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ORIGINAL PAPER

Identification of two blackleg resistance genes and fine mapping of one of these two genes in a *Brassica napus* canola cultivar 'Surpass 400'

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Abstract Blackleg resistant cultivars have been developed through conventional breeding methods and are successfully used globally to control this disease in canola production. To clone blackleg resistance genes and to understand the mechanism underlying the resistance, a blackleg resistant canola cultivar 'Surpass 400' was used to develop a gene mapping population. A previously reported high density genetic map was used to find a resistance gene region that corresponded to linkage group N10 in *B. napus*. Differential interactions between the resistant lines and a pathogen isolate were discovered with two resistance genes *BLMR1* and *BLMR2* identified through linkage analysis of five genome-specific molecular markers. *BLMR1* provides resistance through the hypersensitive response that protects inoculated cotyledons from becoming infected, Unlike *BLMR1*, *BLMR2* slows down the development of individual infection loci. *BLMR1* and *BLMR2* segregated independently in two large F_3BC_2 populations. Fine mapping of *BLMR1* was performed with 12 genome-specific molecular markers. The closest marker with a genetic distance of 0.13 cM to *BLMR1* was identified, which lays a solid foundation for cloning *BLMR1*.

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Introduction

Blackleg, caused by *Leptosphaeria maculans*, is one of the most devastating diseases in *B. napus* canola production. Development of blackleg resistant canola cultivars is an effective method to control this disease in canola production. In particular, blackleg resistance is considered as one of the most important traits in the canola breeding programs of all seed companies in Canada, Europe and Australia. Mapping blackleg resistance genes and eventually cloning these genes will facilitate the transfer and pyramiding in *B. napus* of multiple different resistance genes through molecular marker-assisted selection. Mapping of blackleg resistance genes in *B. napus* has been performed for decades. Delourme et al. (2004) mapped *Rlm1*, *Rlm3*, *Rlm4*, *Rlm7* and *Rlm9* on linkage group N7 and other researchers also identified *LEM1*, *LmR1* and *cRLMm*, *cRLMrb*, *cRLMj* and *aRLMj* on the same linkage group (Ferreira et al. 1995; Mayerhofer et al. 1997; Rimmer 2006; Zhu and Rimmer 2003). Additionally, *Rlm2* in *B. napus* was mapped on linkage group N10 (Ansan-Melayah et al. 1998; Delourme et al. 2006) and also on this linkage group, two of three blackleg resistance genes *LepR1*, *LepR2* and *LepR3* that were introduced from wild *B. rapa* var. *sylvestris* were mapped (Yu et al. 2005, 2008). *LepR1* was mapped on linkage group N2 (Yu et al. 2005). Interestingly, all the currently mapped blackleg resistance genes are located in the A genome of *B. napus* (N2, N7 and N10 corresponding to R2, R7 and R10 in the A genome of *B. rapa*) while no blackleg resistance genes have been identified in the C genome of *B. napus*. Although cloning of *LmR1* and *CLmR1* was attempted in *B. napus* several years ago (Mayerhofer et al. 2005), the results suggested that cloning of these blackleg resistance genes will be very difficult due to the complex genome structure of *B. napus*.

Currently, no blackleg resistance gene has been cloned and characterized in this species.

B. napus (containing A and C genomes) is an amphidiploid species originated from natural interspecific hybridization of two diploid species, *B. rapa* (A genome) and *B. oleracea* (C genome). Genetic map construction in Brassica species and comparative genetic analysis between Arabidopsis and Brassica show a high level of chromosomal and gene duplication and rearrangement in all diploid and amphidiploid Brassica species. In particular, amphidiploid Brassica species have extremely complex genome structure and gene function redundancy with at least two members in each family existing. For example, five *FLC* Brassica homologs controlling flowering time in *B. napus* were isolated and functionally characterized through cDNA library screening with the *AtFLC* gene as probe (Tadege et al. 2001). Since sequence similarity of homologous genes exist extensively within and between the A and C genomes, it is very difficult to develop genome-specific markers.

Since the *B. napus* genome is extremely complex, a map-based cloning method has not been successfully used yet to isolate important genes in *B. napus*. However, fine mapping of Mendelian genes is the starting point to clone important genes of interest in *B. napus*, an amphidiploid species. In this report, two flanking blackleg resistance genes on linkage group 10 have been identified and one of these two genes was fine mapped, which establishes a solid basis for cloning this blackleg resistance gene.

