Genetic Analysis of a Complex Hypersensitive Reaction to Bacterial Spot in Tomato

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ABSTRACT

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Lycopersicon esculentum accession Hawaii 7998 is the only identified source of resistance to *Xanthomonas campestris* pv. *vesicatoria*. This resistance is based on a hypersensitive reaction. Interspecific progenies with *L. pennellii* were generated to analyze the inheritance of a hypersensitive reaction. This wild species is susceptible to bacterial spot and has a large number of allelic differences with respect to Hawaii 7998. Eighteen isozymes and a morphological marker were used to probe about 30% of the tomato genome for hypersensitive reaction factors. A rating scale was developed to evaluate the inoculation responses of the parental genotypes and the segregating progenies; evaluations were performed every 8 h after inoculation. Hourly rates of change in score were used to analyze the inheritance of hypersensitive reaction. Linkage between marker loci and hypersensitive reaction factors was tested with two-way contingency tables. Significant heterogeneity between genotypic classes for the relative proportion of plants that changed the necrosis score was interpreted as linkage between the marker locus and a hypersensitive reaction factor. Linkage to the same chromosome 1 markers was detected in the F_2 and the BC_1 to Hawaii 7998 but not in the reciprocal BC_1; this region did not explain all the variation. These results indicated that the hypersensitive response in Hawaii 7998 is controlled by multiple nondominant factors.

Additional keywords: gene tagging.

Bacterial spot caused by *Xanthomonas campestris* pv. *vesicatoria* is one of the most important diseases of tomatoes in regions where high temperatures and heavy rainfall occur together during the cropping season. The lack of resistant tomato cultivars and effective chemical control has contributed to high incidences of bacterial spot (18). However, development of resistant cultivars is now a distinct possibility after the identification of accession Hawaii 7998 (H7998) as a source of resistance in *Lycopersicon esculentum* Mill. (23). Resistance in H7998 is associated with a hypersensitive reaction (9).

Hypersensitivity-associated resistance to some plant bacterial pathogens conforms to the "gene-for-gene" hypothesis (10,15). According to this hypothesis, the incompatible interaction between an avirulence gene in the pathogen and a corresponding resistance gene in the plant results in a hypersensitive reaction (7). Two lines of evidence suggest that resistance to *X. c. vesicatoria* in H7998 fits Flor's model. First, a clone of the avirulence gene *avrRxv* of *X. c. vesicatoria* has been isolated (32), and second, H7998 leaves display a hypersensitive reaction when infiltrated with high inoculum concentrations of *X. c. vesicatoria* strains that contain *avrRxv* but not with strains that lack the gene (R. E. Stall, *unpublished data*). However, continuous variation in hypersensitive reaction intensity observed in intra- (24) and interspecific crosses indicates that the hypersensitivity associated resistance to bacterial spot in H7998 is a complex trait.

Molecular markers have been used in tomato to study the inheritance and linkage relationships of monogenic disease resistances. For instance, the gene for resistance to the root-knot nematode, *Mi*, was linked to *Aps-1* (21); the gene for resistance to races 1, 2, and 3 of *Fusarium oxysporum* f. sp. *lycopersici*, *I-3*, was linked to *Got-2* (2,4), and the gene for resistance to

tobacco mosaic virus, *Tm-2a*, was found linked to TG101 (34). Molecular markers also have been used to study the inheritance of quantitative traits in tomato (17,25). We report on the use of isozyme markers to study the inheritance of the complex hypersensitive reaction-associated resistance of H7998.

MATERIALS AND METHODS

Plant materials. The low levels of polymorphism at molecular marker loci among *L. esculentum* lines (14) dictated the use of a susceptible wild species to generate informative segregating progenies. *L. pennellii*, accession LA 716, was selected for its susceptibility to bacterial spot and because it is both an inbred and has allelic differences with respect to H7998 at a large number of molecular marker loci (1,30). Unilateral compatibility between the two species required the use of *L. esculentum* as the pistillate parent (19). The following progenies were produced between these genotypes: F_1; F_2; and reciprocal backcrosses: BCP1 = (H7998 × [H7998 × LA 716]), and BCP2 = ([H7998 × LA 716] × LA 716). The sizes of the segregating progenies were as follows: BCP1 = 190 plants, BCP2 = 180 plants, and F_2 = 98 plants. Bonny Best, a susceptible cultivar of *L. esculentum*, was used as an additional control in some inoculation experiments.

