

# Complexities of Chromosome Landing in a Highly Duplicated Genome: Toward Map-Based Cloning of a Gene Controlling Blackleg Resistance in *Brassica napus*

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## ABSTRACT

The LmR1 locus, which controls seedling resistance to the blackleg fungus *Leptosphaeria maculans* in the *Brassica napus* cultivar Shiralee, was positioned on linkage group N7. Fine genetic mapping in a population of 2500 backcross lines identified three molecular markers that cosegregated with LmR1. Additional linkage mapping in a second population colocalized a seedling resistance gene, ClmR1, from the cultivar Cresor to the same genetic interval on N7 as LmR1. Both genes were located in a region that showed extensive inter- and intragenomic duplications as well as intrachromosomal tandem duplications. The tandem duplications seem to have occurred in the Brassica lineage before the divergence of *B. rapa* and *B. oleracea* but after the separation of Brassica and Arabidopsis from a common ancestor. Microsynteny was found between the region on N7 carrying the resistance gene and the end of Arabidopsis chromosome 1, interrupted by a single inversion close to the resistance locus. The collinear region in Arabidopsis was assayed for the presence of possible candidate genes for blackleg resistance. These data provided novel insights into the genomic structure and evolution of plant resistance loci and an evaluation of the candidate gene approach using comparative mapping with a model organism.

**B**LACKLEG caused by the fungus *Leptosphaeria maculans* (Desm) Ces. & de Not is a devastating disease of canola (*Brassica napus* L. and *B. rapa* L.) and has caused extensive yield losses in Europe, Australia, and Canada (GUGEL and PETRIE 1992). Since chemical control of the disease is difficult, is uneconomical, and poses risks to the producer and the environment (AHLERS 1991), the introduction of genetic resistance to blackleg has become a major objective of canola breeding programs. Brassica crop species comprise primary diploids containing the A, B, or C genome, respectively, and amphidiploids, like *B. napus* (AACC), carrying a combination of two diploid genomes (U 1935). A complete hypersensitive resistance to *L. maculans* has been found in Brassica species containing the B genome, which is effective throughout the life of the plant (ROY 1984; RIMMER and VAN DEN BERG 1992). Such resistance loci have been identified and mapped in *B. nigra*, *B. juncea*, and *B. carinata* and a number of attempts have been made to transfer them into *B. napus* breeding lines through interspecific hybridization (ROY 1984; SJÖDIN and GLIMELIUS 1988; RIMMER and VAN DEN BERG 1992; STRUSS *et al.* 1992, CHÈVRE *et al.* 1996; PANG and

HALLORAN 1996a). The results have been promising; however, cotransfer of unwanted traits and low frequencies of recombination between the different species have complicated the development of blackleg-resistant oilseed cultivars by conventional breeding (CHÈVRE *et al.* 1997).

Most modern commercial canola varieties carry sources of seedling and adult plant resistance to *L. maculans*, which originate from the A genome of *B. napus* and *B. rapa*. While adult plant resistance in the field is ultimately the trait that is essential for large-scale oilseed production, the wide pathogen variability and the constraints of field testing make it difficult to assess this resistance genetically. Monogenic and polygenic inheritance of adult resistance under controlled and field conditions has been reported in the literature (CARGEEG and THURLING 1979; SIPPPELL *et al.* 1991; STRINGAM *et al.* 1992; DION *et al.* 1995; FERREIRA *et al.* 1995; PANG and HALLORAN 1996b,c; PILET *et al.* 1998; LI and COWLING 2003). In contrast, cotyledon or seedling-stage resistance is generally believed to be controlled by single dominant genes, which have been shown to confer resistance to specific races of the pathogen. Although numerous studies suggest that seedling and adult blackleg resistances are under different genetic controls, many authors have observed a significant correlation between the two (NEWMAN and BAILEY 1987; MCNABB *et al.* 1993;

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BANSAL *et al.* 1994; LI and COWLING 2003). This could be due to tight linkage of a cotyledon resistance gene to one of several major adult resistance quantitative trait loci (QTL) in a particular variety or allelic variation at a single locus could control both adult and cotyledon resistance. Closely linked loci for adult and seedling resistance were identified in the canola cultivar Major and in the *B. napus* breeding accessions RB87-62 and DH88-752 (FERREIRA *et al.* 1995; ZHU and RIMMER 2003). Similarly, the race-specific cotyledon resistance gene Rlm1 has been shown to explain 70% of the variation for adult plant resistance in the French cultivar Maxol (DELOURME *et al.* 2004). In fact, the strong correlation between seedling and field resistance in the Australian canola cultivar Maluka was exploited to expedite the development of the cultivar Quantum for Western Canadian field conditions (STRINGAM *et al.* 1995). Therefore, markers for seedling resistance can be a great asset for accelerating oilseed breeding programs.

Loci controlling cotyledon resistance to *L. maculans* have been mapped in various *B. napus* cultivars using molecular markers. Single dominant genes were identified in the cultivars Major, RB87-62, Shiralee, and Maluka (FERREIRA *et al.* 1995; MAYERHOFER *et al.* 1997; RIMMER *et al.* 1999) and mapped to the same linkage group LG6, which has been shown subsequently to be equivalent to the A genome linkage group N7 (FERREIRA *et al.* 1995; PARKIN *et al.* 1995). Interestingly, a single major locus controlling field disease resistance in the cultivar Cresor has also been mapped to the same region of N7 (DION *et al.* 1995). More recently, race-specific resistance genes identified in the cultivars Maxol, Quinta, Glacier, Samourai, and Darmor have been mapped to two genomic regions on linkage groups LG10 and LG16 of a consensus linkage map (DELOURME *et al.* 2004). A cluster of five cotyledon resistance genes was suggested on LG10. Due to the absence of common molecular markers this linkage group cannot be associated directly with the previously cited mapping studies but on the basis of comparisons of resistance gene and avirulence gene specificities DELOURME *et al.* (2004) suggest that LG10 corresponds to LG6 of FERREIRA *et al.* (1995) and hence to N7. A concentration of blackleg resistance loci appears to be on N7; however, little is known about the relationship of these genes and much less is known about their role in the defense response. Cloning of such resistance genes would not only allow analysis of the function and structural organization of these loci but also expedite the transfer of resistance into elite breeding lines without the associated linkage drag that is unavoidable in conventional breeding. Moreover, different cloned R genes could be used to "pyramid" the resistance in one cultivar to enhance the durability and range of the resistance.