Materials and methods

Mapping populations and *L. maculans* isolates

A resistant *B. napus* canola cultivar 'Surpass 400' and a susceptible *B. napus* canola cultivar 'Westar' were used to produce gene mapping populations. A total of 908 F_2 and 10,896 F_3 individuals were inoculated and screened with *L. maculans* isolate 87-41, originally collected from *Brassica oleracea* (Yu et al. 2008), at the cotyledon stage. Two F_3 lines showing differential interactions with the *L. maculans* isolate 87-41 were backcrossed to 'Westar'. 16 F_3BC_1 lines were produced and their phenotypic segregation was characterized. After two resistance genes on linkage group N10 were separated, fine mapping was performed with 1,513 F_3BC_2 individuals that segregated at the locus corresponding to a vertical resistance phenotype.

Preparation of *L. maculans* isolate suspension

Pycnidial inoculum of the *L. maculans* isolate 87-41 was prepared according to the method described by Mengistu et al. (1991) with modifications as follows: the cotyledons with lesions were collected and washed three times in sterilized distilled water in a laminar hood. The cotyledons were then treated with 15% (V/V) bleach for 8 min with occasional agitation. After three 2-min washes with sterilized water, the cotyledons were transferred to petri dishes with V8 agar medium (250 ml V8 juice, 0.5 g $CaCO_3$ and 15 g granulated agar per litre). The dishes were placed in a temperature and light-controlled growth chamber. After incubating for a week, the medium was covered with black pycnidia and sometimes pink pycnidiospores were released. The spores were discharged by washing and scraping the agar surface with a sterilized glass slide. The inoculum concentration was adjusted with distilled water to 2×10^7 spores/ml from the stock solution.

Phenotype determination by inoculation

Cotyledons of individual plants were punctured with sharp pointed forceps. 10 µl of spore suspension was placed on each puncture. The plants were kept at room temperature for recovery overnight. The plants were then placed in controlled environmental growth conditions (14 h light at 20°C during day time and 18°C at night). In 12–16 days, disease symptoms were fully developed, and the disease severity was rated according to the classification of 0–9 (Chen and Fernando 2005). Disease severity ratings of 0–4 were classified as resistant while ratings of 5–9 were classified as susceptible. The cultivars 'Westar' and 'Surpass 400' and their F_1 progeny were used as controls for every inoculation run.

DNA extraction and SRAP marker development

A modified CTAB extraction procedure as described by Li and Quiros (2001) was used to extract DNA. SRAP was performed as described by Sun et al. (2007). A five fluorescent dye color set, '6-FAM', 'VIC', NED', 'PET' and 'LIZ', were used for signal detection using an ABI 3100 Genetic Analyzer (ABI, Toronto). The 'LIZ' color was used for the size standard, while the other four colors were used to label SRAP primers. The ultradense genetic map with 13,551 SRAP markers that was constructed with 58 DH lines from a cross of 'Westar' and 'Zhongyou 821' (Sun et al. 2007) was used to develop SRAP markers that were linked to the resistance gene. To use this map, the mapping population of 'Westar' and 'Surpass 400' was screened with the same primer sets as used for the ultradense map construction. DNA samples from eight resistant plants and eight susceptible plants were used to perform an initial round of SRAP marker analysis. After a molecular marker was found to be linked with disease resistance in the small population size, a larger number of individuals

from the same mapping population including another 64 resistant plants and 64 susceptible plants were tested to confirm the SRAP markers that were still linked to the resistance gene. These confirmed SRAP markers were compared with the SRAP molecular markers on the ultradense map (Sun et al. 2007). If a SRAP marker being linked to the resistance gene shared the same SRAP primers and similar fragment size with a SRAP marker on the genetic map, this SRAP marker was considered an anchoring marker. After anchoring the SRAP markers linked to the resistance gene on the ultradense SRAP map, the SRAP molecular markers flanking the anchoring markers were used to confirm the linkage and find increasingly closer SRAP markers using known flanking SRAP markers on the ultradense genetic map to test the blackleg resistance mapping population.

SRAP marker sequencing and identification of Arabidopsis synteny

SRAP PCR products were separated with sequencing gels. The gels were stained with a silver staining kit (Promega, Toronto). The target markers were identified by comparing the band patterns with the marker patterns that were produced with the ABI 3100 Genetic Analyzer. DNA was eluted as described in molecular cloning (Sambrook and Russell 2001). The DNA was reamplified and compared with the original SRAP profile to confirm the correct position by running the PCR products on an ABI 3100 Genetic Analyzer. The confirmed DNA products were sequenced with a BigDye Terminator v3.1 kit following the ABI sequencing instruction (Applied BioSystems, ABI, Toronto, Canada).