Isozyme analysis. The techniques of starch gel electrophoresis and enzyme staining used in this study have been described elsewhere (3,22,27,28). The genotype of F_2 plants was determined at the following isozyme loci: *Prx-1, Skdh-1,* and *Bnag-1* on chromosome 1; *Est-7* and *Prx-2* on chromosome 2; *Prx-7* on chromosome 3; *Pgm-2* on chromosome 4; *Prx-5* on chromosome 5; *Aps-1* on chromosome 6; *Got-2* on chromosome 7; *Aps-2* on chromosome 8; *Est-2* on chromosome 9; *Prx-4* on chromosome 10; *Sod-1* on chromosome 11; and *Est-4, 6Pgdh-2,* and *Pgi-1* on chromosome 12. The morphological locus, *Pn* (chromosome 8), also was examined; this marker controls the accumulation of anthocyanins in the basal cells of large leaf hairs in the cotyledon leaves of LA 716 (31). Plants of the reciprocal backcrosses were genotyped at the same marker loci as those in the F_2 population, except *Prx-2* and *Prx-5* were not determined in BCP1 and *Bnag-1, Prx-2, Prx-5, Pn, Est-2, Sod-1,* and *6Pgdh-2* were not determined in BCP2.

Inoculum source. Strain 90-14 of race 1 of the *X. c. vesicatoria* tomato group (*X. c. vesicatoria*T) was used in inoculations. Inoculum was prepared from cultures that were grown in nutrient broth (DIFCO Laboratories, Detroit, MI) with continuous shaking for 24 h at 30 C. Cultures were centrifuged at 5,200 × g_{max} for 10 min, and the bacterial pellets were resuspended in sterile tap water. The bacterial suspension was adjusted to a concentration of 5×10^8 cfu/ml (OD_{600} = 0.3). Inocula with different concentrations were obtained by dilution.

Rating scale for hypersensitive reaction. The BCP1, BCP2, and F_2 populations were tested independently of each other, and in each case, the parental genotypes were used as controls. All plants were grown in a greenhouse and pruned above the fully expanded fourth true leaf. Approximately 7 days after pruning, plants were moved to a growth room at 24 C and 60% humidity with a 16-h light period. The first distal pair of opposing leaflets of the fourth true leaf was inoculated. An area of about 1 cm^2 was infiltrated on the abaxial side of each leaflet. A bacterial suspension (5×10^8 cfu/ml) was forced into the intercellular spaces with a syringe fitted with a 26-gauge needle (12); the border of the infiltrated area was marked by puncturing the leaf with the needle. The percentage of the total infiltrated area that became necrotic was estimated every 8 h after infiltration. The following rating scale was used: 0 = no necrosis; 1 = 1–20%; 2 = 21–40%; 3 = 41–60%; 4 = 61–80%; and 5 = 81–100% or confluent necrosis. The score given to each plant was based on the evaluation of both leaflets.

Electrolyte leakage and internal bacterial populations. In addition to the rate of necrosis development, two other parameters associated with hypersensitivity (11) were evaluated: 1) electrolyte leakage and 2) the level of internal bacterial populations. Cuttings of H7998, the F_1 (H7998 × LA 716), and LA 716 were evaluated and used as reference points. Bonny Best cuttings were used as additional susceptible controls. The bacterial population in the leaf area infiltrated with bacterial suspension (5×10^5 cfu/ml) was determined daily by the dilution plate method of Hibberd et al (8). Electrolyte leakage was measured every 12 h after infiltration with a bacterial suspension (5×10^8 cfu/ml), according to the procedure described by Hibberd et al (8).

Data analysis. All statistical analyses were performed with the Statistical Analysis System (SAS Institute, Cary, NC). Map distances between linked loci were estimated with the aid of MAPMAKER software (13).

RESULTS

Isozyme analysis. Significant deviations from Mendelian ratios were detected at several loci (Table 1). Most of the deviations were detected in the backcross in which *L. esculentum* was the recurrent parent (BCP1). Eight of 16 markers showed significant skewing: *Bnag-1* (chromosome 1), *Est-7* (chromosome 2), *Est-*

TABLE 1. Analysis of monogenic segregations in BCP1 (Hawaii 7998 × [H7998 × LA 716]), BCP2 ([H7998 × LA 716] × LA 716), and F_2

Locus	Chromosome no.	BCP1[a]			BCP2			F_2			
		e/e[b]	e/p	$\chi^2_{1:1}$	p/p	e/p	$\chi^2_{1:1}$	e/e	e/p	p/p	$\chi^2_{1:2:1}$
Prx-1	1	76	72	NS	102	73	4.8*	9	50	37	16.5***
Skdh-1	1	81	67	NS	88	72	NS	24	42	26	NS
Bnag-1	1	88	54	8.1**	...	...		21	46	25	NS
Est-7	2	120	73	11.5**	76	100	NS	27	53	18	NS
Prx-2	2	...[c]	...		...	...		28	51	18	NS
Prx-7	3	97	87	NS	70	57	NS	20	42	30	NS
Pgm-2	4	93	97	NS	77	83	NS	26	48	23	NS
Prx-5[d]	5	...	...		...	...			72	26	NS
Aps-1	6	85	101	NS	70	80	NS	14	45	37	11.4**
Got-2	7	99	91	NS	80	88	NS	20	47	24	NS
Aps-2	8	99	75	NS	88	66	NS	28	46	21	NS
Pn[d]	8	74	96	NS	...	...		23	74		NS
Est-2[d]	9	106	77	4.6*	...	...		24	73		NS
Prx-4	10	57	130	28.5***	71	68	NS	17	56	23	NS
Sod-1	11	69	118	12.8***	...	...		8	45	34	15.6***
Est-4	12	29	64	13.2***	86	72	NS	17	46	25	NS
6Pgdh-2	12	79	109	4.8*	...	...		18	54	26	NS
Pgi-1	12	77	115	7.5**	80	84	NS	16	62	20	8.2**