Map-based cloning has been successfully employed to isolate disease resistance genes from a number of plant species (BENT 1996). This strategy involves fine-scale map-

ping of the gene in a large segregating population and identification of tightly linked flanking markers that allow quick chromosome walking toward the gene or, preferably, chromosome landing on a large insert clone carrying the resistance gene (TANKSLEY *et al.* 1995). For this approach to be successful, an ample supply of polymorphic markers is required to delimit the gene within a sufficiently small genetic interval of <1 cM.

Brassica species represent the closest crop plant relatives to the model plant *Arabidopsis thaliana* and the presence of chromosomal collinearity between these two species has been established in several studies (reviewed in SCHMIDT *et al.* 2001; SCHMIDT 2002). Large-scale ancestral genome duplications in both *Arabidopsis* and the amphidiploid *B. napus*, as well as the occurrence of rearrangements and insertions/deletions, complicate this collinear relationship (SCHMIDT 2002). Nevertheless, the extensive resources developed for the model plant, including the genome sequence, can be exploited for fine mapping in *B. napus* (SNOWDON and FRIEDT 2004). MUANGPROM and OSBORN (2004) found almost perfect collinearity of 13 RFLP markers between *Arabidopsis* and *B. rapa* around a dwarf gene, enabling them to identify a candidate gene in *Arabidopsis* and subsequently to clone and characterize the corresponding gene in *B. rapa* (MUANGPROM *et al.* 2005). Since *Arabidopsis* accessions are usually blackleg resistant (BOHMAN *et al.* 2004) and sequence homology between the two species in coding regions can be >85% (CAVELL *et al.* 1998), candidate resistance genes could be present in collinear regions of *Arabidopsis* and be identified by similarity to known resistance gene motifs. Indeed, it was shown by somatic fusion experiments that *Arabidopsis* harbors blackleg resistance genes that are functional in *B. napus* (BOHMAN *et al.* 2002).

In this article we present a fine-scale map of the LmR1 locus of the Australian canola cultivar Shiralee, which is positioned in the same genetic interval as a cotyledon resistance gene originating from the French cultivar Cresor. The region was shown to be genetically complex yet displayed strong collinearity with the end of *Arabidopsis* chromosome 1. The intricacies of mapping in a highly duplicated genome and the feasibility of a candidate gene approach are discussed.

## MATERIALS AND METHODS

**Plant material and mapping populations:** Two different mapping populations were used to map the blackleg resistance genes of the canola cultivars Shiralee and Cresor, respectively. All parental lines were doubled-haploid (DH) lines of *B. napus*. A common susceptible parent was used for both mapping crosses: a newly resynthesized *B. napus* line PSA12 [M. Beschorner and D. Lydiate, Agriculture and Agri-Food Canada (AAFC) Saskatoon Research Centre], produced by embryo rescue from an interspecific cross between *B. oleracea* ssp. *alboglabra* line A12DH (G. King, Horticultural Research International) and *B. rapa* line Parkland Sunshine (K. Falk, AAFC Saskatoon Research Centre).

DH12075 was derived from a cross between the blackleg-resistant canola cultivar Cresor and the susceptible cultivar Westar and was shown to be blackleg resistant in Canadian field trials (G. Seguin Schwartz and G. Rakow, AAFC Saskatoon Research Centre). Shiralee is an Australian canola cultivar resistant to the prevalent blackleg isolate found in Canada. Single resistant F<sub>1</sub> plants from the cross DH12075 × PSA12 as well as from Shiralee × PSA12 were backcrossed to PSA12 to generate two backcross (BC)1 populations. Initially 90 and 100 BC1 lines from the DH12075 and Shiralee populations, respectively, were genotyped and assessed for blackleg resistance to identify markers flanking the resistance genes. Another 250 and 2448 lines from each mapping population, respectively, were screened for the presence of recombination events across the interval carrying the resistance loci. This maximized the resolution of marker order within the interval carrying the resistance gene.

**Greenhouse screening for blackleg resistance:** The cotyledon resistance response to blackleg was determined for the BC1 populations as described in MAYERHOFER *et al.* (1997). The resistance phenotype of each backcross line was determined using 16 self progeny from each BC1.

**DNA isolations, Southern hybridizations, PCR reactions, and sequencing:** Genomic DNA preparations from fresh or lyophilized leaf tissue, Southern analyses, and RAPD reactions were carried out as described in SHARPE *et al.* (1995) and MAYERHOFER *et al.* (1997). PCR reactions for sequence-characterized amplified region (SCAR) markers were performed in a Gene Amp 9700 thermocycler (Perkin-Elmer, Norwalk, CT) in 25- $\mu$ l reaction volumes containing 10 mM Tris-HCl (pH 8.0); 50 mM KCl; 0.01% gelatin; 2.5 mM MgCl<sub>2</sub>; 0.4 mM each of dATP, dCTP, dGTP, and dTTP; 0.2  $\mu$ M each of primer; 0.5 units/reaction Taq DNA polymerase (Perkin-Elmer); and 25 ng of template DNA. The cycle parameters were 94°/2 min; 12 cycles of 94°/30 sec, 72° (-0.1°/cycle)/30 sec, 72°/1 min; and 23 cycles of 94°/30 sec, 60°/30 sec, 72°/1 min, followed by 72°/5 min. Sequencing reactions were carried out using a BigDye Terminator v.3.1 cycle sequencing kit (Applied Biosystems, Foster City, CA).

**Molecular markers:** DNA probes with the prefix “est” designate Arabidopsis EST clones purchased from the Arabidopsis Biological Resource Centre (<http://www.arabidopsis.org>). Markers rapdD3 and rapd184 are RFLP probes cloned from gel-purified RAPD bands generated by the primers OPD-03 (5'-GTCGCCGTCA-3'; Operon Technologies, Alameda, CA) and 184 (5'-CAAACGGCAC-3'; University of British Columbia), respectively. RFLP markers with prefixes “mi,” “pO,” “pW,” and “ec” are genomic or cDNA clones from Arabidopsis and Brassica species, respectively, and have been described earlier (FERREIRA *et al.* 1995; SHARPE *et al.* 1995; LIU *et al.* 1996). Markers starting with the designation “BL,” “LL,” “ML,” “RL,” and “es” are *B. napus* EST clones, described on the Brassica/Arabidopsis Genomics Initiative web site (<http://Brassica.agr.gc.ca/>). The SCAR marker AO39 was kindly provided by Daryl Somers (AAFC Saskatoon Research Centre). “BNID,” “BNIH,” “BNIB,” and “TH” designate end probes (from the T7 and R ends) generated from DH12075-derived BAC clones (S. EDES and I. PARKIN, unpublished data). All markers were employed as RFLP probes in Southern analyses except when followed by the designation “(SCAR),” in which case their sequence was used to generate allele-specific PCR primers.