BLAST analysis of the marker sequences was performed with the TAIR Arabidopsis database (http://www.arabidopsis.org). Sequences of some SRAP markers were found to have confident hits ($E < 1e^{-10}$) to Arabidopsis genes and considered be homologous to the Arabidopsis genes.

Development of genome-specific SCAR and SNP markers

BAC clone sequences on linkage group R10 of a *B. rapa* genetic map (http://www.brassica-rapa.org/BRGP/geneticMap.jsp) that corresponds to N10 in *B. napus* were selected to develop genome-specific codominant molecular markers. In total, nine BAC clones were selected and primers were designed in accordance with the BAC sequences. First, these primers were used to amplify *B. oleracea* DNA to obtain corresponding sequences in the C genome. Second, clulstalw (http://www.ebi.ac.uk/Tools/clustalw2/index.html) analysis was used to find the sequence differences between *B. rapa* and *B. oleracea*. Using Primer3 (http://frodo.wi.mit.edu/primer3), the different sequences were used to design the primers that amplify only the A genome DNA in *B. napus*. Then the A genome-specific primers were used to amplify 'Surpass 400' and 'Westar' to identify sequence insertions/deletions and single nucleotide polymorphisms (SNPs). Finally, these sequence differences were used to develop sequence characterized amplified polymorphism (SCAR) or SNP markers.

SCAR and SNP detection

For SCAR marker detection, a M13 primer sequence (CACGACGTTGTAAAACGAC) was added to one of two genome-specific primers of the SCAR markers. The M13 primer was labeled with four of five color fluorescent dyes, 6-FAM, VIC, NED, PET and LIZ (internal standard) (ABI, Toronto, Canada). For SCAR marker detection, two rounds of nested or half-nested PCR are required. First, the genome-specific primers were used to obtain PCR products containing deletion/insertion positions. Then a special PCR with three primers was performed. The first PCR reactions for SCAR marker detection were set up in a 10 µl mixture containing two genome-specific primers, 0.15 µM of each primer, 50 ng of genomic DNA, 0.375 mM dNTP, 1× PCR buffer, 1.5 mM $MgCl_2$ and 1 U of *Taq* polymerase. The PCR running program was 94°C, 3 min; 94°C, 1 min; 57°C with −0.8°C each cycle 1 min and 72°C, 1 min for 6 cycles; 94°C, 1 min; 57°C, 1 min and 72°C, 1 min for 25 cycles. In the second PCR, two primers and one labeled M13 primer were included in the PCR cocktail. The concentrations for one primer without the M13 tail and the labeled M13 were 0.15 µM and the concentration of another primer with M13 tail was 0.05 µM. Other components in the 10-µl reaction mixture included 2 µl of the first round PCR products that was diluted 100 times, 1× PCR buffer, 0.375 mM dNTP, 1.5 mM MgCl and 1 U *Taq*. The PCR running program was 94°C, 3 min; 94°C, 1 min; 55°C, 1 min and 72°C, 1 min for 18 cycles and 72°C, 2 min. The PCR products were separated in an ABI 3100 Genetic analyzer. The data were collected and analyzed with ABI GenScan software and further transferred into images for scoring using Genographer software available at http://hordeum.oscs.montana.edu/genographer.

SNP detection as described by Rahman et al. (2008) was used. The genome-specific primers were used to obtain PCR products containing SNP positions. PCR reactions were performed as described in the first round of PCR of SCAR detection. In SNP detection, detection primers were added with a poly A tail to obtain different sizes of products that were used to pool samples before separation. The SNaPshot multiplex kit (ABI, California) was used following the instructions in the kit. The SNaPshot products were pooled first and 2 µl pooled DNA was mixed with

8 µl formamide containing 120 LIZ size standards (ABI, California). Then the DNA fragments were analyzed with an ABI 3100 Genetic Analyzer. Genotypes were scored manually, using peak color verification.

All primers used in this study were listed in Table 1.

Results

Segregation of blackleg resistance in the gene mapping populations

In the gene mapping populations of 'Westar' and 'Surpass 400', 908 F_2 individuals and 12 plants from each derived F_3 line were inoculated with a *L. maculans* isolate 87-41. In the F_2 population, there were 693 resistant plants and 215 susceptible plants showing a 3:1 segregation ratio (χ^2 test, $p = 0.36$). In the F_3 population, all plants in 232 F_3 lines were resistant, all plants in 209 F_3 lines were susceptible, and the remaining 467 F_3 lines showed segregation in these 12 tested plants in each line, showing a 1:2:1 segregation ratio (χ^2 test, $p = 0.38$). The segregation of the resistance gene in the F_2 and F_3 generations of the 'Westar' and 'Surpass 400' cross showed a 3:1 segregation ratio in the F_2 and a 1:2:1 segregation ratio in the F_3 families, suggesting that one dominant resistance gene controls the blackleg resistance in 'Surpass 400', consistent with the previous reports (Li and Cowling 2003; Yu et al. 2008).

