Inheritance of Resistance to Corynebacterium michiganense in Tomato

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ABSTRACT

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Parent clones, first and second filial generations, and backcross generations from six tomato crosses were tested for resistance to *Corynebacterium michiganense*. Three sources of resistance used in the crosses were *Lycopersicon esculentum* (Bulgaria 12, P. I. 330727), and *L. pimpinellifolium* (Utah 737, P. I. 344103, and Utah 20, P. I. 344102). Seedling tomatoes were inoculated in the 3-leaf stage and evaluated 5 weeks later, by measurement of internal vascular discoloration. Resistance appears to be controlled by several quantitative or incompletely dominant major genes that are influenced by modifying genes.

Additional key words: Lycopersicon glandulosum, L. hirsutum, L. peruvianum, stunting, wilt, internal vascular discoloration.

Resistance to *Corynebacterium michiganense* (E. F. Smith) H. L. Jens., was reported first in *Lycopersicon pimpinellifolium* (L.) Mill. in Utah. (1) *Lycopersicon pimpinellifolium* was used by Elenkov (5) in development of the line 8/12 which I have used and which has been known as 'Bulgaria 12' or P. I. 330727 (11). Resistance also has been reported in *L. hirsutum* Humb. & Bonpl. (7), *L. peruvianum* (L.) Mill., *L. glandulosum* C. H. Muller, and in several cultivars of *L. esculentum* Mill. (12). Elenkov (5) indicated resistance in *L. pimpinellifolium* was dominant, but he offered no details. The level of resistance in Bulgaria 12 was not as high as that in some *L. pimpinellifolium* sources (13, 14). This suggested to me that resistance may be controlled by more than one gene.

The objective of this study was to establish the genetic basis of resistance to *C. michiganense* in one source of *L. esculentum* and two sources of *L. pimpinellifolium* germplasm.

MATERIALS AND METHODS

Six controlled cross-pollinations were made between three sources with resistance to *Corynebacterium michiganense* and three susceptible *Lycopersicon esculentum* accessions. All parental material (Table 1) was tested and selected for reaction to *C. michiganense*. The resistant accessions were tested three times and the susceptible accessions once. Clonal materials from those tests were used in the cross pollinations, reciprocal crosses, and backcrosses. The F_2 and backcross populations for a given cross were all obtained from a single F_1 plant. Populations tested from a given cross were: both parents (100 S_1 plants each), F_1 (100 plants), F_2 (400 plants), and the F_1 backcross to both parents (200 plants each). These were tested concurrently in the greenhouse for their reaction to *C. michiganense*.

Seedlings were grown in round 8-cm diameter peat pots in a peat:perlite:soil mix (1:1:2, v/v). Groups of 25 pots of each population were randomized and placed on trays in approximately 1.3 cm of water (6) to provide even soil moisture. Greenhouse temperature ranged from an average maximum of approximately 27 C to an average minimum of 15 C.

Seedlings were inoculated at the three-leaf stage about 24 days after planting. Inoculation was by excision of the first true leaf at its point of attachment to the stem, and application of a bacterial suspension (10, 11). Inoculum was prepared from 5-day-old petri dish cultures of isolate Cm15 (isolated from a fruit spot, collected in Indiana by W. J. Virgin) by suspending the bacteria in sterile Ringer's solution (ionically balanced salt, Consolidated Laboratories, Inc., Chicago Heights, Ill.) and adjusting to approximately 10^6 cells/ml.

Five weeks after inoculation, plants were measured for: (i) plant height from the point of inoculation, and (ii) linear extent of internal vascular discoloration (LEIVD) from the point of inoculation. From these two parameters the percentage of vascular discoloration was calculated and used as a single measure of resistance; i.e., those plants with the smallest extent of discoloration were regarded as the most resistant. This measure was demonstrated previously to correlate well with the growth of *C. michiganense* in tomato stem tissues (13). Resistance can be measured several other ways such as with measurement of yield, stunting, wilt, total plant damage, or mortality. However, LEIVD is a relatively objective measure, is simple to obtain, and correlates well with growth of the pathogen in host tissues.