[a] *, **, and *** = significant deviations at the 0.05, 0.01 and 0.001 levels, respectively. NS = not significant.
[b] Genotypes: e/e = homozygous for *Lycopersicon esculentum* alleles; p/p = homozygous for *L. pennellii* alleles; e/p = heterozygotes.
[c] Data were not collected.
[d] Homozygotes for either *L. esculentum* or *L. pennellii* alleles could not be distinguished from heterozygotes at these loci. A 1:3 ratio was tested in the F_2.

2 (chromosome 9), *Prx-4* (chromosome 10), *Sod-1* (chromosome 11), and *Est-4, 6Pgdh-2,* and *Pgi-1* (chromosome 12). In contrast, only four of 18 markers skewed significantly in the F_2 population: *Prx-1* (chromosome 1), *Aps-1* (chromosome 6), *Sod-1* (chromosome 11), and *Pgi-1* (chromosome 12). Deviations from the expected ratios have been reported before for interspecific crosses with *L. pennellii* (3,20). However, significant skewing for chromosomes 2 and 9 had not been reported. All other marker loci assorted as previously reported (5,27). BCP1 data were used to estimate distances between linked loci: *Prx-1* was 35.7 cM (centiMorgans) from *Skdh-1*, which was 9.2 cM from *Bnag-1* on chromosome 1; and *Est-4* was 30.6 cM from *6Pgdh-2*, which was 14.3 cM from *Pgi-1* on chromosome 12. The distances were calculated with the Haldane function of MAPMAKER (13), and all adjacent loci had a LOD score greater than 4.0. Previously reported distances from *Prx-1* to *Skdh-1–Bnag-1* were 48 cM, and *Est-4* was 8 cM from *6Pgdh-2*, which was 16 cM from *Pgi-1* (5). Discrepancies between the map distances observed here and those from previous reports were not unexpected, because recombination frequencies depend on the genotypes involved (17).

Segregation of hypersensitive reaction. The dynamics of necrosis development recorded for the BCP1, BCP2, and F_2 populations are shown in Tables 2, 3, and 4, respectively. Each data set was summarized and presented as time-course plots of the fraction of the population that increased in necrosis score during each evaluation period (y-axes of Figs. 1 and 2). Contrasting patterns of necrosis development after inoculation with *X. c. vesicatoria* were displayed by H7998 and LA 716 (Fig. 1A; 10 plants of each genotype). H7998 began developing necrosis 8 h after inoculation and reached confluent necrosis (rating of 5) within 48 h. In contrast, LA 716 began developing necrosis 3 days after inoculation, and necrosis in the inoculated area continued developing beyond the evaluation period of 128 h. Necrosis development of F_1 plants preceded that of *L. pennellii* by 8–16 hours; all 10 F_1 plants reached confluent necrosis (rating of 5) within 128 h after inoculation. An overlap was observed in the periods of necrosis development of the F_1 and the wild susceptible parent LA 716, but no overlap was detected between either of these two parents and the resistant H7998.

Different patterns of necrosis development were detected for the BCP1, BCP2, and F_2 populations in time-course plots similar to those described above (Fig. 1B–D). Two peaks were observed in the BCP1 population (Fig 1B). A small fraction of the population began to develop necrosis soon after inoculation, and approxi-

TABLE 2. Necrosis score frequencies of the BCP1 population at 8-h intervals after inoculation with *Xanthomonas campestris* pv. *vesicatoria*[a]

	Hours after infiltration										
Score	16	24	32	40	48	56	64	72	80	88	96
0	151	115	58	24	20	14	11	4	2	1	0
1	26	41	51	53	42	42	43	31	13	4	2
2	7	12	33	32	27	30	22	27	26	22	2
3	6	11	21	29	31	22	21	19	26	19	20
4	2	9	11	30	29	34	41	48	40	40	34
5	1	5	19	25	44	51	55	64	86	107	135
H7998[b]	3.2	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
F_1[b]	0.0	0.0	0.1	0.1	0.5	0.5	0.8	1.1	1.8	2.8	4.0

[a] BCP1 = (*Lycopersicon esculentum*, Hawaii 7998 × [*L. esculentum*, Hawaii 7998 × *L. pennellii*, LA 716]).
[b] Average necrosis scores of parental genotypes (10 plants each) at the indicated time periods.

