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ORIGINAL PAPER

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Identification and mapping of resistance gene analogs and a white rust resistance locus in *Brassica rapa* ssp. *oleifera*

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Abstract The objective of this investigation was to tag a locus for white rust resistance in a *Brassica rapa* ssp. *oleifera* F_2 population segregating for this trait, using bulked segregant analysis with random amplified polymorphic DNA (RAPD) markers, linkage mapping and a candidate gene approach based on resistance gene analogs (RGAs). The resistance source was the Finnish line Bor4109. The reaction against white rust races 7a and 7v was scored in 20 seedlings from each self-pollinated F_2 individual. The proportion of resistant plants among these F_3 families varied from 0 to 67%. Bulked segregant analysis did not reveal any markers linked with resistance and, therefore, a linkage map with 81 markers was created. A locus that accounted for 18.4% of the variation in resistance to white rust was mapped to linkage group (LG) 2 near the RAPD marker Z19a. During the study, a bacterial resistance gene homologous to *Arabidopsis RPS2* and six different RGAs were sequenced. *RPS2* and five of the RGAs were mapped to linkage groups LG1, LG4 and LG9. Unfortunately, none of the RGAs could be shown to be associated with white rust resistance.

Introduction

White rust, caused by *Albugo candida* (Pers.) Kuntze, is an economically important disease of many crucifers. Yield losses due to white rust on turnip rape (*Brassica rapa*) can range from 30% to 60% in heavily infected fields (Bernier 1972). *A. candida* is classified into ten races based on specificity to different crucifer species (Pound and Williams 1963; Hill et al. 1988). While *B. rapa* is the primary host of race 7 (Pidskalny and Rimmer 1985; Petrie 1988), races do not exhibit an absolute adaptation to a particular host species but can also infect heterologous hosts (Petrie 1988), especially those sharing a common genome. The genetic control of resistance to different races of *A. candida* in oilseed brassicas has been reported to be governed by either a single dominant gene (Delwiche and Williams 1974, Ebrahimi et al. 1976; Tiwari et al. 1988; Liu and Rimmer 1991; Ferreira et al. 1995; Kole et al. 1996; Somers et al. 2002), duplicate dominant genes (Verma and Bhowmik 1989) or minor genes (Edwards and Williams 1987; Kole et al. 1996). In *B. napus*, three dominant genes confer resistance to race 7 (Fan et al. 1983; Liu et al. 1996), whereas either a dominant allele at a single locus or two tightly linked loci control resistance to races 2 and 7 (Kole et al. 2002).

Marker-assisted selection (MAS), which is selection based on marker genotype rather than phenotype, facilitates breeding for resistance because laborious disease tests are unnecessary. The MAS approach is particularly important when several resistance genes need to be identified simultaneously. This combination, or pyramiding of resistance genes, aims at a more durable and broad-spectrum resistance. Markers linked to the desired trait can be found by gene mapping, bulked segregant analysis (BSA; Michelmore et al. 1991) and by the candidate gene approach. The candidate gene approach aims at identifying the gene itself that encodes the desired trait, based on its predicted function. An example of such a group is the resistance genes. Mutations occurring in the gene can be used to develop allele-specific markers (e.g. Tanhuanpää et al. 1998), which are the most useful ones in MAS.

The isolation of candidate resistance genes has been possible via PCR through common motifs in resistance genes. Resistance genes have been cloned from several plant species (for a review, see Hammond-Kosack and Jones 1997). They can be classified into two different classes (Staskawicz et al. 1995): leucine-rich repeat

