

## Clubroot Resistance and Linkage in *Brassica campestris*

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### ABSTRACT

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The genetics of resistance in *Brassica campestris* to *Plasmodiophora brassicae* race 6 (defined with the Williams differential set) and possible linkage of genes for resistance with genes controlling phenotypic markers were investigated with rapidly flowering stocks. Two *B. campestris* members of the European Clubroot Differential (ECD) host series and stocks derived from *B. campestris* ssp. *pekinensis* 'Michihili' were sources of clubroot resistance genes. The resistant reaction in Michihili stocks was due

to a single dominant gene, designated *Pb1*. The resistant reaction in ECD stocks was due to more than one independent dominant gene in each stock. The two single dominant genes *Pb2* and *Pb3* were identified from ECD 02 and 03, respectively. *Pb1*, *Pb2*, and *Pb3* were unlinked. Four recessive marker genes were not closely linked with any of the three genes for resistance although loose linkage may occur between *PB3* and *pkl*, and *Pb2* and *ro*.

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Clubroot is a particularly serious disease in the cole crops (*Brassica oleracea*), swedes (*B. napus*), oriental greens (*B. campestris* ssp. *pekinensis* and *chinensis*), and turnips (*B. campestris* ssp. *rapifera*). The clubroot pathogen, *Plasmodiophora brassicae* Wor., exhibits a high degree of pathogenic specialization on various crucifers. Ignorance of the mechanism of genetic recombination in *P. brassicae* is due, in part, to incomplete understanding of the life cycle (1), and in part to a lack of stocks suitable for the genetic analysis of this obligately biotrophic parasite. Genetic stocks bearing single genes for resistance to

clubroot would be useful for identifying pathogenic specialization within *P. brassicae* populations. Such stocks also would facilitate genetic studies in the pathogen and would enable crucifer breeders to examine the action of resistance genes in the host.

Genetic studies of *Brassica* spp. indicate that resistance in *B. oleracea* is under oligogenic or polygenic recessive control, whereas in *B. campestris* and *B. napus*, resistance is primarily under dominant monogenic control (2,4,7,9,14,18,20). Dominant forms of resistance found in European turnips have been useful in breeding programs and, in a number of cases, resistance from *B. campestris* has been incorporated into *B. napus* via interspecific introgression (8,10). Lines of Dutch stubble turnips with two or more dominant resistance genes resulting in differential interactions to *P. brassicae*

populations in European soils have been used as part of the European Clubroot Differential (ECD) series for characterizing pathogenic specialization in *B. campestris* (3,20).

Misclassification caused by susceptible plants escaping infection is a major problem in scoring seedling progenies segregating for resistance and susceptibility to *P. brassicae*. This problem might be minimized if distinctive phenotypic markers could be identified which were closely linked to specific genes governing clubroot resistance. A knowledge of close linkages also would be of considerable value in identifying the transfer of resistance genes in interspecific and intergeneric crosses. As part of our program on the genetics of *B. campestris*, the linkage relations between genes for clubroot resistance and several phenotypic markers were examined.

## MATERIALS AND METHODS

Stocks derived from *B. campestris* ssp. *pekinensis* 'Michihili' (14) and two members of the ECD series, ECD 02 and ECD 03 (*B. campestris* ssp. *rapifera* lines AAbbCC and AABBcc, respectively [3]) were used as sources of resistance to *P. brassicae* race 6. Crosses were made to isolate single dominant genes for clubroot resistance and to develop stocks homozygous for these genes. Pairs of plants homozygous for potentially different genes were crossed, the F<sub>1</sub> plants were self-pollinated, and the F<sub>2</sub> populations were indexed for reaction to *P. brassicae* race 6 to determine if different genes at different loci had been identified.

*Brassica campestris* plants expressing variant phenotypes controlled by four single recessive genes were chosen from selections from accessions of the United States Plant Introduction Service made by P. H. Williams and B. J. Cours, Department of Plant Pathology, University of Wisconsin-Madison. The marker genes included *cr* (cream corolla—corolla cream-colored rather than the wild-type bright yellow, from PI 175054); *nsep* (narrow sepals—sepals involute in mature bud giving narrow tubular appearance, from PI 183395 × PI 175079); *pk1* (puckered leaf—leaves with numerous concave depressions in interveinal tissue, from PI 175054); and *ro* (rosette—internodal elongation absent, from PI 183395 × PI 175079).

The original inoculum source was roots of cabbage (*B. oleracea* var. *capitata*), infected with *P. brassicae*, obtained from a field at Bear Creek, WI. Inoculum was stored at -23 C. Additional inoculum was obtained by inoculation of susceptible host plant cultivars Michihili and *B. oleracea* var. *capitata* 'Jersey Queen'.