Identification of linked SRAP markers on a consensus map

For marker analysis and gene mapping, DNA samples were prepared from all 908 F_2 plants, one plant from each of the

Table 1 Primers for genome-specific SCAR, SNP and SRAP markers

Marker name	Marker type	Primer name	Primer sequence 5′–3′
80A08a	SNP	80A08A	GGTATCGCATTCTGTGACTA
		80A08B	GGAGATGTGCTTCAACGTGA
		80A08R	A_{28}CATTCTTGGGCCGTAGG
80E24a	SNP	80E24A	GACAAACACAATGGACTCAA
		80E24B	GAGGTAGAGAAAGACGAAGA
		80E24R	A_{20}TCGTTTAAGGAATGTGCCAA
9B23a	SNP	9B23A1	CCACAGTTTCTGGAGAC
		9B23B1	GTAGCAAAGGAATCAATTAA
		9B23R1	A_{19}GGAGACTTATGTCAAATCTCT
9B23b	SNP	9B23A2	GTTTGGGTTCTGCAGT
		9B23B2	GACTCCTGGTAGCTTGAACA
		9B23R2	A_{22}CCTACTCAAAGCAGCATC
9B23c	SNP	9B23A3	GCTTCTAGTGTGGTCTTCAC
		9B23B3	GGAGTAGACCGAGACATGAA
		9B23R3	A_{20}TTTTAGTTCACCCGTAAATC
87B10a	In/del	87B10A	CGTGAAACCTGGAAAGAACA
		87B10B	CCAGATCCATACAGTCGAGA
		87B10R	M_{13}CCGTCACAGCAAGCTATGAA
1J13a	SNP	1J13A1	CTTGGAGATCGATTTGA
		1J13B1	TACAGCTAATGACACCCTATAA
		1J13R1	A_{20}CATGTCCTCTTCCAACGA
1J13b	SNP	1J13A2	CCACTAGTACGTGCATCAGA
		1J13B2	ATCCGAGAGAGCTTCTCTGT
		1J13R2	A_{20}TTGTTATACTCAGACCCACC
10B23a	SNP	10B23A	GAAGTGGTAACCGAGAGACAA
		10B23B	AGGCGAAACTTCATCAGAGCA
		10B23R	$A_{11}M_{13}$GCAACTGTTCTCGTCTTC
12D09a	In/del	12D09A	TCCGATCACACGAGTGTTGA
		12D09B	CAACACAGTACACACAAGCA
		12D09R	M_{13}CCTTAGTACATTGCAATCAGT
R278	SRAP	EM1	GACTGCGTACGAATTCAAT
		BG28	GCTCTCCTGAACCGCTTG

M_{13}, 5′-CACGACGTTGTAAAACG-3′; A_n, n means the number of nucleotide 'A'

pure breeding 232 resistant F_3 lines, two plants from each of the pure breeding 209 susceptible F_3 lines, and 2–4 susceptible plants from each segregating F_3 line and in total 2,992 F_3 plants. For primer screening, eight susceptible plants and eight resistant plants from the F_2 mapping population were used to run SRAP molecular markers. 384 primer pairs were used for the initial screening and two SRAP markers R269 and G278 were found to co-segregate with the resistance gene in the 16 plants tested. By comparing these two SRAP markers with the SRAP molecular markers on the ultradense genetic recombination map, it was found that R269 corresponded to SRAP marker 1217Ar269 on N10 linkage group (Sun et al. 2007), but there was no corresponding SRAP marker to G278. After searching the polymorphism of the 32 SRAP markers flanking 1217Ar269 marker on N10 linkage group with the 'Westar' and 'Surpass 400' segregation populations, 210Ay442 and 1128BG275 on the map were found to co-segregate with the resistance gene.

Sequencing of SRAP markers and identification of synteny in Arabidopsis genome

The linked SRAP molecular markers G278, 1128BG275, 210Ay442 and 1217Ar269 were sequenced (NCBI accession numbers: G278, HQ158118; 1128BG275, HQ158119; 210AY442, HQ158120; 1217AR269, HQ158121). After BLAST analysis against the Arabidopsis database (http://www.arabidopsis.org), the sequence of SRAP marker 1128BG275 was found to have a match to At5g18840 (196 nt, *E* value: $4e^{-11}$) and that of G278 a match to At5g57345 (234 nt, *E* value: $1e^{-11}$) in Arabidopsis, respectively. Unfortunately, there was no solid hit in Arabidopsis for the sequences of the remaining two SRAP markers 1217Ar269 and 210Ay442. The corresponding genes to the SRAP marker sequences were located in two syntenic regions in Arabidopsis and according to the comparative genetic information, a corresponding region on linkage group R10 of *B. rapa* was found (http://www.brassica-rapa.org/BRGP/geneticMap.jsp).

