Accumulation of Minor Gene Resistance to *Peronospora trifoliorum* in Diploid Alfalfa

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ABSTRACT

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The inheritance of diploid *Medicago sativa* resistance to *Peronospora trifoliorum*, because of genes with small, cumulative effects, was studied. Infection types (ITs) were described on a 0 (no conidial production) to 5 (copious conidial production) scale. Plants P1 and P2, whose S_1 populations were 0 and 3% resistant (IT = 0) to isolate I-7, were crossed and their F_1 populations were selected for resistance to I-7. Progeny of the selected plants had increased resistance not only to I-7, but also to pathogenically different isolates I-5 and I-8, which suggested that the increased resistance was general in nature. Two F_1 plants of P1 and P2, whose S_1 populations were 59 and 37% resistant to I-7, were crossed. Two of their progeny, whose S_1 populations were 73 and 76% resistant to I-7, also were crossed. Both resistant and susceptible progeny of this cross were selfed. Segregation in S_1 populations of the resistant plants indicated that the apparent general resistance was due to a collection of isolate-specific interactions. Plants with 98% of their S_1 progeny resistant to I-7 were derived from P1 × P2 crosses. However, virtually none of this resistance was expressed in the progeny of a backcross to P1. These results indicated that high levels of minor gene resistance were derived from plants with no readily apparent resistance without fixing the genes for resistance, and that minor gene resistance generally was not expressed in progeny of a resistant-by-susceptible cross. Hence, the expression of minor gene resistance was dependent on genetic background and may have been masked by epistatic susceptibility.

Additional keywords: alfalfa downy mildew, quantitative resistance.

In previous research, we found evidence of five dominant genes that conditioned isolate-specific resistance to *Peronospora trifoliorum* d By. and were distributed between two diploid alfalfa (*Medicago sativa* L.) plants (13). Their effects were recognized by a complete inhibition of conidial production (13). These "major" genes were expressed only in response to inoculation with conidia of certain isolates (13). Therefore, we suggested (13) that *M. sativa* and *P. trifoliorum* share a gene-for-gene (4,10) relationship.

The major genes were not always expressed when they were present (13). In some cases, observed segregation ratios in F_1 populations of plants with the major genes differed from the expected ratios because of an excess of susceptible plants (13). This excess indicated that, in addition to the major genes, other genes were present whose effects nullified or modified the expression of the major genes. A significant, positive correlation of frequency of occurrence and mean infection type of susceptible plants in each F_2 population indicated that the effects of these "other" genes were additive (13). We will refer to host responses conditioned by genes with small, additive effects as "minor gene resistance." This is similar to the definition used by Edwards and Williams (3).

Further investigation revealed that the inheritance of minor gene resistance was complex. The graphical analysis of a six-parent diallel cross revealed that the most resistant parents also had the most dominant alleles (graphed closest to tbe origin of a V_r, W_r plot), indicating that resistance usually was dominant (14). However, the position of a parent on a V_r, W_r graph (a plot of variance of progeny means versus the covariance of progeny means with parental means) is influenced by nonallelic interactions, such as epistasis (1). In a host-parasite interaction, two kinds of epistasis exist (11). Category II epistasis operates among genes within an organism, either the host or the parasite (11). Category IV epistasis operates at the interorganismal level, that is, "epistasis" of the effects of one corresponding host resistance gene and parasite avirulence gene pair over the effects of other present corresponding gene pairs. Category IV epistasis of resistant interaction phenotypes generally is observed in most pathosystems (10). Thus, the position of the most resistant parents

close to the origin of the V_r, W_r graph may have indicated dominance and/or "epistasis" of corresponding gene pairs that resulted in expressed resistance.

In data sets derived by partitioning the six-parent diallel set, resistance did not always cosegregate with apparent dominance (14), suggesting that some resistance was recessive or that susceptibility was epistatic in some cases.

The objectives of this study were to seek further evidence of epistasis of susceptibility, to determine if minor gene resistance was isolate specific, and to determine how much minor gene resistance we could accumulate in a single plant.

MATERIALS AND METHODS

Three monoconidial isolates of *P. trifoliorum* from alfalfa were used: I-5 and I-7 from Kansas and I-8 from California. Monoconidial isolates were derived as described previously (13).