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(LRR) genes with or without a nucleotide-binding site (NBS), and genes with a serine/threonine kinase domain. The NBS is a common protein domain essential for the catalytic activity of various proteins (Saraste et al. 1990; Traut 1994). The majority of resistance genes cloned so far have an NBS and LRR, including the viral resistance gene *N* from tobacco (Whitham et al. 1994), the bacterial resistance genes *RPS2* (Bent et al. 1994) and *RPM1* (Grant et al. 1995) from *Arabidopsis* and the fungal resistance gene *L6* from flax (Lawrence et al. 1995). Resistance genes in the NBS-LRR class can further be divided in two sub-classifications. The NBS of the TIR genes (toll/interleukin-1 resistance; e.g. *N* and *L6*) shares homology with the cytoplasmic domain of the *Drosophila* toll protein and the interleukin-1 receptor of mammals in their amino terminal domain, while others (e.g. *RPS2*) contain leucine zipper motifs in this region (Hammond-Kosack and Jones 1997). In general, the sequences among members in the NBS-LRR class are highly divergent, but short peptide motifs are well conserved. In the NBS region, several conserved structural motifs are found: a phosphate-binding loop (P-loop = kinase 1a), kinase 2, kinase 3 (Traut 1994) as well as the RNBS (resistance nucleotide binding site) A, B, C, and D regions (Meyers et al. 1999). A hydrophobic domain, GLPL, is located downstream of the NBS. The TIR and non-TIR classes can be separated according to their amino acid composition in the motifs (Meyers et al. 1999). Primers based on the conserved sequences amplify a collection of PCR products, so-called resistance gene analogs (RGA), which have been isolated and mapped from several species—e.g. *Arabidopsis* (Aarts et al. 1998), *Brassica* (Joyeux et al. 1999; Fourmann et al. 2001; Vicente and King 2001), soybean (Peñuela et al. 2002) and barley (Toojinda et al. 2000). Some of these have been shown to be linked to actual resistance genes (e.g. Kanazin et al. 1996; Leister et al. 1996, 1999; Yu et al. 1996; Speulman et al. 1998; Collins et al. 2001; Gedil et al. 2001; Shi et al. 2001; Donald et al. 2002).

The purpose of the investigation reported here was to find a marker for resistance to white rust race 7 in *B. rapa* ssp. *oleifera.* Several approaches were taken: BSA with random amplified polymorphic DNA (RAPD) markers, creation of a linkage map and candidate gene (RGAs) analysis. A secondary aim was to also map the RGAs found on the previously created linkage map of *B. rapa* (Tanhuanpää et al. 1996).

Materials and methods

Plant material

All plants of the study were grown in the greenhouse. An F_2 population of 99 individuals segregating for white rust resistance was derived by self-pollinating one white rust-resistant F_1 offspring from the cross between a susceptible (line Bor4206) and a resistant individual (line Bor4109) of spring turnip rape (*Brassica rapa* ssp. *oleifera*). DNAs were extracted by a method slightly modified from Dellaporta et al. (1983) as described by Tanhuanpää et al. (1993).

The F_2 mapping population, which was previously used for creating a linkage map and, in this study, for locating the RGAs, originated from a cross between two individuals of repeatedly selfed spring turnip rape lines Jo4002 and Sv3402 (Tanhuanpää et al. 1996).

White rust evaluation

Seeds were planted in flats (48 cells/flat) and grown in the greenhouse for 6–10 days or until the cotyledons were expanded. Cotyledons were inoculated with a 10 μl droplet (10,000 zoospores/ml) of fresh zoospore inoculum containing white rust strains 7a and 7v. Following inoculation, the flats were grown first for 24–48 h in the dark (18°C, 100% humidity) and then for 24 h in light (18°C, 80% humidity). The plants were scored for the presence or absence of white pustules 8 days after inoculation and re-evaluated on the 14th day. The white rust resistance of F_2 individuals was scored by examining the disease reaction in 20 seedlings from each self-pollinated individual. In parent lines, the reaction was scored in 13 seedlings.

Two bulks based on the disease reaction were constructed from the F_2 individuals. The susceptible bulk contained nine individuals with no resistant offspring, and the resistant bulk nine individuals with the highest proportion of resistant offspring (56–67%).

Cloning and sequencing of RGAs

DNA regions used for the amplification of RGAs included a P-loop motif (amino acid sequence GGVGKTT) of NBS and a second region (amino acid sequence GLPLAL) approximately 160 amino acids downstream. The primers designed to these regions were GGT GGG GTT GGG AAG ACA ACG and CAA CGC TAG TGG CAA TCC derived from the *RPS2* sequence of *Arabidopsis* (Bent et al. 1994) (primers s1 and as1, respectively, in Leister et al. 1996) and degenerate primers GGI GGI GTI GGI AAI ACI AC and IAG IGC IAG IGG IAG ICC (primers s2 and as3, respectively, in Leister et al. 1996). The following PCR programme in a Techne PHC2 Dri-Block cycler was used for amplifying *RPS2*-like sequences: 35 cycles of 30 s at 94°C, 30 s at 64°C and 1 min at 72°C; this was preceded by an initial denaturation step of 2 min at 95°C and followed by a final extension step of 6 min at 72°C. The PCR programme with degenerate primers was the same except that the annealing temperature was 50°C and the number of cycles 40. The PCR reaction was carried out in volumes of 25 μl, with each sample containing 0.4 U of Red Hot DNA polymerase (Advanced Biotechnologies), buffer supplied by the enzyme manufacturer, 2.5 m*M* $MgCl_2$, 100 μM of each dNTP, 200 n*M* (*RPS2* primers) or 500 n*M* (degenerate primers) of each primer and 20 ng DNA.

