Molecular Mapping of a Locus Controlling Resistance to *Albugo candida* in *Brassica rapa*

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ABSTRACT

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White rust, caused by *Albugo candida*, is an economically important disease of crucifers. Genetic analysis for resistance to race 2 of *A. candida* in an F_2 population and a set of F_3 families both derived from a cross between *Brassica rapa* cultivars Per (resistant) and R-500 (susceptible) revealed that resistance is controlled by a dominant allele at a single locus. White rust resistance was associated with leaf pubescence, which also was governed by a dominant allele at a single locus. The resistance locus (*ACA1*) was mapped by linkage analysis with 144 restriction fragment length polymorphism (RFLP) loci segregating among the F_3 families. The *ACA1* locus was mapped to linkage group 4 and was flanked by RFLP marker loci *ec2b3a* (5.4 centimorgans [cM]) and *wg6c1a* (5.0 cM). *ACA1* was linked to the leaf pubescence locus *PUB1* by 13.3 cM. The linked RFLP markers and leaf pubescence may be useful in introgression and map-based cloning of white rust resistance in *B. rapa* and its related species.

White rust, caused by *Albugo candida* (Pers.) Kuntze, is a widespread and destructive disease of cruciferous vegetable (21) and oilseed crops (5,6,8,9,12). White rust appears as prominent white pustules on the cotyledons, leaves, and stems and are frequently followed by 'staghead' galls in the hypertrophied inflorescence (19).

Genetic control of resistance to A. candida has been studied in several Brassica species (2,6,17). Dominant resistance to race 2 of A. candida was reported by Ebrahimi et al. (4) in B. juncea using F1 progenies from crosses between resistant and susceptible accessions. A single dominant gene for resistance to the same race in B. rapa L. also was found by Delwiche and Williams (2), whereas Edwards and Williams (5) observed polygenic control for resistance in a rapid-cycling B. rapa population. Monogenic dominant resistance to A. candida race 2 has been reported in B. carinata and B. nigra (2,3) and in B. juncea (17).

Restriction fragment length polymorphism (RFLP) markers have been used to map a number of morphological and agronomic traits in *B. rapa* (14,15,16). However, mapping of disease-resistance loci has not been reported. Knowledge of the location and number of genes governing white rust resistance would be useful in understanding the genetics and evolution of host-pathogen interactions and in the development of resistant cultivars.

MATERIALS AND METHODS

Host population development and experimental design. Single plants of *B. rapa* cultivar Per from Svalof, Sweden, exhibiting white rust resistance and dense leaf pubescence, and cultivar R-500 from Crucifer Genetics Cooperative, Madison, WI, exhibiting white rust susceptibility and sparse leaf pubescence, were self-pollinated and cross-hybridized. A single F_1 hybrid plant was self-pollinated to produce an F_2 population. F_2 plants were individually self-pollinated to generate the seeds of 56 F_3 families.

In the first experiment, 102 F_2 plants and 24 plants of the self-pollinated parents were evaluated for white rust resistance. Eighteen plants of two control lines also were included: CrGC 1-53, a rapid cycling *B. rapa* line resistant to race 2 (*B. juncea* pathotype) (13) but susceptible to race 7 (*B. rapa* pathotype) (18), and CrGC 4-1, a rapid cycling *B. juncea* line resistant to *A. candida* race 7 and susceptible to race 2. In the second experiment, the parental lines, 12 F_1 hybrids, and 56 F_3 families were tested in a randomized complete block design replicated 12 times. Each replicate contained 1 representative from the parentals and 10 representatives from each F_1 and F_3 family together with 10 plants each from resistant and susceptible controls.

Plant growth and disease evaluation. Plants were grown in Com-pack D812 (T.O. Plastics, Minneapolis) trays filled with Jiffy mix (Jiffy Products of America, Chicago) and kept in the greenhouse at 24°C under continuous cool white irradiation at 250 $\mu mol/m^2/s$. Plants were watered daily with 1× Hoagland's solution. The plants were inoculated 5 days after sowing.