Identification of two blackleg resistance genes on linkage group 10 of 'Surpass 400'

During the screening of F_3 lines with the isolate 87-41 of *L. maculans*, differential interactions were found. Two F_3 lines WS37 and WS45 showing different cotyledon lesions were backcrossed to 'Westar' and 16 F_3BC_1 lines were produced. When the resistance response was observed carefully at the 12–16 DAI (day after inoculation) that was relatively longer than that in the previous testing (10–12 DAI), cotyledon disease lesions in 5 out of 16 F_3BC_1 lines derived from F_3 WS37 and WS45 lines could be classified into three groups (Table 2). The disease symptoms in one group of cotyledons were similar to those of the susceptible parent 'Westar' and infection on the cotyledons resulted in extensive tissue death, accompanied by full sporulation (Fig. 1c, 'S' phenotype). Another group of cotyledons were similar to those of the resistant parent 'Surpass 400' and infection on cotyledons was restricted to a very limited area and blackening occurred only around inoculation sites (Fig. 1a, 'R_1' phenotype). The third group of cotyledons showed 3–5 mm brown necrotic lesions and no sporulation occurred (Fig. 1b, 'R_2' phenotype). The data suggested that there is more than one blackleg resistance gene in the region of linkage group N10.

Since the sequence of some BAC clones on linkage R10 of *B. rapa* is available (http://www.brassica-rapa.org/BRGP/geneticMap.jsp), more genome-specific SCAR and SNP molecular markers were developed according to the BAC clone sequence in the region anchoring the blackleg resistance gene(s). Five genome-specific molecular markers 80A08a, 80E24a, 87B10a, 1J13a, and 10B23a developed with five BAC clone sequence on linkage group R10 were used to analyze five F_3BC_1 lines WS37-1, WS37-2, WS37-3, WS37-4 and WS37-7, of which each consisted of 96 individuals and in total 480 individuals. After these individuals from the five F_3BC_1 lines were screened, 15 recombinants were identified (Table 3). As mentioned previously, three phenotypes were distinguished and designated as 'S' (susceptible), 'R_1' (strongly resistant

Table 2 Phenotypic segregation in sixteen F_3BC_1 lines derived from two F_3 lines WS 37 and WS45

F3BC1 lines from WS37	1	2	3	4	5	6	7	8
Phenotypes	R_1, R_2, S	R_1, S	R_1, R_2, S	R_1, S	S	R_1	R_1, R_2	S
F3BC1 lines from WS45	1	2	3	4	5	6	7	8
Phenotypes	R_1, R_2, S	R_2, S	R_2, S	R_1, R_2, S	S	R_1, R_2, S	R_2, S	S

Eight F_3BC_1 lines derived from each F_3 line were included

'R_1', represents the strong resistance phenotype; 'R_2', weak resistance phenotype; 'S', susceptible phenotype