The PCR fragments of the expected size (approx. 500 bp) amplified from the parents of the population were excised from the agarose gel and purified using a Wizard Preps DNA purification system (Promega, Madison, Wis.). The products amplified with *RPS2* primers were ligated with a Sureclone ligation kit (Pharmacia, Piscataway, N.J.), and TOP10F′ cells were chemically transformed with recombinant plasmids (TOP10F′ One Shot kit; Invitrogen, Carlsbad, Calif.). Putative transformants were checked by hybridization with the respective *RPS2* fragment, and plasmid DNAs were prepared with the alkaline lysis method described in Maniatis et al. (1982). A TA Cloning kit (Invitrogen) was used for the ligation of PCR products amplified with degenerate primers into the pCR2.1 vector (Invitrogen) and for chemical transformation of INVαF′ cells. Plasmid DNAs were purified with the CONCERT Rapid Plasmid Miniprep system (Life Technologies, Gaithersburg, Md.), and the presence of the insert was ascertained by *Eco*RI restriction analysis. Several clones from each transformation were sequenced with either the A.L.F. DNA Sequencer or the ALFexpress (Pharmacia).

Markers

RAPD primers were purchased from Operon Technologies (Alameda, Calif.) (denoted with a letter and a number) or synthesized (denoted with a number only) (primer sequences in Tanhuanpää et al. 1996) on an Applied Biosystems 392 DNA/RNA synthesizer (Foster City, Calif.). The PCR protocol that yielded the best results consisted of 40 cycles of 30 s at 95°C, 30 s at 35°C and 2 min at 72°C in a Mastercycler thermal cycler; this was preceded by an initial denaturation step of 3 min 30 s at 95°C. The 25-μl reaction mixture consisted of 0.75 U *Taq* polymerase (Promega or MBI Fermentas), buffer supplied by the enzyme manufacturer, 1.5 m*M* $MgCl_2$, 100 μM of each dNTP, 600 n*M* of primer and 20 ng of DNA. Different markers produced by the same RAPD primer were designated with lower-case letters followed by the number of the primer. The homology of RAPD markers in two different populations (white rust and mapping population) was ascertained by hybridization.

Restriction fragment length polymorphism (RFLP) analysis was performed using standard methods (Maniatis et al. 1982) with restriction enzymes *Eco*RI, *Eco*RV, *Hin*dIII, *Pst*I, *Sca*I, *Bgl*I, *Bgl*II, *Kpn*I, *Sal*I, *Stu*I, *Xba*I, *Tru*I, *Acc*I (MBI Fermentas or Boehringer Mannheim). The probes included purified plasmid DNA of each RGA class and one genomic *Brassica* DNA clone (WG6C1A) from Dr. T. Osborn (University of Wisconsin-Madison). The RGAs were located, whenever possible, on both the current and previously created linkage map of spring turnip rape (Tanhuanpää et al. 1996). One microsatellite marker, 35D (Szewc-McFadden et al. 1996), was amplified by PCR and visualized with the A.L.F. DNA Sequencer (Pharmacia).

Newly developed PCR-based marker techniques in which primers target intron-exon boundaries were also used (Li and Quiros 2001, Gawel et al. 2002). The PCR programme in the Mastercycler for amplifying SRAP (sequence-related amplified polymorphism) markers was as presented in Li and Quiros (2001). The PCR reaction (25-μl volumes) consisted of 0.75 U *Taq* polymerase (MBI Fermentas), $(NH_4)_2SO_4$-containing buffer supplied by the enzyme manufacturer, 1.5 m*M* $MgCl_2$, 100 μM of each dNTP, 400 n*M* of each primer and 20 ng of DNA. The forward primer was labelled with a fluorescent dye (FAM), thus enabling the scoring of markers with the MegaBACE 500 Sequencer (Pharmacia). The PCR programme used in the Mastercycler for amplifying the markers described by Gawel et al. (2002) was as follows: seven cycles of 40 s at 94°C, 1 min at 60°C, 2 min at 72°C, followed by 33 cycles with the annealing temperature raised to 64°C. This programme contained an initial denaturation step of 3 min 30 s at 95°C and a final extension step of 10 min at 72°C. The 20-μl reaction mixture consisted of 0.4 U of red hot DNA polymerase (Advanced Biotechnologies), buffer supplied by the enzyme manufacturer, 2.5 m*M* $MgCl_2$, 100 μM of each dNTP, 1 μM primer and 40 ng DNA. Markers were run on agarose gels and scored by visual inspection.