**Linkage analysis:** All polymorphic loci detected with RFLP- or PCR-based markers were analyzed independently in the two mapping populations. Linkage analysis was carried out using Mapmaker v3 with a LOD score of 4.0 (LANDER *et al.* 1987). Linkage groups generated in the two populations were subsequently aligned through the identification of common alleles at several loci. The loci were assigned to the linkage groups of *B. napus* through the identification of common al-

les with parents of the mapping populations described in PARKIN *et al.* (1995) and SHARPE *et al.* (1995). Brassica-derived sequences were analyzed for homology to the Arabidopsis genome using the BLASTN program of the National Center for Biotechnology Information (<http://www.ncbi.nlm.nih.gov/>).

**B. napus BAC library screening:** The BAC clones were derived from DH12075 genomic DNA partially digested with either *Hind*III (TH, BNID, and BNIH clones) or *Bam*HI (BNIB) and ligated into pINDIGOBAC-5 (except those named TH, which were ligated into pBELOBAC11). The BAC clones were screened either by hybridization of probes to doubled-spotted macro-arrayed clones as described in RYDER *et al.* (2001) or by PCR amplification from four-dimensional pools of BAC DNA.

## RESULTS

**Strategy for fine mapping blackleg resistance loci:** Initially, the genotype and resistance phenotype were determined for a small number of individuals from each of the two backcross populations. As expected, in both populations the resistance phenotype mapped as a single dominant locus to linkage group N7 (MAYERHOFER *et al.* 1997; RIMMER *et al.* 1999). Further mapping in each population was targeted to the resistance gene regions by assessing only the phenotype of lines with recombination events between the markers flanking the resistance loci, with the most extensive mapping carried out in the Shiralee population. Global comparative mapping between *B. napus* and Arabidopsis had shown that the identified region of N7 displayed some homology to the bottom of Arabidopsis chromosome 1 (PARKIN *et al.* 2005). Arabidopsis EST clones from this region and Brassica EST clones homologous to this region of Arabidopsis chromosome 1 were assayed as potential markers in an attempt to saturate the interval on N7 containing the resistance loci (see below). Concurrently *B. napus* BACs were identified from a library of DH12075 using markers mapped to the region and probes generated from BAC end sequences were used to further target the region. Similarly, clones from a genomic library of Shiralee in  $\lambda$ -DASHII were identified and used to generate probes from both end sequences. Altogether 91 Arabidopsis ESTs, 23 *B. napus* ESTs, 54 *B. napus* BAC end sequences, and 19 *B. napus*  $\lambda$ -DASHII clone end sequences for a total of 187 markers were assayed for their ability to identify polymorphic loci on N7 in the target interval.

**Inter- and intragenomic duplication of the R gene region:** Of the 187 markers tested, 50 identified 109 polymorphic loci in the Shiralee population that were mapped to *B. napus* linkage groups. The majority of the polymorphic loci (57) were mapped to linkage group N7 (Table 1, Figure 1). The subsequent selection of BC1 lines with recombination events between the flanking markers rapdD3 and AO39 allowed the locus order between these markers to be more accurately resolved compared to that of homologous loci mapped to duplicated regions of the *B. napus* genome (Figure 1).

**TABLE 1**  
**Location and number of marker loci on Brassica linkage groups**

Markers	No. of loci per linkage group				
	N7	N2	N12	N16	Unlinked
BNID37E13R	1				
BL2665	1				
BNIH148M04T7(SCAR)	1				
RL2534	2		1		
rapdD3	2				
TH8L20R	2				1
pO10	2				
est179D16	1				1
estM79E02	1				
est148G3	1	1	1		
est119E6	1				
est126M9	2		1		
est32A6	2		1	1	
est149M9	2	1	1	1	1
BNID116A5T7	1				
BNID116A5R	1	1	1	1	
rapd184	1				
BNID7804T7	1				1
es1450	1		1		3
ec2h2	1			1	
est187O3	1				
AO39(SCAR)	1				
est118L9	1	1	1		
est151K13	1				
estM64L03	1				
LL2348	1	1	2		
BNID97N1T7	1				1
BNID92M21T7(SCAR)	1				
estM70H09	1				
BNID12B17T7	1		1		
TH28E21T7	1		3		
ML853R(SCAR)	1				
estYAY175	1	1	2		1
est147H3	1			1	
I-D2	1				
est200P8	1	1	2	1	1
est32F9	1	1	2		
pW134	1				
pW150	1		1		
est144I14	1			1	
est149P9	1			1	
RL1856	1		1		
mi157	1				
est160P22	1				
est205F17	1				
estM67J04	1			1	
estM66B02	1			1	
est155E11	1			1	
est103P6	1				
BNID22D17T7	1		1		

Twenty-nine of the RFLP markers that identified loci on N7 (58%) also revealed duplicated loci. The duplicate loci were mapped to a second location on linkage group N7 (tandem repeat), to three other linkage groups, N2, N12, and N16, and several remained unlinked (Table 1,

Figure 1). All the RFLP markers hybridized to additional monomorphic bands that presumably represented further duplicated loci. The relatively insensitive process of Southern hybridization means that several more loci could remain undetected due to nucleotide divergence or small insertion/deletions of the duplicated sequences. N2 and N7 are derived from the *B. rapa* A-genome progenitor and N12 and N16 are derived from the *B. oleracea* C-genome progenitor of *B. napus* (PARKIN *et al.* 1995). N2 and N7 shared eight homologous marker loci and N12 and N16 shared four loci detected by the same marker, which indicated the presence of intragenomic duplications within the A and C genomes, respectively. Intergenomic duplications between the A and C genomes were also evident. N2/N12 and N7/N16 have been shown previously to be homeologous (PARKIN *et al.* 1995, 2003; SHARPE *et al.* 1995) and shared 8 and 11 homologous marker loci, respectively. However, the most extensive homeology was detected between N7 and N12. A total of 17 markers detected duplicated loci between the two linkage groups and both regions had undergone an intrachromosomal tandem duplication event. An intrachromosomal duplication event has previously been identified for the same region of linkage group O6 (equivalent to N16) of *B. oleracea* (RYDER *et al.* 2001). On the basis of these data, including the tandem duplication, it appears that the R gene region is present in at least three copies in the A genome and possibly in four copies in the C genome of Brassica species. However, the inability to map all detected loci due to lack of polymorphism precludes the absolute level of genome duplication from being determined.