Two diploid alfalfa plants were used. Plant P1 was an individual plant grown from *M. sativa* seed lot PI172984; plant P2 was grown from seed lot PI172989. Seed was supplied by the U.S. Department of Agriculture, North Central Regional Plant Introduction Station, Ames, IA.

Seeds from crosses and selfs of these two plants were produced by hand pollination in a greenhouse. Flowers used as female first were emasculated with ethanol (18).

Procedures from the standard test to characterize downy mildew resistance in alfalfa (16) were used. Seeds were scarified with a razor blade and planted about 8 mm deep in autoclaved mason's sand in aluminum bread pans. Five days later, seedlings were sprayed with an aqueous suspension of 10^5 conidia/ml of water. The plants were enclosed in plastic boxes covered with aluminum foil and were maintained at 20 C for 24 hr. Then covers were removed, and continuous 98.2 μE sec^{-1} m^{-2} cool-white fluorescent light was provided. Six days later, the pans of plants again were enclosed in the aluminum foil-covered plastic boxes to provide the darkness and near 100% relative humidity needed for conidial production (5). Intensity of conidial production on the cotyledons was evaluated 15 hr later under 12 × magnification. The infection types (ITs) were described on a 0 to 5 scale, described elsewhere (14), where 0 = no conidial production and 5 = copious conidial production. Plants rated zero were classified as "resistant." Because of complications introduced by using this kind of scale (discussed below), we limited statistical comparisons to chi-square tests of homogeneity (15) on the two-category (resistant, susceptible) data.

To examine the inheritance of resistance from genes with small, quantitative effects, a crossing and selection scheme (Fig. 1) was initiated with plants P1 and P2, which expressed no major dominant resistance genes to the isolates we used (13). Plants P1 and P2 were crossed and 18 F_1 plants were progeny tested; that is, the F_1 plants were self-pollinated and the resistance of the F_2 plants to isolate I-7 was determined. Plants (P1 × P2)A and (P1 × P2)K, whose S_1 populations had the highest proportion of resistant plants, were crossed, and their F_1 plants were selected for resistance to I-7. Two of these plants, (A × K)B and (A × K)E, which produced large numbers of S_1 plants and crossed well, were crossed, and their progeny were selected for resistance or susceptibility to I-7. Then B × E plants were selfed, and their S_1 plants were examined for resistance to isolates I-5, I-7, and I-8.

To examine the expression of minor gene resistance in a resistant × susceptible cross, a resistant plant from the B × E cross was backcrossed to P1.

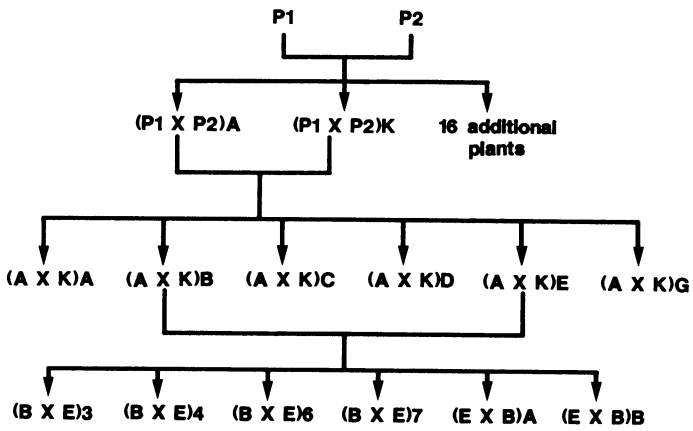

Fig. 1. Crossing scheme used to develop diploid alfalfa plants with minor gene resistance to *Peronospora trifoliorum*. Plants (E × B)A, (B × E)4, and (B × E)6 were selected for susceptibility to isolate I-7. All other descendants of P1 and P2 were selected for resistance to isolate I-7.

RESULTS

All S_1 plants of plant P1 were susceptible to isolate I-7 (Table 1) and were rated as IT 4. Extensive self-pollination of four S_1 plants of P1 yielded 64 S_2 plants, all of which exhibited an IT 4 upon interaction with isolate I-7 (data not shown). Thus, P1 apparently lacked alleles that conditioned resistance to isolate I-7.

Three percent of the S_1 population of plant P2 was resistant to I-7 (Table 1). Therefore, P2 had some alleles that resulted in resistance to I-7 when in particular combinations. A similar proportion of S_1 plants of P2 were resistant to isolates I-5 and I-8 (Table 1). Because the reciprocally produced F_1 populations of P1 and P2 did not differ significantly, the data from the reciprocal crosses were bulked.