Statistical and sequence analyses

Goodness-of-fit between observed and expected F_2 segregation ratios at marker loci was tested with chi-square analysis. The MAPMAKER 3.0 software (Lander et al. 1987) was used for map construction using a LOD score of 3.5 (white rust population) or 4.0 (mapping population) and a maximum recombination fraction of 0.40 as linkage criteria. Genetic distances in centiMorgans were calculated by Haldane's mapping function. Quantitative trait data were analysed using MAPMAKER/QTL 1.1.

CLUSTER W (1.81) (Thompson et al. 1994) service at the European Bioinformatics Institute (http://www2.ebi.ac.uk/clustalw) was used to align deduced amino acid sequences. BLAST searches (Altschul et al. 1997) against Genbank and EMBL databases were performed using the National Center for Biotechnology Information (Bethesda, Md.) website (http://www.ncbi.nlm.nih.gov.)

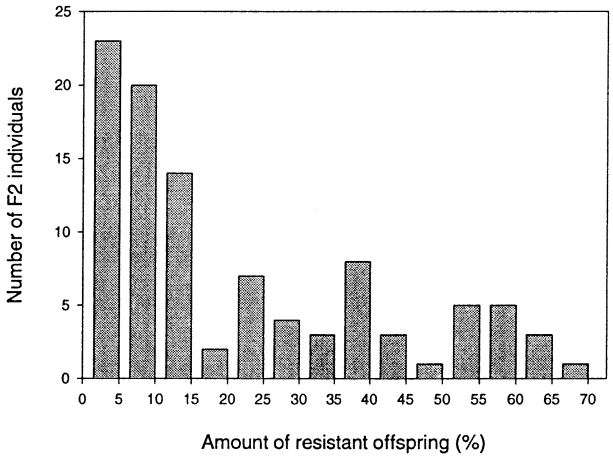

Fig. 1 Frequency distribution of white rust resistance in the F_2 population from the *Brassica rapa* ssp. *oleifera* cross Bor4109 × Bor4206. Resistance percentage was scored in 20 seedlings of each self-pollinated F_2 individual

Results

Disease testing

Twenty-three F_2 individuals were classified as susceptible on the basis of progeny scoring (0% resistant offspring) on the 14th day following inoculation. None of the F_2 plants was 100% resistant, with the amount of resistant offspring varying from 5% to 67% (Fig. 1). The proportion of resistant plants in the resistant parent line was 69%. There was a great deal of variation in sporulation intensity and speed of dissemination among individual plants. This suggests that in addition to genes that control the resistance, other loci are involved in the control of sporulation.

Sequence analysis of RGAs

Seventy-four clones were sequenced from the fragment amplified with *RPS2* primers from the resistant parent and three clones were sequenced from the susceptible parent. Most of the clones were similar, with the exception of some with one to three base substitutions, which were most probably errors made by *Taq* polymerase during amplification. Thus, only the *B. rapa* gene corresponding to the *RPS2* of *Arabidopsis* was amplified. The 468-bp sequence (excluding primer sequences) of *B. rapa RPS2* (AF315087) contained 62 base substitutions compared to the *Arabidopsis RPS2*, leading to 23 differences in their amino acid composition (Fig. 2).