**Intrachromosomal tandem duplication of R gene region:** Seven of the markers linked to the resistance gene on linkage group N7 had duplicated loci that formed a repeat unit contiguous with the interval defined by the flanking markers rapdD3 and AO39 (SCAR). The loci in this second unit cosegregated as a result of the selection of mapping lines (Figure 1). Consequently the orientation of the two repeats with respect to each other could not be established, although the duplication previously identified on the homeologous region of linkage group O6 (RYDER *et al.* 2001) suggests that the tandem duplication may be part of a larger inverted repeat. Five duplicated loci indicated the presence of a similar tandem duplication event on linkage group N12 (Figure 1).

**Correspondence of Cresor and Shiralee R gene region:** Cotyledon resistance to blackleg was mapped independently in the Shiralee- and Cresor-derived mapping populations that were generated using a common susceptible parent. Alignment of the two maps using common marker loci demonstrated that the Shiralee (LmR1) and Cresor (CLmR1) resistance genes mapped to the same genomic location on N7 and were flanked by common loci (Figure 2a). However, the Shiralee resistance gene cosegregated with three markers, BNID116A5T7,

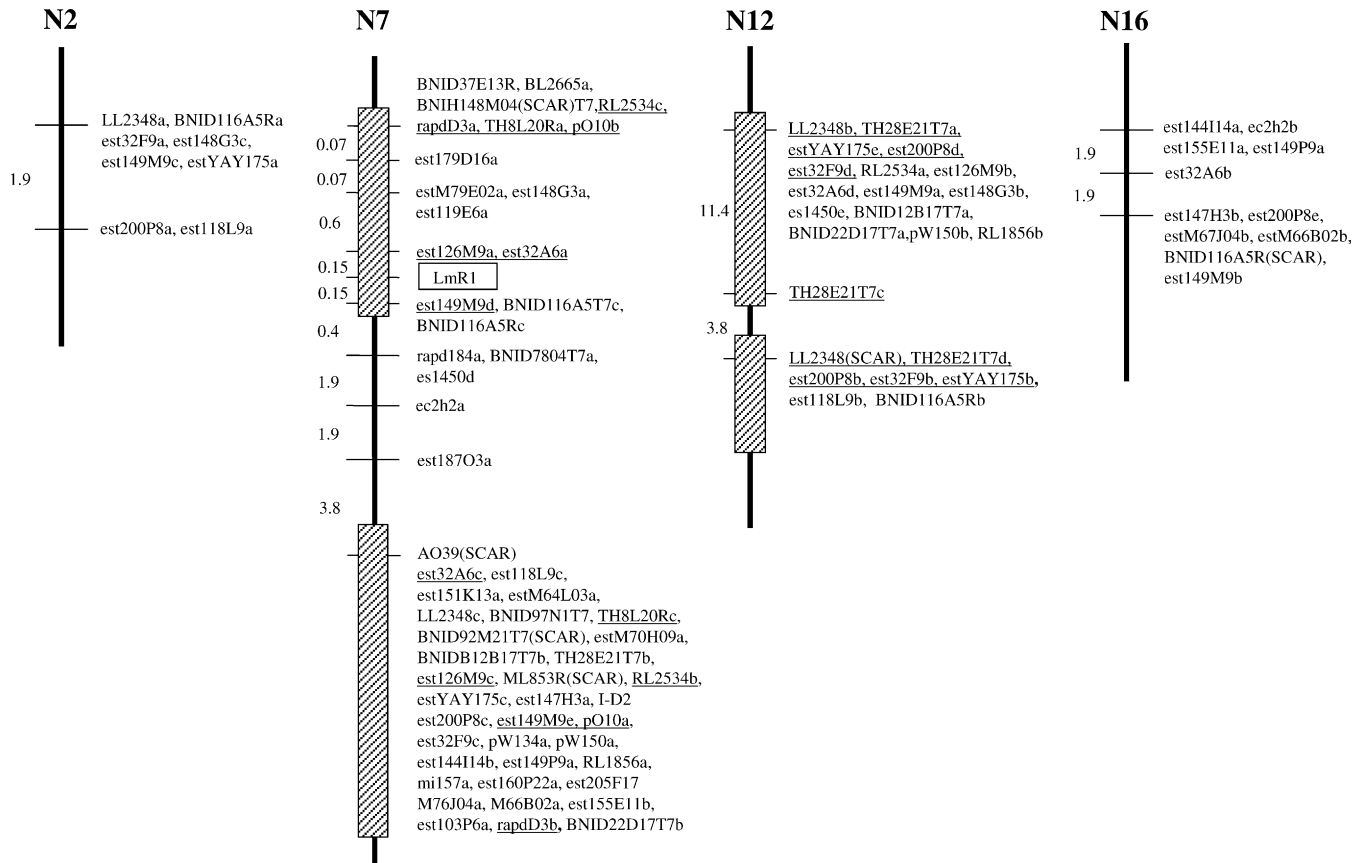


FIGURE 1.—Linkage map of Shiralee × PSA12 population. Marker loci linked to the blackleg resistance gene LmR1 as well as duplicated loci are shown. Vertical bars show linkage groups N2, N7, N12, and N16 of *Brassica napus*. Map distances are given in recombination units. Markers with tandem duplications on linkage groups N7 and N12 are underlined and are adjacent to hatched boxes.

BNID116A5R, and est149M9, which could not be mapped to this region in the Cresor population. The closest flanking markers with common alleles between the two mapping populations were est148G3 and a cluster of markers, rapd184, BNID7804T7, and es1450, which spanned a distance of 1.3 recombination units in Shiralee and 3.2 recombination units in Cresor (Figure 2b). These data suggest that the resistance genes from Shiralee and Cresor could be allelic or members of an R gene cluster.