To prepare inoculum, infected roots were ground in a Waring blender followed by filtration through cheesecloth. The filtrate was then centrifuged at 1,020 g for 5 min and resuspended in water three times. The suspension was quantified by means of a bright-line hemocytometer and stored at 4 C until used (19).

Plants were grown at 21 ± 2 C under continuous illumination of 16,000 lux from mixed Sylvania Cool White and GroLux (1:1) fluorescent light. Seedlings germinated in quartz sand were rinsed in water, dipped in a suspension containing 5 × 10<sup>8</sup> resting sporangia per milliliter, and planted in a pasteurized mix of sphagnum peat and organic muck soil (1:1, v/v) in 5.5-cm-diameter plastic pots or 7.0-cm-diameter clay pots. Four plants were placed in each pot and the pots were placed in a 5-cm-deep bed of sphagnum peat.

The inoculum was classified by using two sets of differential host plants. Tests with Williams' set confirmed the sample of inoculum to be *P. brassicae* race 6 (19). Using the denary classification system of the ECD host series (3) it was designated as race 16/02/30.

Self, F<sub>1</sub>, and testcross seed was obtained by pollination with forceps. Self-fertilizations were made by bud pollination (12). Emasculation generally was performed in crosses because of the variable amount of self-compatibility present in the *B. campestris* stocks used. F<sub>2</sub> seed was obtained by self-pollination of individual plants or by full-sib crosses among several F<sub>1</sub> individuals achieved by pollen transfer with a camel's-hair brush.

Crosses were made to study the relationships between the following pairs of genes: *cr*, *Pb2*; *cr*, *Pb3*; *nsep*, *Pb3*; *pk1*, *Pb1*, *pk1*, *Pb2*; *pk1*, *Pb3*; *ro*, *Pb1*; and *ro*, *Pb2*. Plants homozygous for each of

the resistance genes were used as female parents in crosses with plants containing the marker genes. Susceptible plants homozygous for the marker genes were chosen from clubroot screens and used as pollen parents in these crosses.

To eliminate the possibility that the resistant parents were heterozygous for the recessive marker gene being studied, F<sub>1</sub> plants from these crosses were checked for marker expression, but were expected to be resistant to clubroot and thus were not screened for disease reaction. Those showing the wild-type phenotype for the recessive marker gene were used as female parents in backcrosses with plants homozygous for the marker gene and susceptible to *P. brassicae* race 6.

Backcross populations were inoculated with *P. brassicae* race 6 and indexed for disease reaction. Plants were classified according to marker phenotype at an appropriate stage of development and were rated for disease 22–28 days after inoculation. The disease rating system used was a modification of that of Seaman et al (13) and consisted of four categories of disease severity: 0, no symptoms; 1, very small nodular galls on lateral roots; 2, larger galls on lateral roots or slight swelling of the main root; and 3, substantial swelling of the main root of the plant. Classes 0 and 1 were considered resistant and classes 2 and 3, susceptible. Chi-square tests were performed on the data to determine if significant deviation occurred from the expected ratios for independent segregation of two genes, to determine if either of the two single factor ratios in each population (segregation for the marker regardless of the disease reaction, or segregation for the disease reaction regardless of marker phenotype) varied significantly from the expected, or if linkage between the two genes was indicated (11). Homogeneity  $\chi^2$  values were determined for data from different populations representing the same cross and the data were pooled where differences significant at *P* = 0.05 were not detected.

## RESULTS

**Identification of clubroot resistance genes.** Analyses of testcross and F<sub>2</sub> data confirmed previous tests (14) which indicated that one single dominant gene (*Pb1*) controlled the resistance to *P. brassicae* race 6 reported in stocks derived from cultivar Michihili of *B. campestris* ssp. *pekinensis*. Crosses to susceptible plants suggested that resistance to *P. brassicae* race 6 was controlled by more than one independent single dominant gene in the ECD 02 and ECD 03 stocks. When plants homozygous for *Pb1*, *Pb2*, or *Pb3* were crossed in all possible combinations, the F<sub>2</sub> progeny from each cross showed segregation for resistant and susceptible plants (Table 1), indicating that the three genes were distinct (a lack of segregation in the F<sub>2</sub> progenies from two of the parents would have indicated both sources of resistance were at the same genetic locus).