TABLE 3. Necrosis score frequencies of the BCP2 population at 8-h intervals after inoculation with *Xanthomonas campestris* pv. *vesicatoria*[a]

	Hours after infiltration									
Score	40	48	56	64	72	80	88	96	104	112
0	157	137	113	88	35	12	5	2	0	0
1	18	36	52	71	66	53	41	15	7	5
2	1	2	7	10	41	24	21	22	9	7
3	0	0	2	2	16	24	13	14	9	7
4	0	1	1	1	10	30	18	14	23	4
5	0	0	1	4	8	33	78	109	128	158
F_1[b]	0.0	0.1	0.3	0.6	1.1	2.3	2.7	3.4	4.3	4.9
LA 716[b]	0.0	0.0	0.1	0.3	1.0	1.4	2.1	3.0	3.9	5.0

[a] BCP2 = ([*Lycopersicon esculentum*, Hawaii 7998 × *L. pennellii*, LA 716] × *L. pennellii*, LA 716).
[b] Average necrosis scores of parental genotypes (seven plants each) at the indicated time periods.

TABLE 4. Necrosis score frequencies of the F_2 population at 8-h intervals after inoculation with *Xanthomonas campestris* pv. *vesicatoria*[a]

	Hours after inoculation													
Score	16	24	32	40	48	56	64	72	80	88	96	104	116	128
0	88	71	55	45	42	39	32	27	24	19	15	11	4	4
1	6	20	30	33	26	23	25	27	25	23	25	23	16	8
2	1	1	5	8	13	16	13	11	13	18	15	16	14	8
3	1	2	0	4	5	3	8	11	8	8	8	9	15	11
4	1	0	3	1	3	5	4	3	7	6	8	3	7	13
5	1	4	5	7	9	12	16	19	21	24	27	36	42	54
H7998[b]	0.0	0.9	2.9	4.3	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
F1[b]	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	1.0	2.1	3.2	4.4	5.0	5.0
LA 716[b]	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.2	0.5	1.3	2.7	4.4

[a] F_2 of the cross *Lycopersicon esculentum*, Hawaii 7998 × *L. pennellii*, LA 716.
[b] Average necrosis scores of parental genotypes (10 plants each) at the indicated time periods.

mately 60% of the population increased in necrosis score during the 24- to 32-h period (first peak); this fraction declined to the lowest point during the 56- to 64-h interval. The first peak overlapped extensively with that of H7998. A second but moderate rise in the fraction of the population that was increasing in necrosis score was observed between 56 and 96 h after inoculation. By comparison, the average rating of F_1 plants increased from 0.6 to 4.0 during the rise of the second peak of BCP1. In contrast to BCP1, only 10% of the BCP2 population, the reciprocal backcross, increased in necrosis score during the first 40 h after infiltration (Fig. 1C). This percentage rose to 21% in the next 24 h and then rose abruptly during the 64- to 72-h period, reaching the highest point (75%) during the 73- to 80-h period. During this period, the average rating of the F_1 plants increased from 1.1 to 2.3, and the average rating of *L. pennellii* plants increased from 1.0 to 1.4. The F_2 population displayed two modest increases in the fraction of the population displaying necrosis development (Fig. 1D). The first increase comprised 25% of the population and was observed in the first 32 h after inoculation; the second increase occurred between 104 and 116 h after inoculation and comprised 45% of the population. A baseline of about 20% was observed between the two increases.

Relationships among necrosis scores, electrolyte leakage, and internal bacterial populations. Necrosis scores were chosen to analyze the genetics of the H7998 hypersensitive reaction because of their simplicity. For this reason, it was necessary to first assess the reliability of the rating scale by evaluating the correlations between necrosis scores and electrolyte leakage, and between necrosis scores and internal bacterial populations. These parameters are associated with the hypersensitive reaction and are not subjective (11).

Growth curves of *X. c. vesicatoria* were very different in the parental genotypes. Five days after inoculation the bacterial population reached 5.7×10^7 cfu/cm^2 (SE 1.87×10^7) in LA 716 and only 5.2×10^4 cfu/cm^2 (SE 1.36×10^4) in H7998; the population level in the hybrid was 6.7×10^5 cfu/cm^2 (SE 2.75×10^5). Bacterial population levels in Bonny Best were very similar to those in LA 716. The apparent negative correlation between early necrosis score and population levels attained in the infected leaves of parental genotypes was tested in seven BCP1 segregants selected for their contrasting early score phenotypes: high (similar to H7998), medium, and low (similar to LA 716). Several cuttings of each segregant (more than six per test) were analyzed. The rank order correlation coefficient was calculated because it measures the monotonic association between variables that lack a linear association (16). Significant negative correlations ($r = -0.83$, $P = 0.01$) were detected between bacterial population levels measured 5 days after inoculation and scores assigned 32 h after inoculation and beyond.

Electrolyte leakage increased after inoculation to a greater extent in H7998 than in LA 716. Time-course curves showed that at 48 h after inoculation H7998 had three times as much electrolyte leakage as LA 716, while the F_1 was intermediate. Again, Bonny Best had a response similar to that of LA 716. The apparent correlation between early necrosis scores and electrolyte-leakage levels in the parental genotypes also was tested in the same BCP1 segregants. Significant positive correlations ($r = 0.86$, $P = 0.001$) were observed between electrolyte leakage measured 32 h after inoculation and scores assigned 16 h after inoculation and beyond.