The degenerate primer pair amplified four different PCR fragments from the parents, one of which was of the expected size, 500 bp, and separated the NBS and GLPLAL motifs of the primers. Sixty-two clones of the PCR product from the resistant parent were sequenced. Some sequences were either shorter than expected or of poor quality, thereby decreasing the total number of

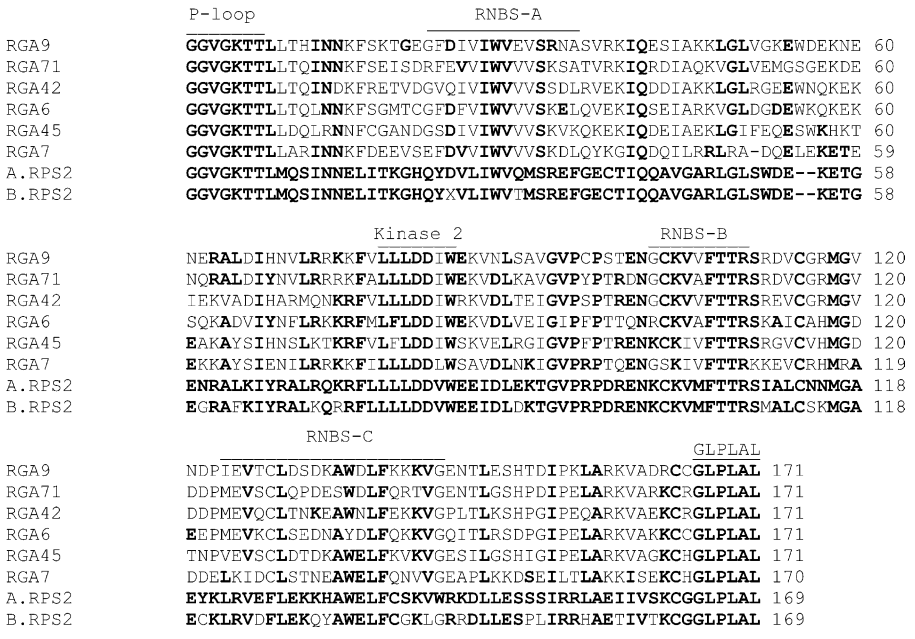

Fig. 2 Alignment of the deduced amino acid sequences of six resistance gene analogs and *RPS2* (*B.RPS2*) from *B. rapa* and, for comparison, *Arabidopsis RPS2* (*A.RPS2*)(U14158) based on CLUSTAL W analysis. Amino acids similar to those in *Arabidopsis RPS2* are in **bold**. RGA9 contains one insertion, and the reading frame has therefore been adjusted by one base. RGA45 contains a stop codon, which has not been taken into account in the analysis. The first seven and last six amino acids were contributed by the degenerate primer sequences. Amino acid motifs according to Meyers et al. (1999) are marked at the *top* of the sequence

Table 1 Resistance gene analogs and *RPS2* amplified from *Brassica rapa*

Name	Sequenced from line	Number of clones	Length[a] (bp)	Mapped in[b]	Linkage group	Accession no.
RGA6	Bor4109, Bor4206	36, 4	475	map	LG1	AF315081
RGA7	Bor4206	2	472	map	LG4	AF315086
RGA9	Bor4109, Bor4206	15, 1	476[c]	map, wr	LG4	AF315082
RGA42	Bor4109	1	475	map, wr	LG9	AF315083
RGA45	Bor4109	1	478[d]	wr	LG4	AF315084
RGA71	Bor4109	1	475	map, wr	-	AF315085
RPS2	Bor4109, Bor4206	74, 3	468	map, wr	LG1	AF315087

[a] Primer sequences not included
[b] Map, Mapping population (Tanhuanpää et al. 1996); wr, population segregating for white rust resistance
[c] Includes an insertion causing a frameshift
[d] Includes a stop codon

readable sequences to 54. Most of the sequences were very similar. However, five different RGA types could be recognized (Table 1, Fig. 2). These five types were labelled RGA, followed by the clone number representing the class. RGA42, RGA45 and RGA71 were only represented by one clone, but RGA6 was represented by 36 clones and RGA9 by 15 clones. RGA6, RGA42 and RGA71 contained full open-reading frames. RGA9 and RGA45 represented pseudogenes: RGA9 included one extra nucleotide (82T), causing a frame shift, and RGA45 included a stop codon (between amino acids 62 and 63). Nine clones were sequenced from the susceptible parent. Two of these were not resistant genes (included the same primer at both ends and several stop codons). Four were RGA6 types and one was the RGA9 type, with some modifications in their nucleotide and amino acid sequences. Two clones (differed from each other by three amino acids) represented a totally new RGA type, which was named RGA7 (Table 1).

