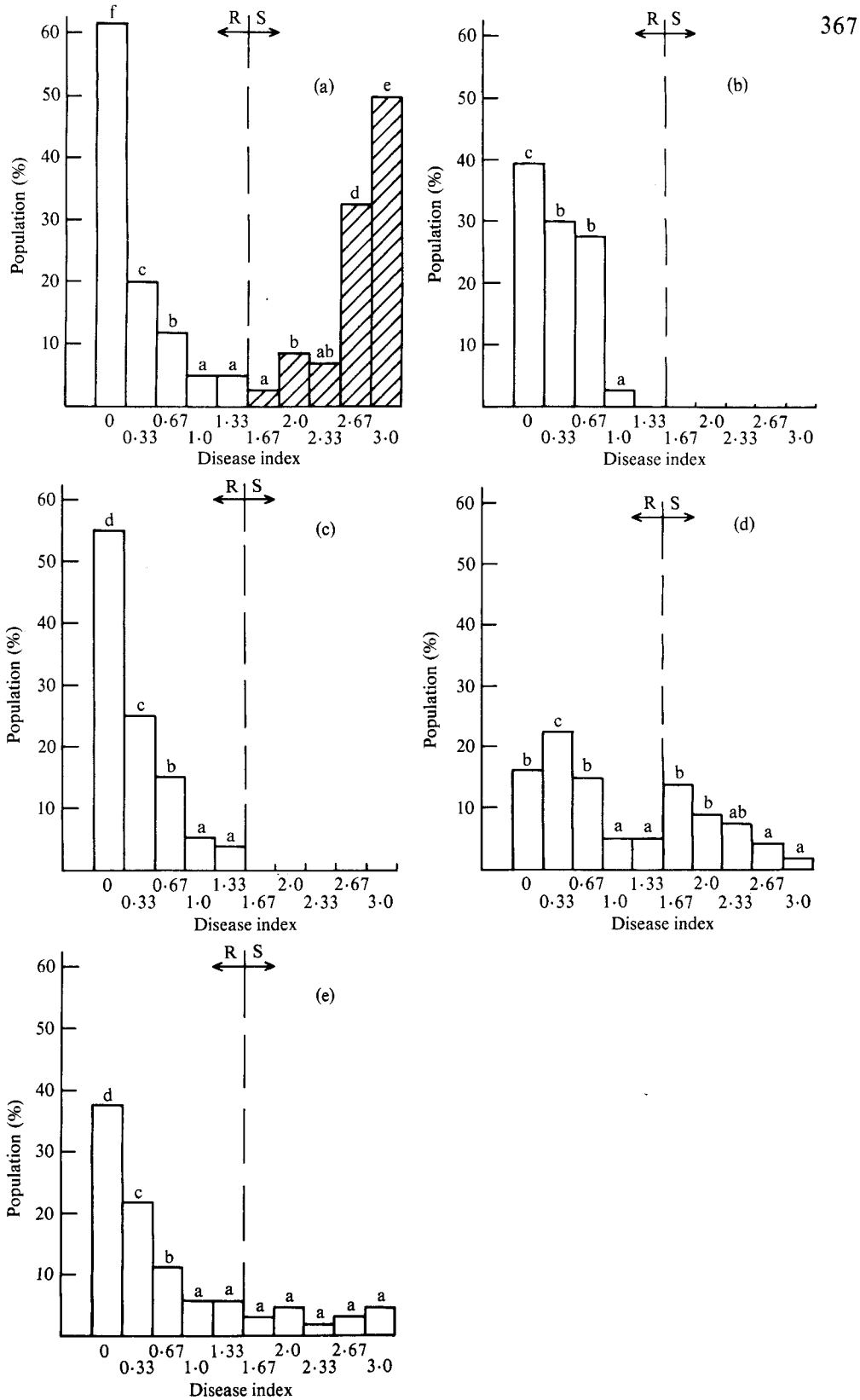

Fig. 1. Distribution of disease severity of tomato plant population infested with *Pseudomonas syringae* pv. *tomato*. (a) Rehovot-13 and VF-198 (hatched). (b) F_1. (c) Backcross F_1 × Rehovot-13. (d) Backcross F_1 × VF-198. (e) F_2. Columns followed by different letters in each sub-figure differ significantly at $P = 0{\cdot}05$.