About 5% of the F_1 population of plants P1 and P2 was resistant to I-7, 1% was resistant to I-5, and 3% was resistant to I-8 (Table 1).

F_2 populations, derived by selfing F_1 plants resistant to isolate I-7, indicated that plants (P1 × P2)A and (P1 × P2)K were the most resistant to I-7 (Table 1). Plant (P1 × P2)A produced an F_2 population with a significantly lower proportion of plants resistant to isolate I-5 than to I-7 or I-8 (Table 1). Nonetheless, there was a marked increase in the proportion of plants resistant to each isolate, relative to the population from which the F_1 plants were selected.

Full sibs (P1 × P2)A and (P1 × P2)K were crossed, and six progeny resistant to isolate I-7 were selected. S_1 plants were produced from each of these plants for evaluation of resistance to each of the isolates. However, two of the plants were poorly self-fertile and did not produce enough S_1 seed for evaluation with each of the three isolates. In three of the four populations that were evaluated with each isolate, a significantly higher proportion of plants was resistant to I-7 than to I-5 and/or I-8 (Table 1).

Full sibs (A × K)B and (A × K)E were crossed. Three progeny plants were selected for susceptibility, and three were selected for resistance to isolate I-7. Of the three selected for susceptibility, the most obvious increase in susceptibility was expressed by plant (E × B)A, whose IT was 3. This plant produced an S_1 population with very few plants resistant to any isolate (Table 1). The two other plants selected for susceptibility, (B × E)4 and (B × E)6, each expressed an IT of 2 and produced S_1 populations that were not significantly more susceptible to I-7 than the S_1 populations of their immediate parents, (A × K)B and (A × K)E. However, they were significantly more susceptible to isolates I-5 and I-8 (X^2 values not shown).

F_1 plants from the cross of (A × K)B and (A × K)E, which were selected for resistance to I-7—plants (E × B)B, (B × E)3, and (B × E)7, Table 1—produced S_1 populations that had high proportions of plants resistant to I-7 but significantly lower proportions of plants resistant to isolates I-5 and I-8, in most cases (Table 1).

Ninety-eight percent of the S_1 population of (E × B)B was resistant to isolate I-7. This plant was backcrossed to P1. In the resulting population, 9% was resistant to isolate I-5, 14% was resistant to I-7, and 0% was resistant to I-8 (Table 1).

DISCUSSION

The scale that we used for conidial production ratings was

TABLE 1. Reactions to three isolates of *Peronospora trifoliorum* by S_1, F_1, and backcross populations of diploid alfalfa plants selected for resistance or susceptibility to isolate I-7