The mean LEIVD and the standard deviation (SD) were calculated for each population and frequency

distribution graphs of the LEIVD were made for each cross. An estimate of the gene number was calculated for each cross by the method of Castle (4) which was modified as suggested by Wright (15). The estimated gene number was calculated as follows:

$$n = \frac{(P_1\bar{x} - P_2\bar{x})^2}{8(\sigma F_2 - \sigma F_1)}$$

where $P_1\bar{x}$ is the mean LEIVD of the susceptible parent, $P_2\bar{x}$ is the mean LEIVD of the resistant parent, σF_2 is the variance of the F_2 LEIVD percentages, and σF_1 is the variance of the F_1 LEIVD percentages.

RESULTS

Statistical analysis failed to reveal any significant differences between reciprocal crosses. This suggested that cytoplasmic factors did not influence gene action. Therefore, reciprocals of all crosses were combined for all statistical analyses.

Resistant parents.—Reactions to *C. michiganense* for the two parental accessions of *L. pimpinellifolium* were similar in their mean LEIVD, with 7% for A134 and 9% for A129 (Table 2). The other resistant accession (A146) responded to *C. michiganense* with remarkable uniformity in all three crosses. Mean LEIVD percentages for A146 were 54, 46, and 45 involving crosses with A171, A265, and A100, respectively. Although all three A146 populations originated from a single plant, the experiments were conducted at three different times. The same is true of both A129 populations. All variances for A146 were comparable as were those for the *L. pimpinellifolium* accessions (Table 2).

Gene number estimates for those crosses involving *L. pimpinellifolium* ranged from about four to 11 (Table 2). Estimates for the crosses between A146 and A171, A265, and A100 were 1.5, 16.2, and 0.7, respectively (Table 2). The first and last numbers suggest the involvement of one gene, whereas the second deviates substantially. The standard deviation of the F_1 in this case is as large as that of the F_2, resulting in a small divisor and hence a large gene number estimate. There were some off-type plants in the F_1, possibly from seed contamination, resulting in more variability. If one substitutes a standard deviation more in accord with the other F_1 populations involving A146, the gene number estimate is approximately 2.

Frequency distributions of the LEIVD are presented in Fig. 1. These graphs illustrate the variation in each population and permit comparison between crosses and also between parents and their progeny within a cross. The graphs also portray the variation in each population which is measured by the standard deviation (Table 2). The two *L. pimpinellifolium* accessions (A129 and A134) show far less variation than did *L. esculentum* (A146). Since plants of both populations of A129 are of one genotype, as are the plants of the three A146 populations, the very small differences among means and standard deviations are predictable.

Susceptible parents.—Two susceptible parents reacted similarly to *C. michiganense*. Accession A171 had mean LEIVD percentages of 100 and 96 in crosses with A134 and A146, respectively. Similarly, A265 mean LEIVD values were 98 and 96 in crosses with A129 and A146 (Table 2). The nearly identical means are predictable because all populations of a given parent are from the same genotype. The other parent (A100) had a considerably larger standard deviation and a mean LEIVD of 77. The frequency distributions illustrate the variation found in each population (Fig. 1). The standard deviation for A171 and A265 ranged from zero to 11, whereas for A100 it was 18. Frequency distributions are not shown for the F_2 and backcross generations for the

TABLE 1. Disease reaction of *Lycopersicon* sp. parents used for determining inheritance of resistance to *C. michiganense*

Line	Species	Accession number	Disease reaction
P. I. 344102 (Utah 20)	*L. pimpinellifolium*	A129	Resistant
P. I. 344103 (Utah 737)	*L. pimpinellifolium*	A134	Resistant
P. I. 330727 (Bulgaria 12)	*L. esculentum*	A146	Resistant
P. I. 283907	*L. esculentum*	A171	Susceptible
VF 145-7879	*L. esculentum*	A100	Susceptible
Highlander	*L. esculentum*	A265	Susceptible