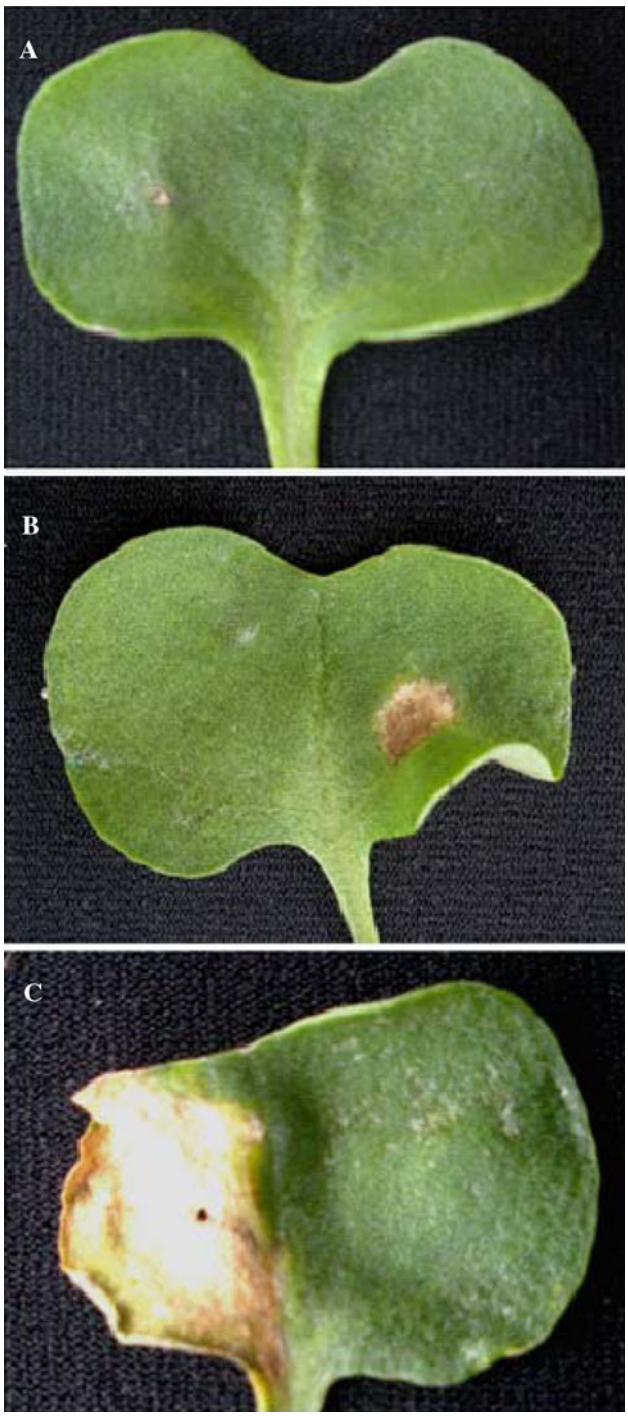

Fig. 1 Phenotypic segregation in F_3 lines of 'Surpass 400' (blackleg resistant parent) and 'Westar' (susceptible parent) showing cotyledons inoculated with *L. maculans* isolate 87-41. **a** Resistant phenotype 'R_1', similar to 'Surpass 400'; **b** resistant phenotype 'R_2', weak resistance; **c** susceptible phenotype 'S', similar to 'Westar')

phenotype, hypersensitive response) and 'R_2' (weakly resistant phenotype, delayed development of inoculation loci). Interestingly, the recombinants corresponding to the 'R_1' phenotypes shared a segregation pattern that

Table 3 Phenotypes and genotypes of recombinants in the F_3BC_1 population detected by five genome-specific molecular markers

Plant no.	Phenotype	80A08a	80E24a	87B10a	1J13a	10B23a
37-1-8	R_1	H	H	H	H	H
37-1-2	S	W	W	W	W	W
37-1-3	S	W	W	H	H	W
37-7-9	R_1	W	H	H	H	H
37-1-7	R_1	H	H	H	W	W
37-3-2	R_1	H	H	H	W	W
37-4-1	R_1	H	H	H	W	W
37-1-12	R_1	H	H	H	H	W
37-2-1	R_1	H	H	H	H	W
37-2-7	R_1	H	H	H	H	W
37-1-1	R_2	W	W	H	H	H
37-1-9	R_2	W	W	H	H	H
37-7-4	R_2	W	W	W	H	H
37-7-5	R_2	W	W	W	H	H
37-7-7	R_2	W	W	W	H	H
37-7-8	R_2	W	W	W	H	H
37-3-3	R_2	W	W	W	W	H

Two parental-like individuals 37-1-8 and 37-1-2 and fifteen recombinants 37-1-3 to 37-3-3 were detected in the F_3BC_1 population. 80A08a, 80E24a, 87B10a, 1J13a and 10B23a are five genome-specific molecular markers

'R_1', represents the strongly resistant phenotype; 'R_2', weakly resistant phenotype, 'S', susceptible phenotype; W, 'Westar'-like genotypes; H, heterozygous genotypes of 'Westar' and 'Surpass 400'

corresponded to a resistance gene and so did the recombinants corresponding to the 'R_2' phenotypes, suggesting that there is another blackleg resistance gene in 'Surpass 400'. Consequently, the data confirmed that there are two blackleg resistance genes in this region of linkage group 10. One of these two blackleg resistance genes is closest to the molecular marker 80E24a and no recombination between this molecular marker and the resistance gene was found in 480 F_3BC_1 individuals. Another blackleg resistance gene is located outside the molecular marker 10B23a. Although one blackleg resistance gene, *LepR3* was mapped in this region (Yu et al. 2008), the current results allowed the identification of two blackleg resistance genes and since it is not possible to tell which one corresponds to the reported *LepR3* locus, these two blackleg resistance genes were designated *BLMR1* and *BLMR2* in the following analysis. The *BLMR1* gene confers the 'R_1' phenotypes and the *BLMR2* gene corresponds to the 'R_2' phenotypes.