**Comparative mapping with *A. thaliana*—search for candidate genes:** Sequences from all the markers linked to the resistance locus were subjected to BLASTN searches against the Arabidopsis genomic sequence. Using a score value of  $\geq 82$  as a cutoff for putative orthology, all the sequences had homologs at the bottom of Arabidopsis chromosome 1 (At1) (Figure 3, Table 2). The 16 markers between BNID116A5R and BNID37E13R were almost perfectly collinear between *B. napus* and Arabidopsis, except for marker est179D16. The order of markers below the R gene from rapd184 to AO39 suggests inversion of a collinear block between the two genomes. The extent of collinearity between this region of At1 and the second duplicated region on N7 could not

be accurately determined since the markers in this region were cosegregating.

The LmR1 gene from Shiralee was found to cosegregate with three markers: est149M9, BNID116A5R, and BNID116A5T7. The sequences of two, est149M9 and BNID116A5R, were homologous to two adjacent annotated Arabidopsis genes, At1g80670 (WD-40 repeat family protein) and At1g80680 (nucleoporin family protein), neither of which has been implicated previously in a plant's response to pathogens. Due to the inversion directly flanking the map position of LmR1, extending the search for candidate genes to an area delimited by the flanking markers, est126M9 and rapd184, identifies three regions on At1 (Figure 3). The first is a collinear region between markers est126M9 and BNID116A5R covering an interval of  $\sim 43$  kb on At1 and containing 15 annotated genes. Two of the 15 predicted genes contain protein motifs that have been associated with disease resistance genes, namely At1g80630 (leucine-rich repeat motif) and At1g80640 (protein kinase motif). The second interval on At1 that could contain candidate genes lies between markers BNID116A5R and YAY175 and the third lies between rapd184 and BNID37E13R

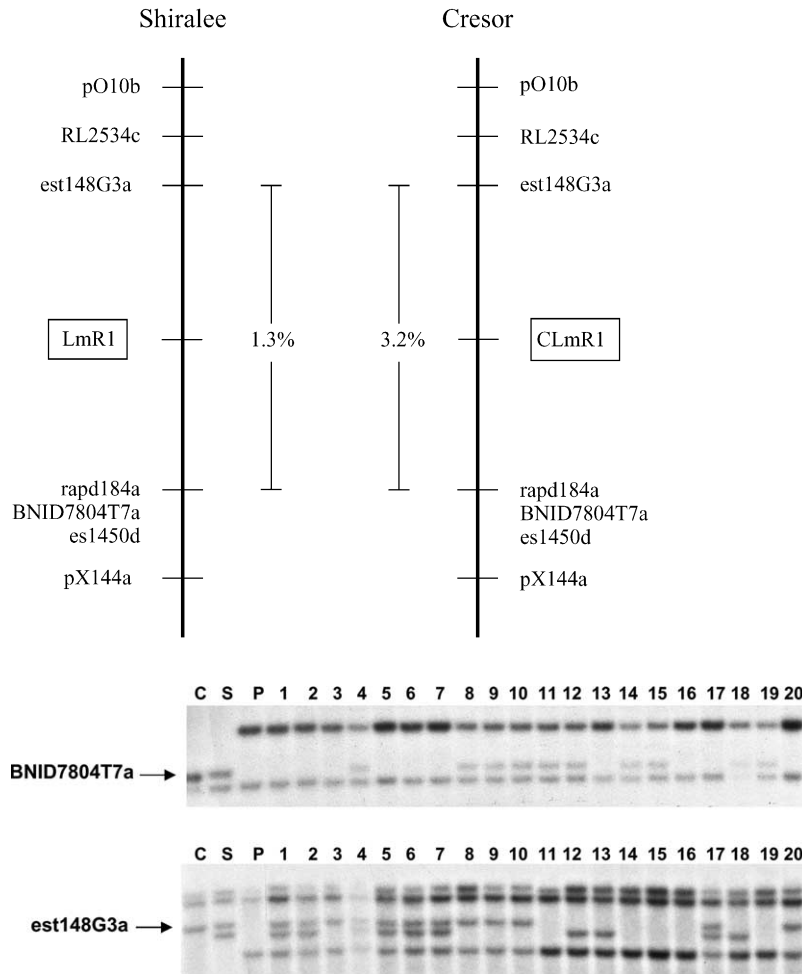


FIGURE 2.—(a) Comparison of flanking markers of the blackleg resistance genes from Shiralee (LmR1) and Cresor (CLmR1) on linkage group N7. Only marker loci with identical Southern bands in both populations are shown. Genetic distances are given in recombination units. (b) Southern analysis of flanking markers BNID7804T7 and est148G3a in segregating populations derived from Shiralee and Cresor (DH12075). The arrows show the bands for loci BNID7804T7a and est148G3a, which are tightly linked to the resistance genes LmR1 and CLmR1. Lanes C, S, and P show parental DNA from DH12075, Shiralee, and PSA12, respectively. Lanes 1–10 show DNA of 10 BCI lines derived from Shiralee × PSA12. Lanes 11–20 show DNA from 10 BCI lines derived from DH12075 × PSA12. The two Southern analyses were generated using different BCI lines. All DNAs were digested with *EcoRV*.

on Arabidopsis chromosome At1. These latter two regions contain 26 and 24 annotated Arabidopsis genes, respectively. Although no characterized R genes have been mapped to these regions, two protein kinases (At1g80870 and At1g79640), two wall-associated kinases (At1g79670 and At1g79680), and one LRR transmembrane protein kinase (At1g79620) were found in these regions.

## DISCUSSION

*A. thaliana* is a close relative of Brassica species and it has been recognized that the sequence information and genomic resources of the extensively studied model organism could be used for marker development, map-based gene cloning, and candidate gene identification in Brassica crops (reviewed in PFLIEGER *et al.* 2001; SCHMIDT *et al.* 2001, 2002; SNOWDON and FRIEDT 2004). The anticipated duplications due to the amphidiploid nature of the *B. napus* genome and the ancestral duplications observed in its diploid progenitors (PARKIN *et al.* 2003), as well as the occurrence of rearrangements within collinear regions, can pose impediments to such

applications. In this study, the fine mapping of the blackleg R gene was further complicated by ancient duplication of the collinear genomic region in Arabidopsis and tandem intrachromosomal duplication of the region containing the R gene in *B. napus*.