It was found (Fig. 1*a*) that disease index (D.I.) of Rehovot-13 ranged between 0 (61% of plants) to 1·33 (3·7% of plants) and mean D.I. was 0·23, whereas the susceptible cultivar VF-198 produced a disease severity ranging from 1·67 (1·7% of plants) to 3·0 (50% of plants) and mean D.I. was 2·73. Disease indices of the parents did not overlap.

To explain the results (illustrated in Figs 1*a* to 1*d*) two possibilities have been considered: the relative resistance of cv. Rehovot-13 might be controlled either by a single factor of incomplete dominance or by a dominant major gene in interaction with minor genes which increase the degree of resistance.

The F_1-population (Fig. 1*b*) was somewhat more susceptible than the resistant parent (Fig. 1*a*), as indicated by its increased disease index (mean D.I. 0·31 compared to 0·23) and the decreased proportion, 39·5%, of completely resistant plants as compared to the 61% of such plants in the resistant parent. Thus, resistance seems incompletely dominant, but the dominant gene plus minor gene hypothesis might lead to similar results.

The backcross BC_1F_1 to the resistant parent (Fig. 1*c*) is essentially intermediate between P_1 and F_1, with a mean D.I. of 0·28. These results could be explained by either of the two hypotheses.

The backcross to the susceptible parent (Fig. 1*d*) gave two distinct populations, one more or less resistant and one susceptible. If resistance were controlled by a single partially dominant factor, the frequency distribution within the resistant group of this backcross should be identical with that of the F_1, and the distribution of the susceptible group of this backcross should be identical with that of the susceptible parent. The ratio of 'resistant' (Rr) to 'susceptible' (rr) should be 1 : 1. It is obvious that this was not the case. The results do, however, fit the second hypothesis, explaining resistance of Rehovot 13 by a major dominant gene strengthened by minor genes. The presence of minor genes might be responsible for the slightly increased 'resistance' of the susceptible group of the backcross (Fig. 1*d*) and the smaller number of minor genes might account for the decreased resistance in the resistant group of the backcross.

Table 1. *Response of 21 tomato lines to artificial inoculation with* Pseudomonas syringae *pv.* tomato

Lines	Disease index* 9 days after inoculation
Ontario 7710 (Canada)	0·32 ± 0·12
Rehovot-13	0·45 ± 0·01
Extra Marmande	1·29 ± 0·09
Saladette	1·37 ± 0·35
Step No. 535	1·85 ± 0·05
Hosen 228-VF	1·99 ± 0·07
Rutgers nor, acc. 364	2·04 ± 0·25
Manalucy	2·05 ± 0·07
Red sherry	2·06 ± 0·04
Kewalo	2·10 ± 0·27
Rutgers rin, acc. 365	2·21 ± 0·16
Italian winter	2·32 ± 0·02
VFN-70T-82-1 (U.C.D.)	2·44 ± 0·16
Tropic-VF	2·58 ± 0·37
VF-198	2·82 ± 0·18
339944-3 (U.C.D.)	1·43 ± 0·23
97-3 (U.C.D.)	2·10 ± 0·41
Lycopersicon pimpinellifolium P.I. 126927	0·51 ± 0·05
Lycopersicon esculentum var. *cerasiforme*	1·61 ± 0·41
Lycopersicon pimpinellifolium P.I. 126932	2·10 ± 0·07

* Disease index, see text. The indices are the means of two experiments.