TABLE 2. Gene number estimates, linear extent of internal vascular discoloration (LEIVD) means (x), and standard deviations (SD), based on LEIVD for six tomato crosses inoculated with *Corynebacterium michiganense*

Cross	Gene number estimate	P_1 (sus.)[a]		P_2 (res.)[a]		F_1		F_2		$P_1 \times F_1$		$P_2 \times F_1$	
		$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD
A171 × A134	7.5	100	0	7	10	19	14	21	18	46	29	10	10
A171 × A146	1.5	96	8	54	19	82	15	73	20	80	19	55	22
A265 × A129	4.4	98	5	9	6	40	14	40	20	76	21	24	12
A265 × A146	16.2	96	11	46	18	62	19	70	19	87	15	60	16
A100 × A129	10.8	77	18	9	4	29	13	27	15	47	21	...	...
A100 × A146[b]	0.7	77	18	45	21	45	16	54	21	64	21	...	...

[a]Susceptible (sus.) and resistant (res.) to *C. michiganense*.
[b]A146 designated as P_3 in Fig. 1.

crosses involving A100 (Fig. 1). However, from the means and standard deviations (Table 2), one can deduce the approximate extent of those distributions. None is appreciably different from comparable crosses.

F_1 and F_2 populations.—All F_1 populations reacted to *C. michiganense* similarly in that their LEIVD percentages were distributed intermediate to those of the resistant and susceptible parents. The single exception was the F_1 of A100 × A146 in which case the mean LEIVD percentage was the same as that of A146. However, the mean LEIVD of the F_2 of this cross was 10% higher and more nearly as one would expect based on the other results. The standard deviations were very similar and nearly as high as that of A146 (Table 2). The mean LEIVD percentages of the F_1 population were slightly higher than those of the resistant parents (P_2 and P_3), indicating that incomplete dominance is involved.

The F_2 populations had mean LEIVD percentages which were similar to the F_1. The standard deviations were slightly higher than those of the F_1 except for one which was the same (Table 2). The frequency distributions for the F_2 appeared similiar to the F_1, but exhibited a slightly wider distribution as indicated by the standard deviations.