The zoospore inoculum of *A. candida* race 2 was prepared from stored sporangia and inoculated on the cotyledons of the test plants as described by Williams (20). After inoculation, the emerging true leaves were pruned every other day to prolong cotyledon retention. Development of white rust was observed daily, and the interaction phenotype (IP) was recorded from 7 to 17 days after inoculation. A rating scale of 0 to 9, in which plants rated 0 show no visible response, those rated 1 show minute hypersensitive flecks without sporulation, and those rated 2 to 9 show increasing amounts of sporulation (20), was used. Plants producing any evi-

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dence of white rust sporulation (IP = 2 to 9) were rated as susceptible.

Segregation and RFLP linkage analysis. Chi-square goodness of fit was applied to test the segregation of white rust resistance and leaf pubescence and also to test for linkage between these traits in the F_2 and F_3 populations. The estimated linkage distance between the two loci based on F_2 data was computed by applying maximum likelihood analysis (11) and was expressed in map units of percent recombination.

The 56 F_3 families screened for white rust resistance were among the 85 F_3 families used previously to develop a linkage map of 144 RFLP loci (16). These RFLP data were combined with disease reaction (homogenous resistant, homogenous susceptible, and segregating) scores to map a single gene controlling white rust resistance using MAPMAKER version 2.0 (obtained from Lander et al. [10]). Distances were expressed in centimorgans (cM) using the Kosambi map function.

RESULTS

Symptoms of white rust were observed on the susceptible genotypes 3 to 4 days after inoculation. Symptoms began as small white pustules that coalesced over time. Most of the susceptible plants attained maximum IP scores 7 days after inoculation. A few plants, however, exhibited delayed sporulation and attained maximum IP scores 10 days after inoculation. The IP scores recorded 10 days after inoculation, therefore, were used for analysis. No sporulation was observed on the cotyledons of the resistant cultivar Per, and all plants had IP scores of 0. The susceptible parent R-500 showed severe symptoms with abundant sori of sporangia forming mostly on the abaxial surface, covering half of the cotyledonary surface (mean IP score 8.0). The F_1 hybrids were resistant, with IP scores of 0. Dense leaf hairs were observed on these F_1 plants, resembling the resistant parent. CrGC 4-1, the susceptible control line, was uniformly heavily infected (mean IP score 9.0). CrGC 1-53, the resistant control line, had a mean IP score of 1.4. After 1 week, severe leaf deformation and gall formation were observed on some plants of R-500 and many plants of CrGC 4-1.

Most of the resistant plants in F_2 or F_3 populations had no symptoms on either cotyledonary surface (IP 0). A few resistant plants had small, pinpoint to large, brown necrotic flecks under the inoculation points (IP 1). The mean IP scores of resistant F_2 individuals and F_3 families were 0.1 and 0.2, respectively. The susceptible F_2 or F_3 plants had many to few pustules on the adaxial surface and many scattered small to large pustules on the abaxial surface (IP 7) or very few to no pustules on the adaxial surface and many large coalescing pustules on the abaxial surface (IP 9). A few susceptible plants had few to many scattered pustules on the adaxial surface and none to few scattered pustules on the abaxial surface (IP 5). The mean IP scores of susceptible F_2 plants and F_3 families were 8.3 and 8.2, respectively. The segregating F_3 families had a mean IP score of 3.1.

The F_2 segregation ratio did not deviate significantly from 3:1 for disease reaction or leaf pubescence (Table 1). Significant linkage was detected for these characters, and the loci controlling them were estimated to be 11.1 ± 4.4 map units apart. The segregation of resistance and leaf pubescence in the F_3 families (Table 2) did not deviate significantly from the expected 1:2:1 ratio, and significant linkage between these two traits also was obtained for this population.

The *ACA1* locus mapped to linkage group 4, which contained 12 RFLP marker loci distributed over 146.9 cM (Fig. 1). This locus was closely flanked by marker loci *wg6c1a* (5.0 cM) and *ec2b3a* (5.4 cM) and was 13.3 cM from the leaf pubescence (*PUB1*) locus.

DISCUSSION

Segregation analysis indicated that resistance to *A. candida* race 2 was controlled by a dominant allele at a single locus in cultivar Per. This locus, named *ACA1*, was tightly flanked by two RFLP marker loci, each about 5 cM apart from *ACA1*. These markers could be used to monitor introgression or incorporation of resistance into susceptible genotypes of *B. rapa* and its related species.