**Linkage studies.** Joint segregation  $\chi^2$  values for data from crosses with *cr* and *Pb2* or *Pb3* did not indicate evidence for linkage (Table 2). Chi-square values indicated a good fit to the expected ratios for independent segregation of *cr* and *Pb2*. Data for *cr* and *Pb3* deviated significantly from the expected. Although the data did not support the hypothesis of independent segregation of *cr* and *Pb3*, this was not interpreted as due to linkage, since only one parental type (resistant with wild-type flowers) predominated, and not both, as would be expected if the genes were linked. The deviation was most likely due to accidental self-pollination of the

TABLE 1. Summary of F<sub>2</sub> data from tests for allelism between three potentially different genes for resistance to *Plasmodiophora brassicae* race 6, *Pb1*, *Pb2*, and *Pb3* in *Brassica campestris*

Genes	Number of plants with clubroot reaction of:				$\chi^2$ <sup>a</sup>	<i>P</i> <sup>a</sup>
	0	1	2	3		
<i>Pb1</i> , <i>Pb2</i>	218	0	5	16	2.66	.25-.10
<i>Pb2</i> , <i>Pb3</i>	58	0	3	3	1.07	.50-.25
<i>Pb1</i> , <i>Pb3</i>	258	0	5	8	0.96	.50-.25

<sup>a</sup>Based on an expected ratio of 15R:1S with classes 0 and 1 grouped as resistant and classes 2 and 3 grouped as susceptible.

F<sub>1</sub> plants when they were backcrossed to the clubroot-susceptible parent containing *cr*.

Narrow sepals (*nsep*) and clubroot resistance (*Pb3*) were apparently each governed by a single gene, and were not linked (Table 2).

Ratios for crosses pairing *pk1* with each of the clubroot resistance genes deviated significantly from those expected for independent segregation of two genes. In all cases, more plants had the puckered leaf characteristic than had been expected and this unexpected inheritance of puckered leaf appeared to cause the deviation rather than close linkage of *pk1* with *Pb1*, *Pb2*, or *Pb3*. Genes *Pb2* and *pk1* may be loosely linked (Table 2), with a recombination fraction, *p*, of 0.45. Segregation of *Pb2* or *Pb3* alone fit expected ratios of 1:1, but segregation of *Pb1* did not.

In crosses involving *ro* and *Pb1* or *Pb2*, *ro* appeared to be loosely linked with *Pb2* (recombination fraction, 0.46), but not with *Pb1*. There was no evidence to reject the hypothesis that clubroot resistance was due to a single dominant gene in each case.

## DISCUSSION

Understanding of the mechanisms underlying variability shown by *P. brassicae* is limited by our lack of knowledge of genetics of the pathogen and a lack of genetic stocks with single genes for resistance to clubroot. Development of *B. campestris* stocks with single resistance genes to one population of *P. brassicae* and investigation of linkage relationships of host resistance factors are important first steps in studying the genetics of host resistance and pathogen variation.

Although the ECD *B. campestris* stocks had genotypes assigned based on their reaction to European *P. brassicae* populations (3,20), these were not assumed to apply to the North American *P. brassicae* population used in this study. The genes designated *Pb2* and *Pb3* may therefore be equivalent to genes previously named although not studied individually. Use of stocks containing single genes as defined by differential hosts could separate field populations of the pathogen into their constituent races, and provide a more accurate assessment of pathogen variation. Pathogen races selected by their interaction with specific genes could then be used to study the properties of additional host genes for resistance and to assess the capacity for variability within *P. brassicae* populations.

In addition to use in differential hosts, *Pb1*, *Pb2*, and *Pb3* may be useful as sources of resistance in commercial cultivars of *B. campestris* or other *Brassica* crops since the ease with which many species of *Brassica* intercross makes possible interspecific gene transfer (6). The usefulness of single genes, however, is dependent upon the variability within pathogen populations and the capacity for variation in the pathogen.

Marker genes serve in genetic studies to mark new cultivars, to demonstrate certain types of gene action, and to monitor pollen contamination and interspecific gene transfer (15,16). Dominant factors expressed at an early stage of plant development are most useful if expression of the marker gene is used to indicate the presence of a resistance factor to which it is linked.

The recessive genes used in this study were chosen because their expression was generally reliably recognized and basic information about the genetics of *B. campestris* could be gained from them. Rosette (*ro*) is easily recognized, but may be difficult to work with due to detrimental effects of the gene. Deviation from the expected 1:1:1:1 segregation ratio in crosses involving *ro* and *Pb1* or *Pb2* was primarily due to the shortage of rosette plants in backcross populations. Decreased stem elongation slowed seedling emergence, increased chances of loss during inoculation, and put the very short plants at a competitive disadvantage with normal plants, resulting in a scarcity of rosette plants. Puckered leaf (*pk1*) is expressed over most of the period of plant development, making it potentially useful in stocks which do not have factors that complicate its inheritance. Highly significant deviation for inheritance of *pk1* made it difficult to determine if indications of linkage were accurate. Since the wild-type plant was used as the female parent, self-pollination would not result in an increase of plants expressing the puckered leaf characteristic. Additional genes producing a phenotype similar to *pk1* in the resistant parent or partial expression of the *pk1* gene in the heterozygous condition in a new genetic background could have contributed to the deviation. Cream corolla (*cr*) and narrow sepals (*nsep*) although generally easily distinguished in the proper genetic background, are less useful because of their late expression in plant development.