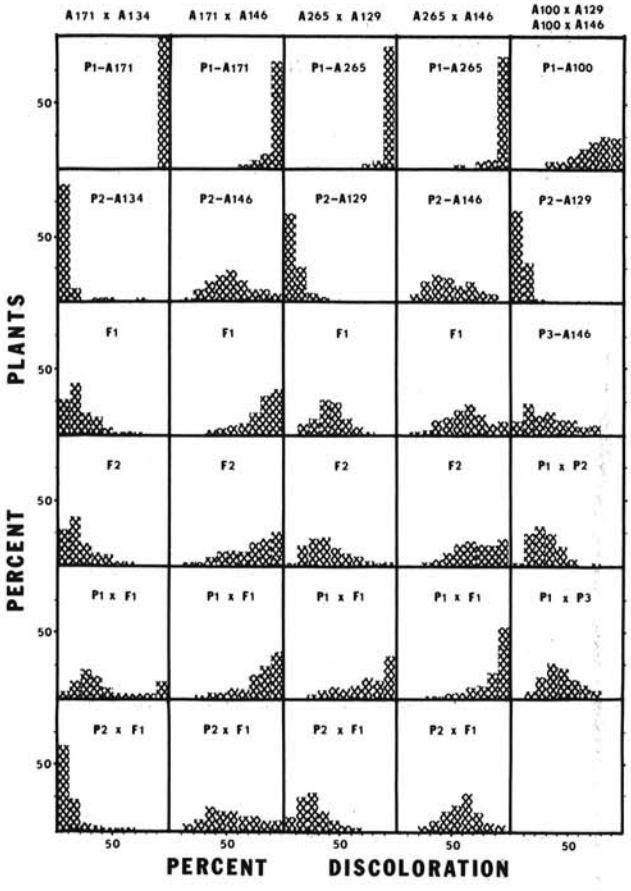

Fig. 1. Frequency distribution of percent linear extent of internal vascular discoloration (LEIVD) in populations of tomato crosses: A171 (susceptible) × A134 (resistant); A171 (susceptible) × A146 (resistant); A265 (susceptible) × A129 (resistant); A265 (susceptible) × A146 (resistant); A100 (susceptible) × A129 (resistant); and A100 (susceptible) × A146 (resistant), arranged in 10-percentile increments.

Backcross populations.—The F_1 backcross to P_2 (resistant parent) was not made in either cross A100 × A129 or A100 × A146. However, the F_1 was backcrossed to P_1 (susceptible parent) in both crosses, and those backcross populations reacted to *C. michiganense* with mean LEIVD percentages of 47 and 64, respectively (Table 2). The mean LEIVD percentages (Table 2) and the frequency distributions of the backcrosses (Fig. 1) suggest that more than one gene is involved.

DISCUSSION

The level of resistance in crosses with *L. pimpinellifolium* (A129 and A134) was consistently higher than that of Bulgaria 12 (A146). The gene number estimates for resistance to *C. michiganense* in *L. pimpinellifolium* ranged from 4 to 11. This clearly indicates that more than one major gene is involved. The mean LEIVD percentages of 19-40 for the F_1 populations of the same crosses suggest that genes for resistance are characterized by incomplete dominance, or are quantitative because the means are intermediate to both parents. Crosses involving A146 as the resistance source point to one or two incompletely dominant major genes controlling resistance and there are indications of the presence of some modifying genes in some cultivars classed as susceptible (Fig. 1) as well as in A146. For example, the cross A100 × A146 appears to indicate a single major gene (gene number estimate of 0.7), whereas the LEIVD percentage of 45 for both the P_2 and F_1 populations, respectively, strongly suggests dominance. However, the frequency distributions for P_1 and F_1 (Fig. 1), their high standard deviations (Table 2), and the response of the F_2 all suggest the possibility of modifying genes accompanying one or two incompletely dominant genes. The frequency distributions of the other Bulgaria 12 crosses also support such a conclusion (Fig. 1). The gene number estimate for *L. pimpinellifolium* ranging from 4 to 11 appears somewhat high considering the relative ease of transferring the resistant character; however, the low end of that range may be realistic. Resistant accession A146, with a lower level of resistance than the *L. pimpinellifolium* accessions, fits my expectations with a lower gene number estimate than A129 or A134. This is expected if one assumes that several genes for resistance are involved and that they have additive effects.

The reaction of tomato to *C. michiganense* appears to be similar to that of tetraploid alfalfa (*Medicago sativa* L.) to *Corynebacterium insidiosum* (McCulloch) H. L. Jens. Brink et al. (3) show that the level of resistance in plants of alfalfa to *C. insidiosum* ranges continuously from a very low to a very high level. My results with *C. michiganense* on tomato show a similar distribution (Fig. 1). The genetics of resistance in alfalfa to *C. insidiosum* has not been completely resolved; estimates of gene number range from many (3) to but a few (8, 9). Barnes et al. (2) suggested that possibly two different genetic systems were operable with the two alfalfa populations they examined with recurrent selection.

The results of my experiments allow for more than one explanation; however, the most likely one is that resistance in both *L. pimpinellifolium* accessions and in

Bulgaria 12 is controlled by quantitative or incompletely dominant genes with one to four major genes controlling resistance in these accessions. The continuous range of resistance seen in hybrid populations could be explained by additional minor genes acting independently to modify the major gene action.

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