Linkage relationships between isozyme markers and hypersensitive reaction genes. Scores from a rating scale commonly are used to evaluate and compare responses of different genotypes to pathogenic attack and, by extension, to evaluate progenies segregating for resistance. However, instead of scores, rates of score change were used in this analysis for several reasons. The necrosis observed in bacterial spot never develops beyond the inoculated area. The only visual distinction between H7998 and the wild species was the timing and rate of necrosis development in the inoculated area. Furthermore, the progenies analyzed (Fig. 1), and analysis of other progenies obtained from intraspecific crosses with H7998, clearly indicated the quantitative nature of the hypersensitive response. Necrosis in H7998 developed within a short period of time, whereas early necrosis development in

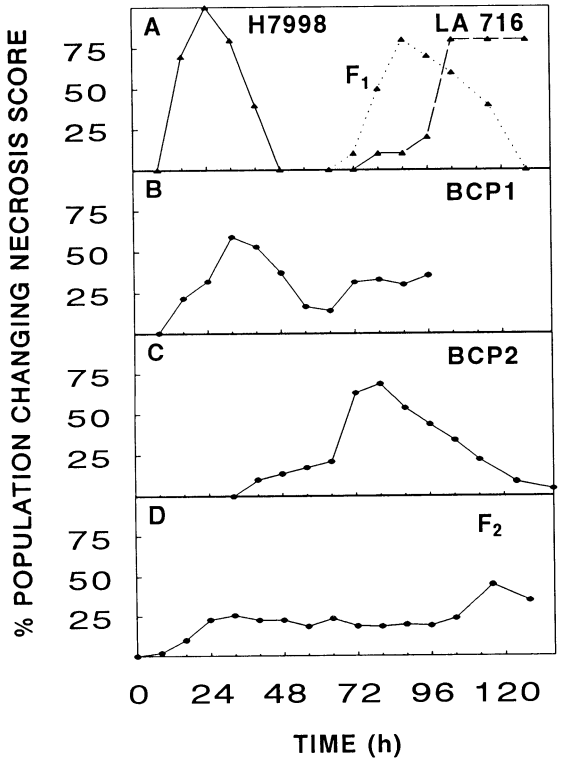

Fig. 1. Time-course changes in the fraction of the population displaying an increase in necrosis rating during the evaluation period: **A,** Hawaii 7998, LA 716, and their F_1 progeny (dotted line); **B,** backcross population to Hawaii 7998 (BCP1); **C,** backcross population to *Lycopersicon pennellii* (BCP2); and **D,** the F_2 progeny.

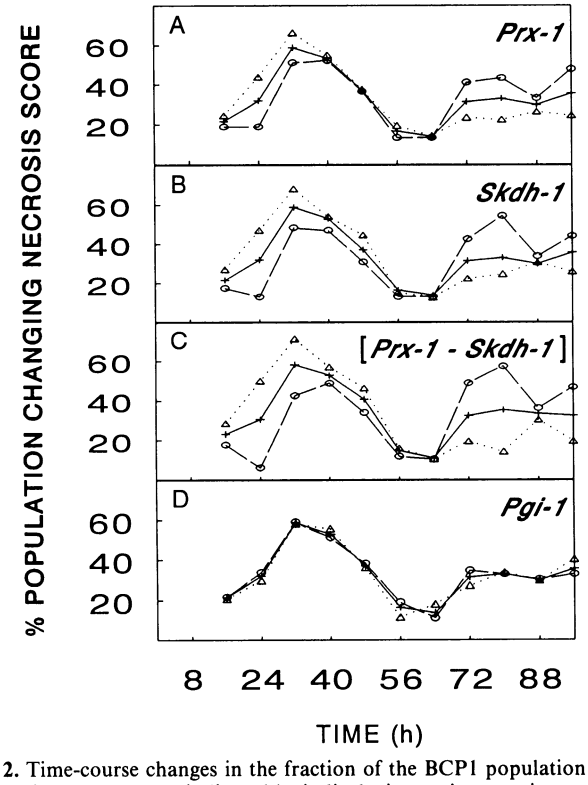

Fig. 2. Time-course changes in the fraction of the BCP1 population and genotypic classes, at the indicated loci, displaying an increase in necrosis rating during the evaluation period: **A,** *Prx-1*; **B,** *Skdh-1*; **C,** *Prx-1–Skdh-1*, parental classes ; and **D,** *Pgi-1*. Solid lines = entire population; dotted lines = percentage of all homozygotes for Hawaii 7998 alleles; dashed lines = percentage of all heterozygotes.