Data from crosses between the four marker genes and the genes for clubroot resistance indicated two possibilities of loose linkage, between *Pb3* and *pk1*, and *Pb2* and *ro* (Table 2). In both cases, highly significant deviation occurred in the observed segregation ratios for each marker, thus the suggestion of linkage is not conclusive. Data indicating that *Pb1*, *Pb2*, and *Pb3* were three genes for resistance to *P. brassicae* race 6 at three different loci (Table 1) also suggested that these genes were not closely linked, since the segregation expected for independent genes did occur. Linkage relationships between the marker genes used in this investigation (*cr*, *nsep*, *pk1*, and *ro*) have not been studied and populations obtained from several of the crosses were too small to detect anything but very close linkage, so it is reasonable that no closely linked genes were identified from the small sample of gene combinations investigated in the present study. Increased knowledge of the inheritance of the markers, the presence of genes in other stocks expressing phenotypes similar to the markers used, as well as effects of modifying genes in different genetic backgrounds is needed.

TABLE 2. Pooled data from backcrosses to detect linkage between four recessive marker genes and three genes controlling resistance to *Plasmodiophora brassicae* race 6 in *Brassica campestris*

Genes	Number of plants observed with phenotype <sup>a</sup>								Segregation for two factors (expected ratio 1:1:1:1)		Segregation for marker gene (expected ratio -- 1:1)		Segregation for clubroot reaction (expected ratio -- 1:1)		Joint segregation <sup>b</sup>	
	wild type				variant				$\chi^2$	<i>P</i>	$\chi^2$	<i>P</i>	$\chi^2$	<i>P</i>	$\chi^2$	<i>P</i>
	0	1	2	3	0	1	2	3								
<i>Pb2, cr</i>	71	0	23	54	75	0	23	35	3.11	.50-.25	0.80	.50-.25	0.43	.75-.50	1.88	.25-.10
<i>Pb3, cr</i>	103	0	57	34	80	0	29	31	11.99	.01-.005	8.73	<.005	3.06	.10-.05	0.19	.75-.50
<i>Pb3, nsep</i>	131	0	51	74	132	0	47	78	0.33	.975-.95	0.002	.95-.90	0.33	.75-.50	0.002	.95-.90
<i>Pb1, pk1</i>	41	0	13	35	124	0	28	51	60.53	<.005	46.40	<.005	5.52	.025-.01	8.62	<.005
<i>Pb2, pk1</i>	52	0	22	29	95	0	31	63	25.34	<.005	25.33	<.005	0.01	.95-.90	0.00	1.0
<i>Pb3, pk1</i>	67	0	28	20	96	0	50	57	27.38	<.005	24.35	<.005	0.20	.75-.50	2.83	.10-.05
<i>Pb1, ro</i>	213	0	17	203	99	0	42	90	64.88	<.005	61.45	<.005	2.41	.25-.10	1.02	.50-.25
<i>Pb2, ro</i>	94	0	34	40	40	0	24	18	32.82	<.005	29.58	<.005	1.30	.50-.25	1.94	.25-.10

<sup>a</sup>Classes 0 and 1 were grouped as resistant and classes 2 and 3 as susceptible for chi-square analysis.

<sup>b</sup>Based on the expected backcross ratio of 1:1 for plants expressing parental phenotypes (resistant, wild type; or susceptible, variant type) and non-parental phenotypes if the two factors segregate independently. Rejection of this hypothesis due to a low value of *P* would indicate linkage.

Knowledge of the basic genetics of *B. campestris* can be gained from studies of other combinations of the marker and resistance genes used in this study and their relationships with linked marker genes reported in this species (5,16,17). Increased knowledge of basic host genetics will provide a better context in which to study resistance genes. Identification, inheritance, and transfer of resistance genes would be facilitated by knowledge of their linkage relationships. This increased knowledge of specific resistance genes would increase their usefulness to study and differentiate pathogen populations, leading to greater understanding of the genetics of resistance and pathogenicity in this host-pathogen combination.

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