We detected intergenomic duplications of A-genome N7 loci on N12 and N16 of the C genome. Previous analyses showed that N7 and N16 are primary homeologs, but in the region containing the blackleg resistance locus we found that the highest number of duplicated loci was shared between N7 and N12. A more extensive interval from these two linkage groups has also been shown to be homologous by identifying the collinear region on Arabidopsis chromosome 1 (PARKIN *et al.* 2005).

A further layer of complexity was added through intragenomic duplication of marker loci within the A and C genomes. Such duplications within the diploid Brassica genomes have been described in previous mapping studies and suggest recent evolution of the diploid genomes from a common polyploid ancestor (LAGERCRANTZ 1998; LAN *et al.* 2000; O'NEILL and BANCROFT 2000; PARKIN *et al.* 2002). The majority of markers, linked to the blackleg resistance locus, were localized on two

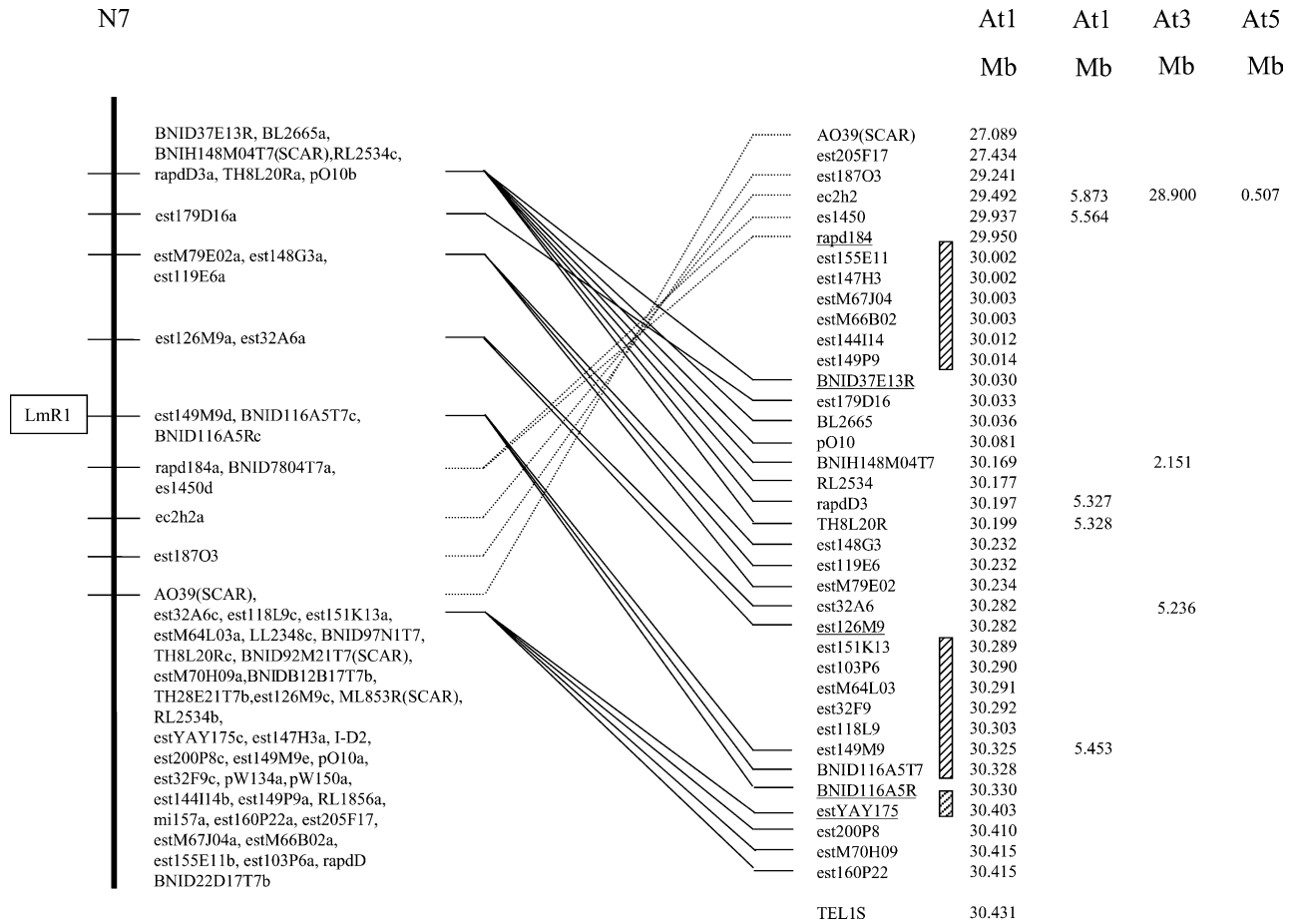


FIGURE 3.—Comparison of the LmR1 locus on linkage group N7 of *Brassica napus* with the physical maps of Arabidopsis chromosomes At1, At3, and At5. Averaged positions of markers on Arabidopsis chromosomes are given in megabases as described on the Arabidopsis Information Resource web site (<http://www.Arabidopsis.org/>). Only orthologous loci on the repeat unit carrying the resistance gene are connected by lines for better clarity. Dotted lines depict the inversion of marker orders between the two genomes. Hatched boxes flanked by underlined markers show the areas on At1 that were analyzed for possible candidate resistance genes.

linkage groups of the A or C genome (N2/N7 and N12/N16, respectively), suggesting chromosomal duplication of the region. A large number of monomorphic loci remained unmapped, which may reflect the choice of mapping parents and/or the fact that only a small genetic interval was analyzed; however, this made it difficult to estimate the copy number for this region. Additionally, the R gene was found to lie within an intrachromosomal tandem duplication on N7. The presence of these internal duplications at homologous regions on the A and C genome (N7 and N12) indicates that they took place before the divergence of *B. rapa* and *B. oleracea* into separate species. Tandem and segmental duplications of resistance genes have frequently been observed in other plants. Genomewide analyses of the organization of NBS-LRR genes in Arabidopsis suggest that tandem duplications distribute and separate this class of resistance genes in the genome and contribute to the diversification of gene families (ARABIDOPSIS GENOME INITIATIVE 2000; reviewed in LEISTER 2004). Linkage group N7 (alternative nomenclature LG6) has

already been shown to be a valuable source of blackleg resistance loci, harboring at least five different loci (FERREIRA *et al.* 1995; MAYERHOFER *et al.* 1997; RIMMER *et al.* 1999). It seems likely that the tandem duplication could harbor a homolog of LmR1, which although nonfunctional in Shiralee, might reveal the identity of one of the additional blackleg loci already assigned to N7. The detected tandem segments are restricted to a small genetic interval and were uncovered only during the fine-scale mapping of the LmR1 locus. It is expected that the genomic structure around the resistance locus will prove to be even more complex; this will be determined through sequencing of *B. napus* BAC clones spanning the R gene.