S_1 or cross[w]	Isolate	No. of plants	Percentage of plants with infection type (IT)[x] 0	1	2	3	4	5	X^2 separation[y]
P1	I-5	82	0	0	0	0	100	0	a
	I-7	76	0	0	0	0	100	0	a
	I-8	117	0	0	0	0	100	0	a
P2	I-5	121	4	4	9	64	17	2	a
	I-7	172	3	7	12	74	1	2	a
	I-8	83	1	6	1	89	2	0	a
P1 × P2	I-5	217	1	6	22	30	39	1	a
	I-7	392	5	6	8	10	29	40	a
	I-8	132	3	5	29	10	52	0	a
(P1 × P2)A	I-5	66	26	8	9	29	27	1	a
	I-7	93	59	5	14	14	5	1	b
	I-8	38	37	5	21	16	21	0	b
(P1 × P2)K	I-5	21	19	5	9	48	9	9	a
	I-7	30	37	3	33	20	7	0	a
	I-8	29	21	0	7	41	31	0	a
(P1 × P2)A × (P1 × P2)K	I-5	43	28	7	35	19	12	0	a
	I-7	43	28	7	30	21	14	0	a
	I-8	35	37	9	23	14	17	0	a
(A × K)A	I-5	...	...	...	...	...	...	...	
	I-7	27	67	0	18	15	0	0	a
	I-8	7	57	0	14	28	0	0	a
(A × K)B	I-5	70	56	7	24	11	1	0	a
	I-7	140	73	7	13	6	0	0	a
	I-8	78	70	13	8	8	1	0	a
(A × K)C	I-5	67	75	7	10	7	0	0	a
	I-7	64	95	5	0	0	0	0	b
	I-8	64	72	16	12	0	0	0	a
(A × K)D	I-5	62	95	1	1	1	0	0	a
	I-7	84	98	1	1	0	0	0	a
	I-8	76	71	10	10	5	3	0	b
(A × K)E	I-5	57	67	9	14	10	0	0	a
	I-7	109	76	10	8	4	1	0	a
	I-8	107	50	9	17	18	6	0	b
(A × K)G	I-5	...	...	...	...	...	...	...	
	I-7	25	100	0	0	0	0	0	a
	I-8	19	63	16	21	0	0	0	b
(A × K)B × (A × K)E	I-5	61	58	11	14	16	2	0	a
	I-7	29	83	7	0	7	3	0	b
	I-8	45	7	20	27	38	9	0	c
(E × B)A[z]	I-5	30	7	0	37	40	17	0	a
	I-7	35	11	20	23	31	14	0	a
	I-8	23	13	13	30	35	9	0	a
(E × B)B	I-5	53	73	6	15	6	0	0	a
	I-7	70	98	2	0	0	0	0	b
	I-8	45	58	15	11	13	2	0	a
(B × E)3	I-5	9	22	11	22	33	11	0	a
	I-7	11	82	9	0	9	0	0	b
	I-8	15	13	7	33	33	13	0	a
(B × E)4[z]	I-5	32	12	3	28	47	9	0	a
	I-7	45	51	15	20	11	2	0	b
	I-8	40	37	10	35	15	2	0	b
(B × E)6[z]	I-5	53	38	7	24	28	2	0	a
	I-7	45	78	9	13	0	0	0	b
	I-8	54	18	5	33	31	11	0	a
(B × E)7	I-5	54	35	13	30	15	7	0	a
	I-7	56	93	5	2	0	0	0	b
	I-8	51	47	10	27	10	6	0	b
(E × B)B × P1	I-5	23	9	0	17	30	35	9	a
	I-7	49	14	8	24	35	18	0	a
	I-8	31	0	0	0	19	64	16	a

[w] Plants below dashed line were progeny of cross immediately above the line, indicated by portion of the name of the plant in parentheses.

[x] IT 0 = no conidia produced. Numbers of conidia produced per seedling in infection types 1 through 5 were ($\times 10^{-3}$): IT 1 = 0.027–0.135, IT 2 = 1.4 ± 0.6, IT 3 = 21 ± 16, IT 4 = 80 ± 11, and IT 5 = 185 ± 34.

[y] IT distributions followed by the same letter were not significantly different according to a chi-square test on the categorical data. Comparisons were made only among isolates within populations.

[z] (E × B)A, (B × E)4, and (B × E)6 selected for susceptibility to isolate I-7.

arbitrary. We agree with Edwards and Williams (3) that such a scale may not accurately reflect the biological scale underlying the expression of gene effects. They (3) have shown that the same quantitative data, expressed on different scales, can lead to very different conclusions. The appropriate scale in the present study was not apparent. Therefore, we limited statistical comparisons to the qualitative difference of asexual parasite reproduction present (plant susceptible) versus asexual parasite reproduction absent (plant resistant). This method is more conservative than a six-category comparison or a quantitative analysis even if the appropriate scale was known because all susceptible plants are assumed genetically similar, as are all resistant plants. Therefore, the number of genetic differences indicated (Table 1) is the minimum.

Because no resistance was detected in S_1 or S_2 plants of P1, it appeared that all of the resistance in the plants included in this study was derived from plant P2. Possibly, P1 had genes or gene combinations that conditioned susceptibility that was epistatic to resistance. In S_1 populations of P2, the proper combination of alleles necessary to express resistance to I-7 occurred in only 3% of the plants. Plants whose S_1 populations were nearly all resistant were obtained by selecting for resistance in the progeny of full-sib matings. Thus, these plants were selected from initially rare phenotypes and from increasingly inbred populations. Both the selection of rare phenotypes and inbreeding increase homozygosity (9). Therefore, plants (A × K)B and (A × K)E should have been homozygous at many of the loci controlling reactions to isolate I-7. However, a single cycle of selection for susceptibility in the F_1 population of (A × K)B and (A × K)E yielded plant (E × B)A, which had very little resistance to any isolate (Table 1). Therefore, the genes responsible for minor gene resistance were not homozygous, suggesting that some of the alleles that conditioned resistance were at least partially dominant but were not expressed in S_1 plants of P1 and only rarely in S_1 plants of P2. This implied that genes, or gene combinations, in P1 and P2 conditioned susceptibility, which was epistatic to resistance. This epistasis may be in category II or category IV (11). The near total loss of resistance as a result of the (B × E)B × P1 backcross (Table 1) was consistent with the hypothesis of epistatic susceptibility in P1.

