

Mapping of a Locus Controlling Resistance to *Albugo candida* in *Brassica napus* Using Molecular Markers

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ABSTRACT

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White rust, caused by *Albugo candida*, is an important disease of crucifers, vegetables, and oilseeds in many countries. A backcross population, BCP₂, as well as F₁-derived doubled-haploid (DH) and F₂ populations obtained from the same single F₁ plant, produced from the cultivar Major × Stellar, were used to study the genetic control of

resistance to white rust in *Brassica napus*. The control of resistance to white rust in this cross was attributed to a dominant allele at a single locus, designated *ACA1*. Other loci may be involved in the control of the intensity of sporulation of the fungus in the plant. The *ACA1* locus was mapped with respect to restriction fragment length polymorphism (RFLP) loci in the F₁DH population. Linkage between the *ACA1* locus and nine RFLP loci was observed on linkage group 9 of a *B. napus* RFLP linkage map.

White rust of crucifers, caused by the pathogen *Albugo candida* (Pers.) Kuntze, is a disease of economic importance in various cultivated crucifers (10-13). The pathogen has widespread distribution in tropical and temperate climates (9,20). The disease is of particular importance on radish (*Raphanus sativus* L.), brown mustard (*Brassica juncea* (L.) Czernj.), and oilseed rape (*B. rapa* L.) seed production (10,17,19). In *B. napus* L., the disease is of primary importance to Asian cultivars (2,6). Most North American cultivars of rapeseed are resistant (11). White rust disease is characterized by the formation of white blisters (sori) on the cotyledons, leaves, stems, and inflorescences. Systemically infected inflorescences become hypertrophied, causing the characteristic staghead galls (13).

The genetic control of resistance to *A. candida* has been studied in several *Brassica* species (1). Three loci were found to condition resistance in *B. napus* cv. Regent to race 7 of *A. candida* (2,6). Two loci also controlled resistance in *B. napus* to *A. candida* race 2 collected from *B. juncea* (18). Each of these studies indicated that the presence of only one allele for resistance was sufficient to condition an incompatible reaction. Regent also has one locus controlling resistance to an *A. candida* isolate (collected from *B. carinata* A. Braun) (7). No information is available regarding linkage relationships among the white rust resistance loci in *B. napus*.

Since genetic maps based on restriction fragment length polymorphisms (RFLPs) have been developed for *B. napus* (3,5), knowledge about the location and number of genes controlling traits such as white rust resistance is of importance for understanding of the genetics of host-pathogen interactions and for breeding programs aimed at the development of resistant cultivars and for breeding strategies.

MATERIALS AND METHODS

Host population. F₁-derived doubled haploid (DH) and F₂ populations were obtained by microspore culture and by self-pollinating of a single F₁ hybrid plant derived from a cross of a white rust resistant plant of Major, a biennial rapeseed cultivar, and a DH line of Stellar, an annual canola cultivar (15). A backcross population (F₁ plant backcrossed to Major) also was developed for this study. These three populations—self-pollinated progenies of the parents, reciprocal F₁s, and controls—were evaluated for white rust resistance. Major was the female in all crosses. The control cultivars Regent and Tower are closely related by pedigree to Stellar, and Primor and Jet Neuf are related to Major (16).

The plants were grown in Com-pack D812 trays filled with autoclaved compost soil:sand:Jiffy Mix (1:1:1). The trays were maintained in a growth chamber at 24 C and under continuous cool-white irradiance of 220 μE · m⁻² · s⁻¹. Plants were watered daily with 1.0× Hoagland's solution. Six days after sowing, the cotyledons were inoculated with an *A. candida* zoospore suspension.

Inoculum preparation and plant inoculation. The *A. candida* isolate ACcar-1 from Ethiopia (7) was maintained and increased on cotyledons of Stellar for two inoculation cycles. This isolate is virulent on seedlings of *B. carinata* and *B. napus* cultivars (7). Zoosporangia were harvested 12 days after inoculation from the eruptive white rust pustules using a vacuum cyclone spore collector attached to a 1.5-ml microcentrifuge tube. Zoosporangia from approximately 32 infected cotyledons were harvested into each tube and kept on ice throughout the procedure. The tubes were immediately placed in a -20 C freezer and stored until inoculation. For zoospore production, the microfuge tube was filled with water at 12 C, gently shaken to suspend the zoosporangia, and incubated for 3 h at 12 C. After incubation, the suspension was immediately placed on ice to prevent zoospore encystment and filtered through a 250-μm-mesh nylon screen, and zoospore concentration was adjusted to 10⁵ spores per milliliter (21).

Six-day-old seedlings were inoculated with a 10- μ l drop of zoospores on each cotyledon, held for 24 h in a dark dew chamber at 20 C and 100% relative humidity, then returned to the growth chamber.