Collinearity between the region carrying the resistance gene and the TEL1S end of chromosome 1 of Arabidopsis was established and this information was exploited to saturate the R gene region with markers. However, due to limited polymorphism in the Brassica populations only 26% of the analyzed markers could be genetically mapped and only a fraction could be

**TABLE 2**  
**Location and score values of markers on Arabidopsis chromosomes**

Markers	Arabidopsis chromosome	Arabidopsis BAC clone	Arabidopsis BAC position (Mb)	BLASTN score
BNID37E13R	1	F20B17	29.942–30.032	143
BL2665	1	F19K16	29.99–30.109	218
BNIH148M04T7(SCAR)	3	F3E22	2.102–2.166	125
	1	F18B13	30.068–30.185	119
RL2534	1	F5I6	30.177–30.310	129
rapdD3	1	F18B13	30.068–30.185	133
	1	T16N11	5.296–5.387	103
TH8L20R	1	F5I6	30.177–30.310	287
	1	T16N11	5.296–5.387	145
pO10	1	F18B13	30.068–30.185	216
est179D16	1	F19K16	29.99–30.109	482
estM79E02	1	F5I6	30.177–30.310	212
est148G3	1	F5I6	30.177–30.310	256
est119E6	1	F5I6	30.177–30.310	218
est126M9	1	F5I6	30.177–30.310	618
est32A6	1	F5I6	30.177–30.310	777
	1	MJK13	5.185–5.268	712
est149M9	1	F23A5	30.320–30.430	228
	1	F7H2	5.387–5.464	123
BNID116A5T7	1	F23A5	30.320–30.430	222
BNID116A5R	1	F23A5	30.320–30.430	434
rapd184	1	F20B17	29.942–30.032	115
BNID7804T7	1	F25O15	15.315–15.425	86
	1	F8L2	14.294–14.386	86
	3	T28G19	13.689–13.810	84
es1450	1	F3O9	5.544–5.659	254
	1	T8K14	29.864–29.942	149
ec2h2	1	F3F9	29.435–29.531	196
	1	F20D23	5.808–5.923	76
	5	T1E22	0.451–0.547	208
	3	F3L24	2.803–2.910	94
est187O3	1	T32E8	29.197–29.279	761
AO39(SCAR)	1	F17M19	27.031–27.111	86
est118L9	1	F5I6	30.177–30.310	517
est151K13	1	F5I6	30.177–30.310	609
estM64L03	1	F5I6	30.177–30.310	480
LL2348	1	F5I6	30.177–30.310	480
BNID97N1T7	1	F5I6	30.177–30.310	168
BNID92M21T7(SCAR)	1	F23A5	30.320–30.430	82
estM70H09	1	F23A5	30.320–30.430	246
BNID12B17T7	1	F18B13	30.068–30.185	218
TH28E21T7	1	T21F11	30.233–30.323	121
	3	T28G19	13.689–13.810	80
	1	T18N24	14.196–14.294	80
	1	T25F15	13.592–13.673	80
	1	F25O15	15.315–15.425	80
	3	T18B3	13.822–13.900	80
	1	F8L2	14.294–14.386	80
	1	T17D15	21.320–21.445	80
	1	T2P3	17.102–17.181	80
ML853R(SCAR)	1	F5I6	30.177–30.310	113
estYAY175	1	F23A5	30.320–30.430	706
	1	T24D18	5.464–5.545	280
est147H3	1	F20B17	29.942–30.032	676
est200P8	1	F23A5	30.320–30.430	533
est32F9	1	F16N3	17.396–17.568	394
	1	F5I6	30.177–30.310	505

(continued)



**TABLE 2**  
(Continued)

Markers	Arabidopsis chromosome	Arabidopsis BAC clone	Arabidopsis BAC position (Mb)	BLASTN score
	5	MUA22	4.524–4.589	82
	1	F28K20	11.088–11.165	80
pW134	1	F1N21	25.140–25.255	157
pW150	1	F5A8	25.050–25.152	147
est144I14	1	F20B17	29.942–30.032	519
est149P9	1	F20B17	29.942–30.032	202
RL1856	1	F23A5	30.320–30.430	68
mi157	1	F23A5	30.320–30.430	1203
est160P22	1	F23A5	30.320–30.430	462
est205F17	1	F3N23	27.401–27.518	638
estM67J04	1	F20B17	29.942–30.032	468
estM66B02	1	F20B17	29.942–30.032	515
est155E11	1	F20B17	29.942–30.032	535
est103P6	1	F5I6	30.177–30.310	490
BNID22D17T7	1	F18B13	30.068–30.185	113
	1	T18N24	14.196–14.294	78
	3	T28G19	13.689–13.810	78
	1	T17D15	21.320–21.445	78
	3	T25F15	13.592–13.673	76
	1	F25O15	15.315–15.425	76
	1	T18B3	13.822–13.900	76
	1	F8L2	14.294–14.386	76
	1	T2P3	17.102–17.181	76

separated by recombination events, resulting in a cluster of markers on the N7 linkage group. Ancient segmental duplications of large portions of the genome exist in Arabidopsis and the bottom of Arabidopsis chromosome 1 shares homology with a region on the upper arm of chromosome 1 (ARABIDOPSIS GENOME INITIATIVE 2000). This duplicated region on chromosome 1 has been shown to be collinear with six different regions of the *B. napus* genome, but not with N2/N12 or N7/N16 (PARKIN *et al.* 2005), and no comparable tandem repeat of the region has been identified in Arabidopsis. These data suggest that the tandem duplication took place in the Brassica lineage after the separation of the two genera. Although the presence of such duplications has been suggested previously (PARKIN *et al.* 2002), this is the first substantial evidence of a segmental duplication that postdates the evolution of the Brassica and Arabidopsis lineages. It is possible that the presence of such tandem duplications will become more evident as further physical mapping of the Brassica genomes takes place. Such events could contribute not only to the expansion of the genome size in Brassicas but also to the extensive phenotypic variation found within the diploid Brassicas.