BCP1 and the F_2 appeared to occur during a longer period. This variation in the timing of necrosis development would not be observed in H7998, because the action of the earliest acting factor would preclude detection of late-acting factors. Therefore, using the rate of necrosis score change in each 8-h interval can facilitate the identification of factors active during that interval. In contrast, if the linkage analysis was performed using the scores, then segregants that attain a high score at an early stage would continue to be counted on the hypersensitive group at all times and might obscure the effect of other factors.

The average hourly rates of necrosis development (Rh_t) were calculated for each 8-h interval and were used to analyze the inheritance of the hypersensitive reaction. The rates permitted us to assess the extent of the change in necrosis ratings and the period of time in which significant change occurred. The small range of the necrosis rating scale (0–5) allowed for only five possible rates and, therefore, yielded a discrete distribution. This type of data precluded a statistical comparison of genotypic means (Student's t test or analysis of variance). For this reason, two-way contingency tables were used to compare the frequency distributions of Rh values between genotypic classes at each segregating marker locus. Plants were classified into two groups according to Rh value: 1) Rh = 0 (no change) and 2) Rh > 0 (some change). This classification was used to reduce the number of cells with small sample size (<5). The sample size of all cells was large enough (>30) to apply the central limit theorem (16) in the reciprocal backcrosses. If the marker locus was not associated with a hypersensitive response factor, then a homogeneous distribution of Rh values between the genotypic classes would be expected. Significant deviations from this pattern were interpreted as linkage. When linkage was detected, z tests were used to compare rate means. These tests provided information about both the magnitude of the effect and the mode of gene action inferred from the sign of the differences in rate means between genotypic classes. Analysis of BCP1 detected significant differences in frequency distributions for 12 of the 16 marker loci in at least one evaluation period. However, in light of the responses of the parental genotypes, an additional criterion must be met to assume linkage to a hypersensitive reaction factor: Significant differences in frequency distributions should be detected in at least two evaluation periods, and the difference of the mean rates for the genotypic classes should change sign between the two evaluation periods. Consider the behavior of the parental genotypes. Rates of the resistant genotype H7998 are >0 during the first 48 h after inoculation, whereas those of the susceptible genotype are 0 because necrosis does not develop in this genotype during this period. Subtracting the mean rate of the susceptible from the resistant genotype gives a positive value. In contrast, H7998 rates are 0 80 h after inoculation because confluent necrosis (100%) was reached earlier; however, this is the time when the susceptible genotype begins to develop necrosis in the inoculated area. Subtracting the mean rate of the susceptible from the resistant genotype gives a negative value at this time. Following this rationale, one morphological locus and five enzyme loci were detected in linkage to hypersensitive reaction-related genes (Table 5). They were *Prx-1* and *Skdh-1* on chromosome 1; *Pgm-2* on chromosome 4; *Got-2* on chromosome 7; *Pn* on chromosome 8; and *Sod-1* on chromosome 11. The effects of the genes linked to *Got-2* and *Pn* were observed during the 0- to 16-h period, those linked to *Pgm-2* and *Sod-1* during the 16- to 24-h period, and those linked to *Prx-1* and *Skdh-1* during the 16- to 24- and 24- to 32-h periods. Homozygotes for H7998 alleles at *Prx-1, Skdh-1-1, Got-2,* and *Pn* had significantly higher rates than did the heterozygotes. In contrast, heterozygotes at *Pgm-2* and *Sod-1* had significantly higher rates than did homozygotes. The effects detected by linkages to *Got-2, Pn,* and *Sod-1* were lower than those detected with the other linkages. None of the linkages detected in BCP1 were detected in the backcross to *L. pennellii* (BCP2). Nevertheless, two additional linkages were identified in this backcross (Table 6): *Est-7* on chromosome 2 and *Prx-4* on chromosome 10. The effect of both linked genes was observed during the 72- to 80-h period. During this period, the homozygotes

TABLE 5. Results of the linkage anlayses between marker loci and genes associated with the hypersensitive reaction in BCP1

Locus	Genotype[a]	Evaluation periods					
		Rh_{0-16}[b]	Rh_{16-24}	Rh_{24-32}	Rh_{40-48}	Rh_{64-72}	Rh_{72-80}
Prx-1	*e/e*		8.2	13.2		2.6	3.6
	e/p		2.6	7.3		5.7	8.3
	χ^2[c]		12.1****[d]	5.2*		8.3**	14.6****
	z[e]		3.9****	3.6***		−3.1***	−3.4***
Skdh-1	*e/e*		8.2	12.8		2.9	3.7
	e/p		2.6	7.3		5.6	6.0
	χ^2		19.0****	7.0**		5.7*	14.4****
	z		4.1****	3.4***		−2.6**	−4.4****
Pgm-2	*e/e*		3.9				6.7
	e/p		7.6				3.7
	χ^2		14.6****				5.6*
	z		−2.6**				2.5**
Got-2	*e/e*	3.0			3.9		
	e/p	1.4			6.0		
	χ^2	4.0*			5.8*		
	z	2.1*			−2.2*		
Pn	*e/e*	3.6				3.2	
	e/p	1.6				5.1	
	χ^2	6.7**				4.7*	
	z	2.0*				−1.8*	
Sod-1	*e/e*		4.0				8.2
	e/p		7.0				3.5
	χ^2		4.9*				11.9***
	z		−2.1*				3.5***