Fine mapping of the blackleg resistance gene *BLMR1*

The recombinant individuals WS37-1-7, WS37-3-2 and WS37-4-1 identified in the F_3BC_1 population were used as female parent to backcross to 'Westar' to produce a large

F_3BC_2 population for fine mapping of *BLMR1* since only *BLMR1* existed in these recombinants judged by the flanking molecular markers. In the F_3BC_2 population, 1,513 individuals were inoculated and 749 resistant to 764 susceptible, obtained, fitting a 1:1 segregation ratio ($\chi^2 = 0.1487$, $p = 0.70$). To fine map *BLMR1*, another five genome-specific SNP and SCAR markers were developed. These new molecular markers were combined with five genome-specific SCAR and SNP that were used to separate the two blackleg resistance genes. According to preliminary mapping, *BLMR1* was located between markers 80A08a and 87B10a and *BLMR2*, in a region covering molecular markers 1J13a, and 10B23a. To fine map *BLMR1*, 1,513 F_3BC_2 individuals were screened with eight markers. Two recombinants out of 1,513 individuals between 80E24a and *BLMR1* were identified, which was equal to a genetic distance of approximately 0.13 cM between the marker and the resistance gene. Therefore, a genetic map containing the blackleg resistance genes *BLMR1* and *BLMR2* was constructed (Fig. 2).

Discussion

Since an ultradense genetic recombination map constructed with over 13,500 SRAP markers is available (Sun et al. 2007), a strategy of anchoring SRAP markers linked to a blackleg resistance gene(s) on the ultradense map was implemented in this study. The results demonstrated that the ultradense map is very useful for map-based gene cloning. Although the gene-tagging population for the blackleg resistance was different from the mapping population, some of the SRAP markers on the ultradense genetic recombination map also showed polymorphism in the gene-tagging populations that allowed anchoring of the SRAP markers developed with other populations, such as the SRAP marker R269 from 'Westar' × 'Surpass 400' population. Using the anchoring of a SRAP marker as a starting point, flanking SRAP markers on the ultradense map were used to pinpoint the region that contained the gene of interest. Among the SRAP markers tested in the new population, approximately one-third of the SRAP markers showed polymorphism and sequencing these SRAP markers allowed the identification of syntenic regions in Arabidopsis.

Since the conserved sequences between Arabidopsis and Brassicas are mostly located in the open reading frame region, it is important for a marker system to detect these gene regions. As reported by Li and Quiros (2001), nearly half of SRAP markers are located inside genes. Consistent with previous results, two of four sequenced SRAP markers