Arabidopsis-derived resistance against *L. maculans* has been transferred to *B. napus* through somatic hybridization (BOHMAN *et al.* 2002). Two regions on chromosome 3 of Arabidopsis were found to be associated with adult-leaf resistance in *B. napus*. An Arabidopsis EMS

mutant, severely compromised in its resistance to the blackleg fungus, was identified in the same laboratory (BOHMAN *et al.* 2004). Genetic analysis suggested the presence of a recessive gene linked to an area of Arabidopsis chromosome 2, which did not contain any previously characterized disease resistance genes. The resistance gene from Shiralee was mapped to a small region collinear with Arabidopsis chromosome 1, which has not been implicated previously in blackleg resistance. Although no characterized R genes were identified in the region, several genes were found that encode proteins containing either protein kinase or leucine-rich repeat domains; such structural motifs have been suggested as characteristic features that can be used to identify plant resistance genes. T-DNA insertional mutants for most of the 65 genes in the collinear region are available and were obtained from Arabidopsis Biological Resource Center. None of the mutant lines analyzed to date, including knockouts for two of the protein kinase domain proteins, have shown susceptibility to blackleg infection compared to wild type (data not shown).

Since both *B. napus* and Arabidopsis have highly duplicated genomes it is crucial that orthologous rather than paralogous sequences are compared when looking for regions with the same gene content. Although some Brassica loci from the R gene region identified sequences on other regions of the Arabidopsis genome, all the Brassica sequences linked to the resistance locus were homologous to an area between 27 and 30 Mb on At1

with a minimum BLASTN score of 82, an empirically determined cutoff for orthology as determined by LUKENS *et al.* (2003). This supports the assumption that candidate genes should be found in this region of the Arabidopsis genome; however, it is probable that fine-scale rearrangements of this complex Brassica locus will have occurred with respect to Arabidopsis. BROWN *et al.* (2003) exploited collinearity with Arabidopsis to clone the Rfo restorer gene from radish. Interestingly the Rfo-equivalent gene was not present at the collinear location in Arabidopsis but was found ~40 kb from this site. Similar events could be imagined at plant resistance loci that have been shown to contain multiple, tightly linked genes that are under constant adaptive pressure to create novel resistance specificities and are prone to gene duplication and intergenic recombination. Thus, the blackleg R gene may have originated from another region of the Arabidopsis genome and been transposed into the region on N7 that is homologous to Arabidopsis chromosome 1. Such changes in the microsynteny will be determined only through sequencing of *B. napus* BAC clones spanning the region. Another possibility for the absence of a classical candidate R gene for blackleg resistance in the region could be more fundamental, that Arabidopsis in fact displays a nonhost response to *L. maculans*. BOHMAN *et al.* (2004) found that susceptibility among Arabidopsis ecotypes is extremely rare and the disease symptoms were delayed compared to those of *B. napus*, suggesting a resistance mechanism independent from known host defense pathways.

Single resistance genes corresponding to the prevalent avirulent race within the pathogen population can be effective in the field but the resistance usually breaks down fairly quickly due to the rapid evolution of the matching *L. maculans* race or directed selection of a virulent race. "Pyramiding" of resistance genes could delay such a breakdown since more mutational events will be needed within the pathogen population to overcome the new resistance and there will be no selective advantage for any one race. We originally intended to use the markers generated during the fine mapping of the Shiralee and Cresor R genes for this purpose. However, our results indicated a very close proximity for the two genes, which makes such an approach impractical. This find is perhaps not surprising since both the adult-plant resistance of Cresor and the cotyledon resistance of Shiralee have been mapped to linkage group LG6/N7 in separate studies (MAYERHOFER *et al.* 1997; RIMMER *et al.* 1999), and although of very different geographical origin, their breeding pedigrees suggest that there may be a common link. Since Cresus\*3 was used in the generation of the French variety Cresor (Canbra × Cresus\*3) and Cresus-o-Precose, a seed stock derived from Cresus, was used in the generation of the Australian variety Shiralee (Haya// Zephyr/ Bronowski/3/RC33/// BJ168/ Cresus-o-Precose) (SERNYK 1994), our data suggest that the resistance genes from Shiralee and Cresor could

be alleles of the same resistance gene or members of the same gene cluster. Classical and molecular genetic studies on a wide array of plant-pathogen systems have demonstrated that R loci can be single genes with multiple alleles (like the 13 alleles for the L locus in flax) or, more commonly, clusters of genes that can affect resistance to different pathogens (reviewed in MICHELMORE and MEYERS 1998). A similar close relationship has been suggested in earlier reports between cotyledon resistance genes from Shiralee and Maluka (MAYERHOFER *et al.* 1997), between those from Maluka and RB87-62 (RIMMER *et al.* 1999), and between the resistance genes Rlm3, Rlm4, Rlm7, and Rlm9 present in the *B. napus* lines Maxol, Quinta, 23.1.1., and Darmor, respectively (DELOURME *et al.* 2004).

A sequence inversion between the collinear regions of the *B. napus* and Arabidopsis genomes close to the resistance gene, together with the recent intrachromosomal duplications on N7, caused complications in the early stages of the mapping project, frustrating the efforts to identify suitable markers. Once these fine-scale rearrangements were elucidated, Arabidopsis proved to be an invaluable source of markers, and the saturation of the R locus was greatly accelerated. This emphasizes the need for fine-scale mapping before collinear relationships can be exploited for chromosome walking or candidate gene approaches.

We have initiated physical mapping of the blackleg resistance locus from Cresor and have identified BAC clones carrying the flanking markers and are in the process of sequencing these clones. Sequence data from the Cresor region and from the equivalent region from Shiralee will help to elucidate the structure, evolution, and specificities of R gene complexes in Brassica species. The duplication of the region containing LmR1 on linkage group N7 and the clustering of independent blackleg resistance genes on N7 suggest that the cloning of LmR1 and the subsequent capture of LmR1 homologs may identify additional blackleg resistance genes.

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