[a] Genotypes: *e/e* = homozygotes for *Lycopersicon esculentum* alleles; *e/p* = heterozygotes.
[b] The average rate of necrosis development (Rh = [increase necrosis rating × 10^3]/h) for each genotype.
[c] Comparison of the frequency distributions of Rh between genotypic classes. Plants were separated into two groups: one with no increase in necrosis rating and the other with an increase.
[d] *, **, ***, and **** significant at the 0.05, 0.01, 0.001, and 0.0001 levels, respectively.
[e] The z statistic was used to compare the mean rates of the two genotypes, $z = (\bar{y}_1 - \bar{y}_2)/\sqrt{s_1^2 + s_2^2}$.

at *Est-7* had significantly higher rates than did the heterozygotes, whereas the opposite was true for *Prx-4*.

Only linkages to *Prx-1* and *Skdh-1* were detected in the F_2 progeny (Table 7). The activities of the linked genes were detected in both cases between 24 and 40 h after inoculation, the time when confluent necrosis was observed in plants of H7998. Analysis of this progeny was limited to the use of contingency tables. No attempt was made to compare the rates of the genotypic classes because sample sizes of the homozygous groups were too small (<30) and the distribution too discrete to apply the central limit theorem (16). This problem resulted from the fact that, compared to BC_1, F_2 progenies have an additional genotypic class; in addition, the experimental F_2 progeny was half the size of the reciprocal backcrosses.

DISCUSSION

Results from the inoculation experiments showed that H7998 and LA 716 clearly differ in the rate and timing of necrosis development in response to inoculations with *X. c. vesicatoria* (Fig. 1A). In addition, population-level counts and electrolyte-leakage measurements were correlated with early necrosis score in the parental genotypes. The first two parameters are associated with the hypersensitive reaction (11) and were correlated with early necrosis scores in a sample of BCP1 segregants that were selected for their contrasting early necrosis scores. These results proved that the necrosis scores used in these analyses were reliable estimators of the hypersensitive reaction.

Comparisons of the time-course responses of the parental genotypes and those of the segregating progenies strongly suggest the hypersensitive reaction of H7998 was not inherited as a dominant trait. Although most reported resistances associated with hypersensitive reactions are controlled by a single dominant gene, recessive genes governing hypersensitive reactions have been reported in several pathosystems (6). The responses of both F_1 and BCP2 (the backcross to the susceptible parent) progenies were very close to that of the susceptible parent (LA 716) but not exactly the same. In contrast, approximately 60% of BCP1 plants (the backcross to the resistant parent) and 25% of the F_2 population displayed a response similar to that of the resistant parent. These results suggest a single recessive gene may control the hypersensitive reaction in H7998, and the results presented in Table 5 suggest that this gene is located on chromosome 1 near *Prx-1–Skdh-1*. However, if this were the case, plots similar to those from Figure 1 would show single peaks for each genotypic class of either *Prx-1* or *Skdh-1*. The plots in Figure 2A and B clearly show that this is not the case, because the profiles of the two genotypic classes still display two peaks like those detected in the entire BCP1 population. The homozygous class has a relatively greater proportion of plants developing necrosis within 48 h after inoculation than does the heterozygous class; this distribution is reversed at a later time. This phenomenon is not observed in plots of marker loci for which linkage was not detected (e.g., *Pgi-1*, Fig. 2D). It could be argued that the residual peaks of the *Prx-1* (or *Skdh-1*) genotypic classes represent recombinants between the marker and the hypersensitive reaction-controlling gene and that this gene lies between *Skdh-1* and *Prx-1*, because single-locus analysis indicated that *Bnag* was not linked to this factor. To remove the effect of putative recombinants between either marker and the hypersensitive reaction-controlling factor, necrosis activity of the parental genotypes for the locus pair *Prx-1–Skdh-1* was plotted (Fig. 2C). This plot reveals that the two genotypic classes, double homozygous and double heterozygous, still yield two peaks of necrosis development similar to those of the entire population. The proportion of individuals that yields the first peak in the heterozygous class exceeds what could be expected of double recombinants between the marker loci. Therefore, additional locus/loci control the hypersensitive reaction in H7998. Further evidence suggesting the presence of additional hypersensitive reaction factors is provided by linkages detected with two other independent marker loci.