Disease evaluation and experimental design. In order to delay cotyledon senescence, true leaves of the seedlings were removed every other day. Symptoms of white rust were evaluated 8 days after inoculation and re-evaluated 1 wk later. Susceptibility and resistance were measured based on the ability of the pathogen to infect and sporulate on the host. Inoculated plants that showed the development of sporangia either on the adaxial or abaxial cotyledonary surface were considered susceptible, and those with no sporangia development were considered resistant.

A randomized complete block design was used to test 104 DH lines for resistance to *A. candida*. Each inoculated plant of a DH line was treated as an experimental unit and replicated six times. For the parents and F₁s, each experimental unit consisted of two plants, also replicated six times. Four plants per plot with six replicates were used for the controls, since the plot variance was expected to be higher for these cultivars. Two samples of the F₂ population (101 and 124 plants) and one sample of the backcross population (16 plants) were tested. The experimental entries were classified as resistant or susceptible based on a consistent reaction across replicates, or on the reaction of single plants for the F₂ and backcross populations.

RFLP and disease resistance segregation and linkage analysis. Chi-square contingency tables were used to test the hypothesis that one locus controls resistance in the cross, according to the segregation ratios expected for the DH lines (1:1) and the F₂ population (3:1). Gene action was tested by comparing the scores of the reciprocal F₁ hybrids, as well as the segregation ratios of the backcross and F₂ populations, with the parent scores.

A single gene controlling white rust resistance was mapped by linkage analysis to 138 RFLP markers that showed polymorphism between the two parents and that were used previously to develop an RFLP linkage map for the DH population (3). Segregating RFLP loci were scored by recording the genotype of each line in the DH population as homozygous for alleles from Major (M/M) or for alleles from Stellar (S/S). This classification also was used for the interaction phenotype of DH lines with *Albugo*. Tests for association between *A. candida* resistance with RFLP marker loci were first performed by analysis of chi-square values by orthogonal functions (8), and the results were considered significant if the chi-square for linkage was greater than 6.63 ($P < 0.01$). This was confirmed by maximum likelihood analysis using the computer program Mapmaker v2.0 (4). A minimum LOD (log of the odds ratio of linkage vs. no linkage) score of 3.0 and a maximum recombination frequency of 0.35 were used to group the linked marker loci, and the most probable locus order was obtained by three-point and multipoint analysis. Recombination frequencies were corrected based on Kosambi's map distance function as executed by Mapmaker.

RESULTS

Symptoms of white rust were easily observed on susceptible genotypes 3–4 days after inoculation. Symptoms began with small white pustules that coalesced over time. Plants within each of

TABLE 1. Observed segregation ratios for resistance to *Albugo candida* isolate ACcar-1 and chi-square analysis of segregating populations derived from the *Brassica napus* cross Major \times Stellar

Population	Resistant	Susceptible	Total	Chi-square	P value
DH lines	46	58	104	1.38 ^a	0.25–0.35
F ₂ plants					
Sample 1	75	26	101	0.44 ^b	0.50–0.60
Sample 2	87	37	124	2.32 ^b	0.10–0.25
BC1(M)	32	0	32

^aTested for deviation from 1:1.

^bTested for deviation from 3 (resistant):1 (susceptible).

the DH lines had a uniform reaction to the pathogen. All lines that supported sporulation to any degree were considered susceptible. The susceptible parent Stellar produced sori of sporangia only on the abaxial surface of the cotyledons, as did most of the DH susceptible lines. Some highly susceptible DH lines produced abundant sori over the entire half of a cotyledon in a shorter time than did Stellar and exhibited extensive cotyledon deformation. Other susceptible lines (6.7% of the total) supported only small pustules (1–2 mm diameter) on the adaxial surface. Resistant lines behaved as did the parent Major, exhibiting no symptoms on either cotyledonary surface, or revealed minute spots of purple pigmentation in the area where the spore suspension had been placed. Scores made at 8 days were corroborated when plants were re-evaluated 15 days after inoculation. The range of symptoms observed in the DH lines also was observed in the F₂ population.

The control lines Primor and Jet Neuf are genetically related to Major by pedigree and were found to be uniformly resistant to *A. candida*. Among the lines related to Stellar, Tower was heterogeneous for resistance to ACcar-1, some plants being highly susceptible while others were resistant. Regent was found to be uniformly resistant.

The observed segregation ratios of resistant and susceptible genotypes in the DH and F₂ populations were not significantly different than 1:1 and 3:1, respectively (Table 1). Both reciprocal F₁ hybrids were resistant to ACcar-1, suggesting that maternal effects did not influence the interaction phenotype. The backcross population was found to be uniformly resistant. These data indicate that a single dominant allele from Major (*ACA1*) conditions resistance to *A. candida* ACcar1.