The amino acid sequences of the six different RGA types and *B. rapa RPS2* were 40–71% similar to each other (individual results not shown) and contained the RNBS-A motif that is typical of non-TIR genes (Meyers et al. 1999) (Fig. 2). The DNA sequences of these six RGAs were compared against DNA databases using BLASTN. The three best hits were found to *Brassica* sequences identified by Fourmann et al. (2001) (Table 2), which had been amplified mostly with primers similar to those used in the present study, namely s2 and as3. It is interesting that *B. napus* RGA5 homologous to RGA9 and *B. napus* RGA19 homologous to RGA45 also contain the frame shift and the stop codon, respectively, but in a different position.

Mapping of RGAs and a white rust resistance locus

The original idea was to use BSA to identify markers linked to white rust resistance. The screening of 391 RAPD primers revealed 15 markers polymorphic between the bulks. However, when these were scored in the individual plants separately, none of the markers seemed to be linked with resistance. Consequently, two different strategies in searching for a white rust resistance locus

Table 2 Three best BLAST hits of the *B. rapa* RGA sequences identified. All of the homologies are according to Fourmann et al. (2001)

	Homologies	E-value[a]	Identity[b]
RGA6	*B. napus* RGA21 (AF209490)	0.0	464/475=97%
	B. oleracea RGA12 (AF338960)	e^{-178}	339/348=97%
	B. napus RGA43 (AF263326)	e^{-163}	306/311=98%
RGA7	*B. napus* RGA1 (AF107545)	0.0	428/447=95%
	B. rapa RGA1 (AF338965)	0.0	337/343=98%
	B. oleracea RGA1 (AF338949)	e^{-153}	323/339=95%
RGA9	*B. napus* RGA5 (AF209501)	0.0	448/466=96%
	B. napus RGA34 (AF223317)	e^{-164}	300/301=99%
	B. oleracea RGA2 (AF338950)	e^{-154}	315/327=96%
RGA42	*B. rapa* RGA3 (AF338967)	0.0	446/450=99%
	B. oleracea RGA5 (AF338953)	0.0	435/450=96%
	B. napus RGA36 (AF263319)	e^{-165}	318/326=97%
RGA45	*B. napus* RGA19 (AF209488)	0.0	445/478=93%
	B. rapa RGA42 (AF263325)	e^{-165}	308/313=98%
	B. napus RGA20 (AF209489)	e^{-141}	425/481=88%
RGA71	*B. napus* RGA6 (AF107549)	0.0	468/475=98%
	B. rapa RGA2 (AF338966)	0.0	397/399=99%
	B. napus RGA35 (AF263318)	0.0	400/405=98%

[a] Number of similar matches expected by chance alone
[b] The extent to which two sequences are invariant