Table 2. *Disease development in detached tomato leaves inoculated* with* Pseudomonas syringae *pv.* tomato *according to the leaf enrichment method and in plants incubated under periodic mist*

	Days after inoculation									
	Disease index†	Total no. of bacteria in 1 g of leaf (fresh weight) in mist		No. of bacteria within 1 g of leaf (fresh weight) in mist		No. of specks per leaf after enrichment§	Total no. of bacteria in 1 g of leaf (fresh weight) after enrichment‡		No. of bacteria within 1 g of leaf (fresh weight) after enrichment	
Tomato line	8	2	8	2	8	5	2	8	2	8
Lycopersicon esculentum VF-198	2·96a**	$6{\cdot}4 \times 10^{2}$a	$7{\cdot}3 \times 10^{7}$a	$1{\cdot}1 \times 10^{2}$a	$9{\cdot}3 \times 10^{6}$a	28·4a	$2{\cdot}1 \times 10^{3}$a	$8{\cdot}6 \times 10^{8}$a	$7{\cdot}6 \times 10^{2}$a	$6{\cdot}7 \times 10^{6}$a
Lycopersicon pimpinellifolium P.I. 126927	0·48b	$4{\cdot}9 \times 10^{2}$a	$6{\cdot}7 \times 10^{7}$a	10^{1}b	$4{\cdot}1 \times 10^{1}$b	2b	$4{\cdot}6 \times 10^{3}$a	$5{\cdot}1 \times 10^{8}$a	10^{1}b	$8{\cdot}1 \times 10^{1}$b

* Plants were inoculated with 10^{9} CFU/ml and detached leaves with 10^{7} CFU/ml of *P. syringae* pv. *tomato*.

† In 52 plants of each line maintained in mist chamber.

‡ Number of bacteria inside and outside the leaf; means of five replicates.

§ Fifteen leaves per replicate, five replicates.

** Numbers followed by different letters in each column differ significantly at $P = 0{\cdot}05$.

These results fit the hypothesis if we assume that the resistance of at least some of the individuals with D.I. 1·00 and 1·33 is based on minor genes only.

The F_2 population (Fig. 1*e*) lends support to the dominant plus minor genes hypothesis. The F_2 can be divided into two distinct groups. The distribution within each group and especially in the susceptible one, is undoubtedly different from that of the respective parents (Fig. 1*a*). The resistant sub-group (with D.I. ranging from 0 to 1·33) with minor genes, is similar to the F_1. Moreover, in the susceptible sub-group (D.I. 1·67 to 3·0) minor genes should exist, making this group more resistant than the susceptible parent (P_2).

In greenhouse tests, *L. pimpinellifolium* P.I. 126927 was resistant to bacterial speck as were the previously known resistant cultivars Rehovot-13 and ONT-7710. Cvs Extra-Marmande, Saladette, 339944-3 and the wild type *L. esculentum* var. *cerasiforme* were moderately resistant, whereas 14 other lines tested, especially VF-198 and Tropic-VF, were highly susceptible (Table 1).

The apparent resistance of *L. pimpinellifolium* P.I. 126927 observed in mist chamber experiments was checked by inoculating plants in several different ways simultaneously. Eight days after inoculation (Table 2) *L. pimpinellifolium* P.I. 126927 plants showed a mean disease index of 0·48, compared to 2·96 for VF-198 plants. No infection was recorded in uninoculated control plants maintained in the same conditions. Bacterial concentration was assessed in both cultivars within the leaves and on their surface. Bacterial populations on leaves of both varieties were high throughout the period of incubation in the mist chamber. However, the internal bacterial population of VF-198 increased to high levels with time, whereas *L. pimpinellifolium* P.I. 126927 bacterial counts remained steady with time (Table 2). The different endogenous populations are due to different levels of disease development in resistant and susceptible plants (Bashan, Sharon, Okon & Henis, 1981). Disease indices of VF-198 and *L. pimpinellifolium* P.I. 126927 were similar in both tests.

In an additional trial, leaves from *L. pimpinellifolium* P.I. 126927 and VF-198 plants were inoculated according to the leaf enrichment method (Sharon *et al.*, 1981). Total bacterial numbers and bacteria within the leaf were counted separately. It was found (Table 2) that leaves of VF-198 were infected, with symptoms appearing within 5 days, whereas leaves of *L. pimpinellifolium* P.I. 126927 showed only few visible symptoms. Differences in the endogenous bacterial population between the susceptible and the resistant cultivars were also observed using this method (Table 2).

These findings should encourage further work on genetical improvement of tomato cultivars for resistance to bacterial speck of tomato.

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