A single locus controlling leaf pubescence was linked with white rust resistance. Leaf pubescence is an easily detectable character

TABLE 1. Segregation and linkage analysis[a] of a *Brassica rapa* F_2 population for resistance to *Albugo candida* race 2 and for leaf pubescence

	Disease reaction		
Pubescence	Resistant	Susceptible	Total
Dense	69	9	78
Sparse	2	22	24
Total	71	31	102

[a] Segregation for disease reaction ($\chi^2 = 1.58$, $P = 0.21$) and pubescence ($\chi^2 = 0.12$, $P = 0.73$) tested against 3:1; joint segregation ($\chi^2 = 61.35$, $P \leq 0.01$) tested against 9:3:3:1.

TABLE 2. Segregation and linkage analysis[a] of *Brassica rapa* F_3 families for resistance to *Albugo candida* race 2 and for leaf pubescence

	Disease reaction			
Pubescence	Resistant	Segregating	Susceptible	Total
Dense	7	0	0	7
Segregating	1	29	1	31
Sparse	0	2	16	18
Total	8	31	17	56

[a] Segregation for disease reaction ($\chi^2 = 3.53$, $P = 0.17$) and pubescence ($\chi^2 = 4.96$, $P = 0.08$) tested against 1:2:1; joint segregation ($\chi^2 = 92.06$, $P \leq 0.01$) tested against 1:2:1:2:4:2:1:2:1.

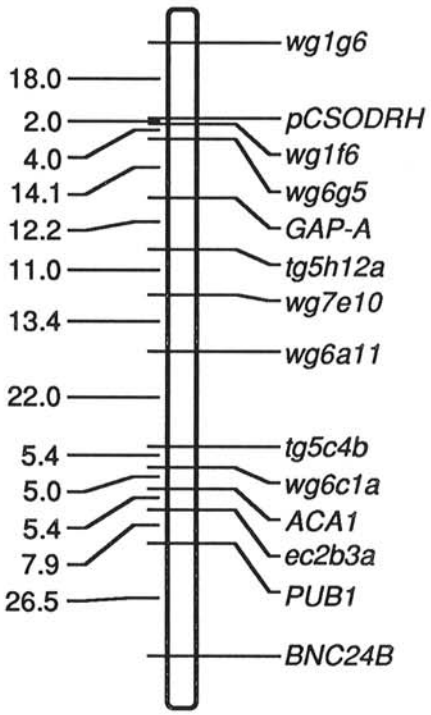

Fig. 1. Linkage map of *Brassica rapa* group 4 from analysis of F_3 families derived from cvs. Per × R-500. Locus *ACA1* controls resistance to *Albugo candida* race 2 and is linked to restriction fragment length polymorphism loci detected by *Brassica* genomic (*wg* and *tg*) and cDNA (*ec*) clones and heterologous probes. Locus *PUB1* controls leaf pubescence. Genetic distances, to the left, are in centimorgans.

that can be scored as early as during initiation of the first foliar leaf. This trait may be used as a morphological marker, along with the molecular markers, to tag and track the *ACA1* gene.

The relationships between *ACA1* and other loci controlling white rust resistance in *Brassica* are unknown, but it is possible that some may be homologous. The linkage relationship of RFLP loci used in this study are conserved across other *Brassica* species (15), providing evidence for homologous chromosomal regions. For example, the two DNA clones detecting markers flanking *ACA1* in *B. rapa* also detect linked RFLP loci on linkage group 4 in *B. napus*. Mapping resistance to *A. candida* in different species using common marker loci could provide evidence for homology of resistance genes among *Brassica* species.

Although a single major gene appeared to condition susceptibility of *A. candida* race 2 in the *B. rapa* population studied, some variation in intensity and timing of sporulation was observed. This indicates the possible involvement of other loci in the control of sporulation. The intensity of sporulation of *A. candida* race 2 in *B. rapa* has been reported to be under polygenic control (5). Ferreira et al. (7) mapped a single major gene, *ACA1*, for resistance to a *B. carinata* pathotype of *A. candida* in *B. napus* using RFLP markers; however, they also observed variation in sporulation in the mapping population and attributed this to other loci. A dominant gene at a single locus, *RAc1*, conditioning resistance to *A. candida* has been mapped with RFLP markers in *Arabidopsis thaliana* (1). Crute et al. (1) also suggested the existence of another *RAc* allele to explain several different response phenotypes to *A. candida* in their accessions. Quantification of interaction phenotypes by several measurements under different screening conditions could be used to identify genomic regions associated with quantitative variation for this trait.

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