The other two hypersensitive reaction-related factors linked to *Got-2* and *Pn*, detected in BCP1, had an effect similar to that produced by the *Prx-1–Skdh-1* linked gene. The small effect observed for these factors and the low level of significance of the statistical tests could be due to loose linkages between the markers and the factors. Failure to detect these linkages in the F_2 population could be due to a combination of loose linkages and greater variability expected in this progeny. For a trait controlled by *n* independent loci, the number of genotypes per class at each locus is a power base higher in the F_2 than in the BC_1 progeny (29). In addition, the F_2 was half the size of BCP1, consequently the sample size of some classes was too small and rendered the test results unreliable. The observation that the activities of the *Got-2*- and *Pn*-linked factors were detected 16 h after inoculation suggests that neither of these had a significant contribution to the major response observed in BCP1, because the peak of activity of the BCP1 population was detected 32 h after inoculation. Other hypersensitive reaction factor(s) located in regions of the genome not marked by the isozyme loci may have gone undetected in this survey.

Minsavage et al (15) demonstrated in peppers that each gene-for-gene system could yield hypersensitive reactions of different intensities. If several resistance genes are present in H7998, only the effect of the gene-for-gene system that gives the earliest and strongest hypersensitive reaction would be detected in H7998, but semiquantitative variation would be detected in populations segregating for genes of varying hypersensitive reaction intensities. It is possible that H7998 carries several independently acting genes responsible for the hypersensitive reaction to *X. c. vesicatoria*, and the strain used in these experiments also could have several *avr* genes capable of interacting with those present in H7998. Whalen et al (33) recently determined that *avrRxv* elicits a hypersensitive response in H7998. RFLP markers (26) that cover the entire tomato genome are being used to determine with greater accuracy the location of these genes and any others that may

TABLE 6. Results of the linkage analyses in BCP2 between marker loci and genes associated with the hypersensitive reaction

Locus	Genotype[a]	Evaluation period	
		Rh_{72-80}[b]	Rh_{96-104}
Est-7	*p/p*	17.5	3.5
	e/p	12.6	7.6
	χ^{2c}	6.5*[d]	6.4*
	z^e	2.6**	−3.3***
Prx-4	*p/p*	12.7	7.9
	e/p	18.1	3.9
	χ^2	4.2*	9.2**
	z	−2.5**	2.6**

[a] Genotype: *p/p* = homozygous for *Lycopersicon pennellii* alleles; *e/p* = heterozygotes.

[b] The average rate of necrosis development (Rh = [increase in necrosis rating × 10^3]/h) for each genotype.

[c] Comparison of the frequency distributions of Rh between genotypic classes. Plants of each genotype were separated into two groups: one with an increase in necrosis rating and the other with no increase.

[d] *, **, and *** significant at the 0.05, 0.01, and 0.001 levels, respectively.

[e] The *z* statistic was used to compare the mean rates of the two genotypes.

TABLE 7. Results of two-way contingency tables to evaluate the association between marker loci and genes associated with hypersensitivity in the F_2

Locus	χ^2			
	Rh_{24-32}[a]	Rh_{32-40}	Rh_{40-48}	$Rh_{116-128}$
Prx-1	7.3*[b]		8.8*	6.4*
Skdh-1	6.7*	7.6*	6.4*	

[a] Comparison of the frequency distributions of rates of necrosis development (Rh) between genotypic classes. Plants of each genotype were separated into two groups: one with an increase in necrosis rating and the other with no increase.

[b] * significant at the 0.05 level.

have gone undetected. Marker-assisted selection is being used to generate near isogenic lines for each of these markers in both H7998 and LA 716. The response of these lines will be evaluated with a virulent strain of *X. c. vesicatoria* (race 2, 89-1) that has been converted to avirulence on H7998 by transfer of *avrRxv* (33).

In contrast to the *Prx-1–Skdh-1–*, *Got-2–*, and *Pn*-linked factors, those linked to *Pgm-2* and *Sod-1* appeared to be dominant factors and, therefore, are carried by the susceptible *L. pennellii*; these factors seemed to require homozygous recessive factors from H7998 for detection because they were detected in BCP1 but not in BCP2, the backcross to the susceptible *L. pennellii*. Frequency distribution analysis indicated that the dominant factors did not have an effect on the action of the recessive factor, *Prx-1–Skdh-1* linked. As expected, contingency tables between either *Prx-1* or *Skdh-1* and *Pgm-2* did not reveal any significant skewing. However, if the dominant factors had affected the *Prx-1–Skdh-1*–linked factor, a significant excess of homozygotes at the *Prx-1* or *Skdh-1* locus and heterozygotes at the *Pgm-2* locus would have been detected in the category of plants that showed an increase in necrosis during the 24- to 36-h period. No significant skewing was detected for this genotypic class. Thus, the locus that interacts with the *Pgm-2*–linked factor remains unidentified. The ability of dominant factors to improve on the hypersensitive reaction of H7998 will be tested in near isogenic lines of H7998. Results from these tests also would have practical implications in a breeding program. They could open the possibility of transferring to modern cultivars, not only the major gene(s) responsible for hypersensitive reaction, but others that may improve performance. The fact that neither the F_1 nor the backcross (BCP2) to the susceptible genotype yielded progeny with a response identical to the susceptible genotype suggests that H7998 may carry a minor gene or genes with either incomplete dominance or a late-acting reduced response.

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