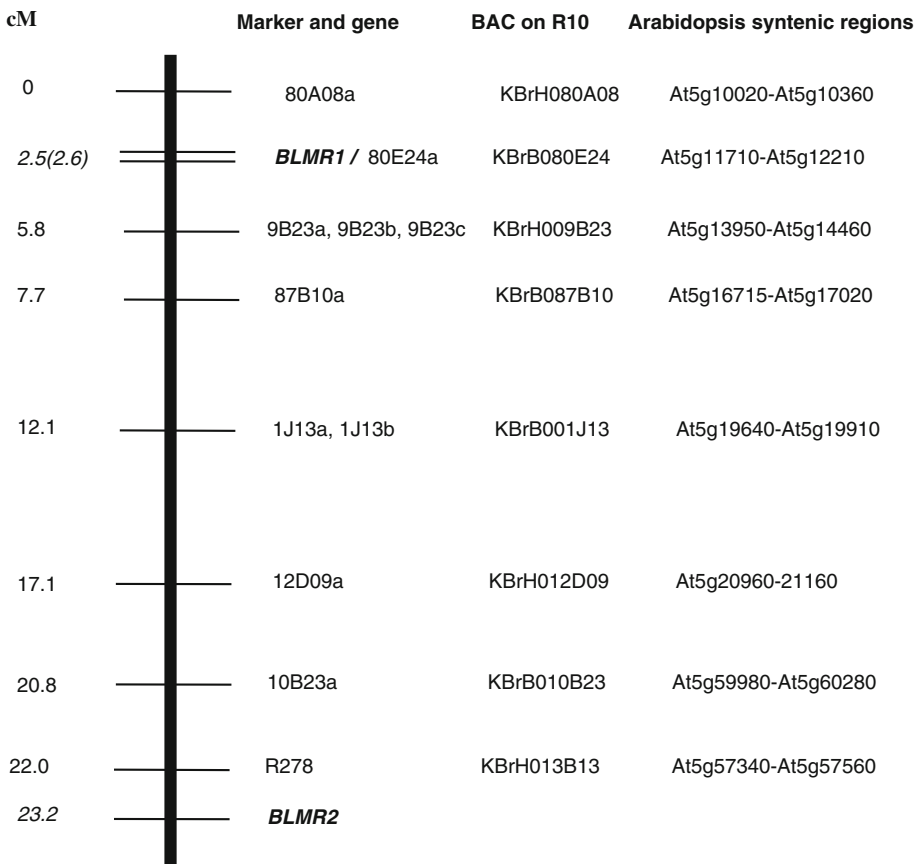

Fig. 2 Mapping of two blackleg resistance gene *BLMR1* and *BLMR2* on a part of linkage group N10 in *B. napus*. The BAC clones on the *right panel* are located on R10 of *B. rapa* (available at http://www.brassica-rapa.org/BRGP/geneticMap.jsp)

in this report corresponded to Arabidopsis-annotated genes that allowed the identification of a chromosomal region in *B. rapa* map (http://www.brassica-rapa.org/BRGP/geneticMap.jsp). There were two syntenic regions inferred with the BLAST analysis of the sequences of SRAP markers on the ultradense map against the corresponding genes in Arabidopsis. The following analysis suggested that each of those two syntenic regions contained one disease-resistant gene.

Two blackleg resistance genes on linkage group N10

In the *B. napus* canola cultivar 'Surpass 400', one blackleg resistance gene *LepR3* was mapped on linkage group N10 in a previous report (Yu et al. 2008). According to the preliminary segregation data in this report, one gene was suggested to be segregating in the mapping population, consistent with a previous report. However, when the plants were kept longer after inoculation (14–16 DPI), careful observation allowed the discovery of differential interactions among the F_3 individuals, which resulted in the identification of three phenotypes in the segregating population. Actually, the '*R2*' phenotype corresponds to the weak resistance that is controlled by the BRLM2 locus and this resistance gene was shown to slow down the development of symptoms or the growth of the pathogen. Eventually, precise genomic specific molecular markers were developed using the BAC clone sequence of a *B. rapa* genetic map that allowed the separation of *BLRM1* from *BLMR2*. In the previous report, *LepR3* was mapped using a segregating population derived from a cross and backcross of 'Westar' and 'Surpass 400' (Yu et al. 2008). In the current report, the same parents and the same *L. maculans* isolate 87-41 were used to separate and map two blackleg resistance genes in the similar region. According to the gene order list in the previous paper, *LepR3* is located in a region of At5g16870 to At5g13640 in Arabidopsis. Compared with the current data, it is found that *LepR3* was located in between *BLMR1* and *BLMR2*. Therefore, it suggests that the *LepR3* may represent the resistance combination of *BLMR1* and *BLMR2* in 'Surpass 400'. On linkage group N10, two other blackleg resistance genes *LepR2* and *Rlm2* were mapped previously (Ansan-Melayah et al. 1998; Delourme et al. 2004; Yu et al. 2005). *LepR2* was suggested to be different from *LepR3* on the basis of genetic analysis (Yu et al. 2008). Since there is no gene information of the molecular markers that are linked to *LepR2*, it is difficult to judge the relationship between *LepR2*, *BLMR1* and *BLMR2*. Similarly, *Rlm2* was mapped in different cultivars and it is not easy to compare with *BLMR1* and *BLMR2*. The comparison of *BLMR1* and *BLMR2* from 'Surpass 400' and *Rlm2* from 'Glacier' is underway using the genome-specific markers and the fine mapping information in this report.

Two blackleg resistance genes are located in two syntenic regions in Arabidopsis

Brassica genomes contain conservative blocks that correspond to parts of the Arabidopsis genome. The sequences of SRAP markers linked to blackleg resistance genes have confident BLAST hits to two Arabidopsis genes that are located in two regions of Arabidopsis chromosome 5. The blackleg resistance genes *BLMR1* was fine mapped with the genome-specific SCAR and SNP markers that were derived from the sequences of BAC clones on linkage group R10 in *B. rapa*. The genes in the BAC clone sequences are in two syntenic regions and the Brassica genes located to the *BLMR1* gene region have a similar order to their corresponding homologs in Arabidopsis. The *BLMR1* gene is located in a region that corresponds to Arabidopsis chromosome 5 from At5g10360 to At5g13950 (Fig. 2). Actually, *BLMR1* was identified to be located on the BAC clone KBrB080E24 and there is no corresponding Arabidopsis gene in the syntenic region. The candidate gene codes for a leucine-rich repeat receptor-like protein and was functionally confirmed through complementary transformation (unpublished data).

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