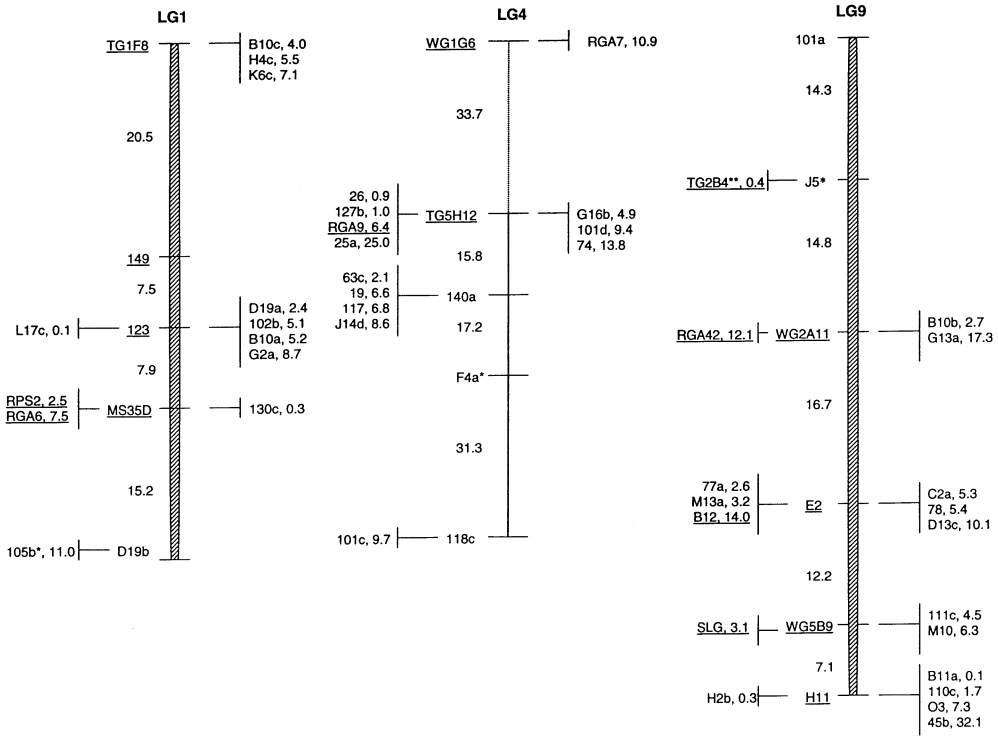

Fig. 3 The position of RGAs and *RPS2* in the linkage groups constructed from the F_2 population of a *B. rapa* ssp. *oleifera* cross Jo4002 × Sv3402. A LOD score threshold of 4.0 was used for grouping, except for WG1G6, which was attached to the framework using a LOD score of 2.0 (*broken line*). For ordering, a LOD score difference greater than 3.0 (*wide line*) or greater than 2.0 (*narrow line*) in favour of the best map was used. Dominant RAPD markers *on the framework* and *on the left side* are derived from Jo4002; *on the right side*; from Sv3402. Marker distances are shown in centiMorgans; for markers not included in the framework, two-point map distances between the marker and the nearest framework locus are shown. The nomenclature of markers is described in the Materials and methods and in Tanhuanpää et al. (1996). Codominant markers are *underlined*. Loci exhibiting aberrant segregation are indicated with *one asterisk* ($P<0.05$) or *two asterisks* ($P<0.01$). Linkage groups are not in scale

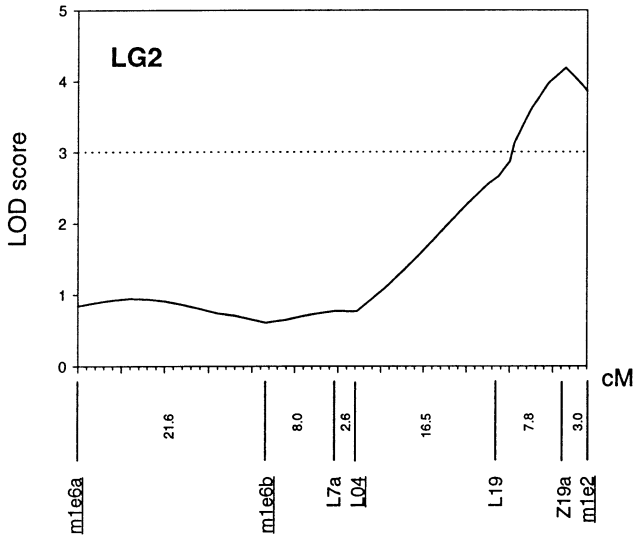

Fig. 4 LOD profile for a locus controlling white rust resistance in the F_2 population from the *B. rapa* ssp. *oleifera* cross Bor4109 × Bor4206. The LOD scores were computed using MAPMAKER/QTL VER. 1.1. The *horizontal dashed line* indicates a LOD score threshold of 3.0. SRAP markers are marked with the *prefix m*; the other markers are RAPDs. For dominant RAPDs, the visible allele is derived from the susceptible parent. Codominant markers are *underlined*

was adopted: quantitative trait locus (QTL) analysis—from a map built mainly with arbitrary markers—and the candidate gene approach using RGAs. The arbitrary markers included 44 RAPDs and 16 markers amplified with intron-exon primers. In addition, 13 RAPD markers and one microsatellite (MS35D), the locations of which were known (Tanhuanpää et al. 1996), were used to identify linkage groups (LG): 149 and MS35D from LG1; L4, L7a and L19 from LG2; F4a, 127b and 101c from LG4; G2b and 90b from LG5; L14a from LG6; 93c and L7b from LG8; 78 from LG9. RFLP marker WG6C1A and RAPD marker WR3 were scored because they have been reported to be located near the white rust resistance locus of race 2 in *B. rapa* (Kole et al.1996) and in *B. juncea* (Prabhu et al. 1998), respectively.

RPS2 and each RGA type were mapped either in the white rust population (RGA45) or in the mapping population (RGA6, RGA7), or in both (*RPS2*, RGA9, RGA42, RGA71), depending on their polymorphism (Table 1). Their position (excluding RGA45 and -71) in the linkage groups of the mapping population is shown in Fig. 3. RGA45 was not polymorphic in the mapping population, but the distance between RGA9 and RGA45 was 1.2 cM in the white rust population. RGA71 remained unlinked in both populations.