Seven RFLP marker loci were linked to the *ACA1* locus (Table 2). These loci map to linkage group 9 of the *B. napus* map (3). The most probable order of these loci, and two other marker loci in the same linkage group, was obtained by multipoint analysis (Fig. 1). The closest associations found were between *ACA1* and marker loci *tg6a12a* (11.8% recombination frequency) and *wg5a5* (17.4% recombination frequency). The *ACA1* locus maps to one end of linkage group 9 (Fig. 1).

DISCUSSION

The classification of resistant and susceptible interaction phenotypes based on the ability of the pathogen to infect and sporulate in the host was facilitated in this study by the easy assessment of the disease symptoms under controlled conditions. Each DH line reacted homogeneously to the pathogen, making the phenotypic scoring and RFLP mapping procedures easy and precise. Compatible reactions were characterized by sporulation of the pathogen, and incompatibility was characterized by the absence of visible signs or symptoms other than minute purple flecks. Segregation analysis indicated that resistance to the *A. candida* isolate ACcar1 was controlled by a dominant allele at one locus, designated *ACA1*. Several RFLP marker loci in linkage group 9

TABLE 2. Linkage tests between restriction fragment length polymorphism (RFLP) marker loci and the *ACA1* locus that controls resistance to *Albugo candida* isolate ACcar-1 in *Brassica napus*

Marker comparison	n ^a	Genotypic classes ^b		Chi-square	RF ^c
		MM:MS:SM:SS			
<i>ACA1</i> –WG3G11	89	26:20:12:31		7.02	0.359
<i>ACA1</i> –WG7F5a	92	26:23:09:34		8.52	0.347
<i>ACA1</i> –WG7F3a	90	29:20:09:32		11.38	0.322
<i>ACA1</i> –WG6B10	95	35:17:07:36		23.25	0.253
<i>ACA1</i> –WG8G1b	84	29:18:07:30		13.76	0.298
<i>ACA1</i> –WG5A5	92	37:13:03:39		39.13	0.174
<i>ACA1</i> –TG6A12a	85	37:08:02:38		49.71	0.118

^aNumber of informative doubled haploid lines in the comparison.

^bMM = alleles from Major at the *ACA1* and RFLP loci, respectively; SS = alleles from Stellar at both loci; and MS and SM = recombinant classes.

^cRecombination frequency.

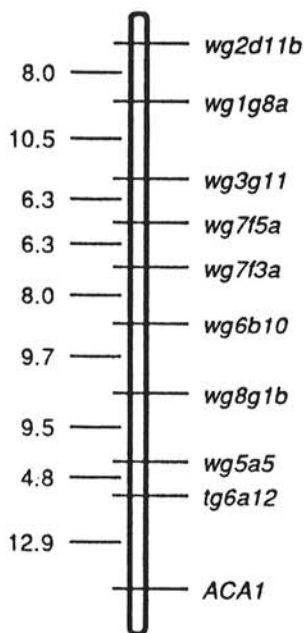


Fig. 1. Linkage map of *Brassica napus* group 9 from analysis of doubled haploid lines derived from (Major \times Stellar) F₁. Locus *ACA1* controls resistance to *Albugo candida* isolate ACcar1 and is linked to restriction fragment length polymorphism loci detected by genomic DNA clones and designated *tg* and *wg* on right. Genetic distances (left) in centimorgans.

were linked with *ACA1*, and *ACA1* was mapped to the end of this group.

The relationship between *ACA1* and other resistant loci described (2,6,18) is not known. A single locus controlling resistance to ACcar-1 was also identified in a population derived from the two annual cultivars Stellar and Regent (7); however, the resistant parent Regent is unrelated to the resistant parent Major. The RFLP probes used here, particularly those that map to linkage group 9, could be used to map white rust resistance genes from other *B. napus* sources to determine if the loci are related.

Although a single major gene appears to condition basic compatibility of *A. candida* ACcar1 on the *B. napus* populations studied, variation in sporulation intensity and rapidity exhibited among individual genotypes suggests that other loci are involved in the control of sporulation. Further research is needed to understand the genetics underlying sporulation of *A. candida* on *B. napus*. In *B. rapa*, the intensity of sporulation of *A. candida* appears to be controlled by a polygenic system (1). The DH population can be used to study the genetic control of sporulation differences in *B. napus*. Quantification of the interaction phenotype by several measurements under different screening conditions could be used with interval mapping analysis (4) to identify genomic regions associated with the trait.

The use of DH lines in genetic studies of host-pathogen interaction has various attendant advantages when evaluating the role of the environment in the expression of the interaction phenotype. Completely homozygous host lines enable more precise evaluation of the interaction phenotype, facilitating the assessment of the

genetic and environmental components of the phenotypic variation. Further work with the DH lines developed in this study could provide relevant information toward understanding the genetics of host-parasite interactions between *B. napus* and *A. candida*.

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