The selections for resistance to isolate I-7 yielded plants that produced S_1 populations with increased proportions of plants resistant to all three fungus isolates tested, suggesting that the minor gene resistance was general in effect. However, some selections produced S_1 populations with significantly lower proportions of plants resistant to isolates I-5 and/or I-8 than to I-7, indicating that some of the resistance was isolate specific. In fact, it is very likely that all of the resistance was isolate specific and that the apparently general resistance was due to an accumulation of isolate-specific effects. Isolate specificity of minor gene resistance has been demonstrated in several pathosystems and has been discussed as part of a review by Johnson (6).

Our investigations of minor gene resistance, following the scheme outlined in Figure 1, yielded the following results:

1. It was possible to select extremely high levels of resistance from plants that expressed virtually no resistance. This result, considered alone, is explicable by an hypothesis of recessive resistance genes with small, quantitative effects and a threshold effect necessary for observed resistance.

2. The above hypothesis was not supported by selection for susceptibility from plants with minor gene resistance. If recessive genes were the only genetic factors involved in resistance, then resistance would have been fixed in the resistant plants. It was not.

3. The above two results, considered together, implied that resistance was due to alleles showing some dominance. However, all of the plants involved in this study were direct descendants of P1 and P2, in which no, and virtually no, resistance was detected, respectively (Table 1). Thus, resistance was present in P1 and P2, was not expressed, and was not explicable by recessive alleles. Therefore, we suggest that epistatic susceptibility was present in P1, and probably in P2.

This kind of epistasis may be thought of as inhibition of resistance. This has been observed in other pathosystems and has been reviewed by Crute (2). However, the present case seems to be less explicable than those documented examples. In our study, resistance apparently was inhibited, then, as a result of selection, was expressed, and then, as a result of further selection, was again inhibited. The last step implied that susceptibility was inhibited in the plants selected for minor gene resistance. All of this occurred in plants that apparently were quite homozygous; thus, few genes were actually segregating. Therefore, we suggest that selection in this pathosystem at this level of inbreeding, in plants with no major resistance genes, may have acted on regulatory loci or on structural genes in a complex pathway necessary for resistance expression. Resistance is a complex phenomenon involving the products of many genes (17). Therefore, it is unlikely that the entire set of genes necessary for resistance could have been assembled from the genotypes of P1 and P2, if the set was not already more or less in place. It seems much more likely that selection acted on a few regulatory genes, or key structural genes, that had been responsible for the inhibition of resistance. P1 apparently had only alleles that resulted in the inhibition of resistance, as evidenced by the near total loss of resistance in the (B × E)B × P1 backcross (Table 1). Thus, successful selection back to susceptibility would require a change at one, or a few, loci. Tepper and Anderson (17) have discussed the possibility that selection acts on regulatory loci.

Edwards and Williams (3) investigated minor gene resistance of *Brassica campestris* L. to *Albugo candida* (Pers. ex Chev.) Kuntze. Their study (3) was limited to a single fungus isolate and, therefore, did not address the question of isolate specificity, but otherwise it was similar to ours. Their investigation (3), and other investigations (7,8,12), yielded results similar to ours for each isolate. This similarity of genetic effects involved in minor gene resistance in unrelated pathosystems suggests that minor gene resistance behaves similarly wherever it occurs.

We conclude that the resistance of alfalfa to *P. trifoliorum* is a complex phenomenon involving genes that are distinguishable by their individual effects and are probably structural (genes PtR_1 – PtR_5 [13]) and genes with small, cumulative, isolate-specific effects, which may be structural or regulatory. A complex system of category II and/or category IV epistasis is present, in which susceptibility may be epistatic in some genotypes and resistance may be epistatic in other genotypes. This complex system results in the appearance of a large amount of genetic diversity, whereas the actual number of genes may not be exceptionally large.

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