A total of 81 markers, including mixed coupling and repulsion markers, were analysed in the white rust population. All segregated with the expected ratios. Owing to the low number of markers, all of the linkage groups could not be identified. A QTL for white rust resistance with a LOD score of 4.2 (Fig. 4) was identified in LG2 which accounted for 18.4% of the variation in white rust resistance. When the possible modes of inheritance of this QTL were tested, free genetics had the highest likelihood (4.2), followed in succession by additive (4.1), recessive (3.5) and dominant genetics (1.8). This QTL was situated 0.1 cM from the RAPD marker Z19a. The resistance percentage in the 75 F_2 individuals with the visible allele of this marker (homo- and heterozygotes) was 15.8±18.1%, and in the 24 individuals without it, 34.3±20.8%. The difference is statistically significant at $P<0.001$ (*t*-test).

Discussion

The aim of this investigation was to find a marker for resistance to white rust race 7 in *B. rapa* ssp. *oleifera.* Because the construction of a linkage map is laborious, RAPD markers for the resistance were sought using BSA. However, no markers linked with resistance were found. The reasons for this failure may include an inadequate number of primers tested, the probable presence of heterozygotes in the bulks and an incorrect bulking of individuals (especially since it was later shown that the white rust resistance gene segregating in the population only accounts for a minor portion of the variation). Consequently, a linkage map with 81 markers had to be created.

A white rust resistance locus against race 7 was mapped to LG2. Kole et al. (2002) mapped a single major QTL locus (or two tightly linked loci), *Aca1*, which controls resistance against races 2 and 7, to the *B. rapa* LG4 and a minor QTL to LG2. In *B. napus*, a white rust resistance locus designated *ACA1* was mapped to LG9 (Ferreira et al. 1995). The isolate used in this study was ACcar-1 from Ethiopia (Liu and Rimmer 1991). *B. napus* LG9 is homologous to *B. rapa* LG2 (Teutonico and Osborn 1994). Kole et al. (2002) suggested that a locus might exist at the end of *B. rapa* LG2 that is homologous to *ACA1* on *B. napus* LG9. Because the three resistance loci found in Ferreira et al. (1995), Kole et al. (2002) and in the present study confer resistance to different isolates of the fungus, it might be that they do not represent homologous genes but a cluster of resistance genes. The possible existence of white rust loci other than the one in LG2 in the present study cannot be excluded because all linkage groups could not be identified owing to the small number of markers. On the other hand, no white rust resistance QTL could be found in LG4 where the *Aca1* locus exists (RFLP WG6C1A in LG4 has been shown to be linked to *Aca1,* Kole et al. 2002).

One approach to searching for the white rust resistance locus is to use candidate genes, in this case RGAs. During this study, a bacterial resistance gene homologous to *Arabidopsis RPS2* and six different RGA types were found. RGA6 and RGA9 consisted of several clones (36 and 15, respectively), and there were nucleotide substitutions among members of the classes. Some of these might be errors that arose during PCR or sequencing, but others might represent real variants inside the class, e.g. a

variant RGA41 of class RGA9 contained six amino acid differences (results not shown). Five RGA types could be mapped to *B. rapa* linkage groups LG1, -4, and -9. *RPS2* was also mapped to LG1. The same RGA types were identified earlier in *Brassica* (Fourmann et al. 2001), but their correlation with resistance traits was not tested nor have they been mapped in *B. rapa*. Unfortunately, none of the RGAs were associated with the white rust resistance locus although they represent resistance loci against some other diseases (needs to be tested) or are pseudogenes (RGA9 and RGA45).

The white rust resistance gene in LG2 is located near the RAPD marker Z19a. The visible allele of this marker is derived from the susceptible parent, meaning that the RAPD marker is in repulsion phase with respect to the desired allele. MAS with a repulsion-phase marker is as effective as with a codominant marker (Haley et al. 1994). In addition, a RAPD marker can be converted into a SCAR (sequence characterized amplified region) marker with specific primers to increase reproducibility. However, because the gene found in the present study accounts only for 18.4% of the variation in white rust resistance, selecting for it alone is perhaps not justified although it could be used when pyramiding resistance genes.

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