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ORIGINAL PAPER

Identification and mapping of a novel blackleg resistance locus *LepR4* in the progenies from *Brassica napus* × *B. rapa* subsp. *sylvestris*

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Abstract Blackleg, caused by *Leptosphaeria maculans*, is one of the most economically important diseases of *Brassica napus* worldwide. Two blackleg-resistant lines, 16S and 61446, were developed through interspecific hybridization between *B. napus* and *B. rapa* subsp. *sylvestris* and backcrossing to *B. napus*. Classical genetic analysis demonstrated that a single recessive gene in both lines conferred resistance to *L. maculans* and that the resistance alleles were allelic. Using BC_1 progeny derived from each resistant plant, this locus was mapped to *B. napus* linkage group N6 and was flanked by microsatellite markers sN2189b and sORH72a in an interval of about 10 cM, in a region equivalent to about 6 Mb of *B. rapa* DNA sequence. This new resistance gene locus was designated as *LepR4*. The two lines were evaluated for resistance to a wide range of *L. maculans* isolates using cotyledon inoculation tests under controlled environment conditions, and for stem canker resistance in blackleg field nurseries. Results indicated that line 16S, carrying *LepR4a*, was highly resistant to all isolates tested on cotyledons and had a high level of stem canker resistance under field conditions. Line 61446, carrying *LepR4b*, was only resistant to some of the isolates tested on cotyledons and was weakly resistant to stem canker under field conditions.

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Introduction

Resistance to blackleg disease, caused by the ascomycete fungus *Leptosphaeria maculans* (Desmaz.) Ces. et De Not. [anamorph: *Phoma lingam* (Tode ex Fr.) Desmaz.], has been identified in *Brassica* species including *B. rapa* L. (genome AA) (Mithen et al. 1987; Leflon et al. 2007), *B. napus* L. (genome AACC) (Rimmer and van den Berg 1992) and several B-genome (Chèvre et al. 1996, 1997; Christianson et al. 2006; Plieske et al. 1998) and C-genome (Mithen and Lewis 1988) species. Extensive efforts to map blackleg resistance genes have been carried out in *B. napus*, and both race non-specific and race-specific resistance have been widely used in *B. napus* breeding programs. Race non-specific resistance is quantitative, reduces the severity of symptoms and may be durable (Delourme et al. 2006). It is controlled by many genetic factors, some of which have been mapped through studies of quantitative trait loci (QTL). Two QTLs responsible for blackleg resistance in *B. napus* cultivar Crésor were identified using RFLP markers (Dion et al. 1995). Ten QTLs controlling blackleg resistance in *B. napus* cultivar Darmor were detected by analysis of RFLP and RAPD markers (Pilet et al. 1998), and 16 genomic regions were revealed for blackleg resistance in the crosses Darmor-*bzh* × Yudal and Darmor × Samourai (Pilet et al. 2001). The QTLs identified in Darmor were further validated through association mapping (Jestin et al. 2011). Multiple QTLs were identified across the four mapping populations derived from blackleg-resistant Australian cultivars, accounting for 13–33 % of the phenotypic variance (Kaur et al. 2009).

In contrast, race-specific resistance is qualitative and controlled by single resistance (R-) genes (Delourme et al. 2006). Delourme et al. (2004) mapped five R-genes, *Rlm1*, *Rlm3*, *Rlm4*, *Rlm7* and *Rlm9*, to *B. napus* linkage group N7 and one gene, *Rlm2*, to N10. *Rlm1*, *Rlm2* and *Rlm7* were also identified in *B. rapa* (Leflon et al. 2007). The R-genes *Rlm5* and *Rlm6*, derived from *B. juncea* (L.) Czern., *Rlm8* from *B. rapa* and *Rlm10* from *B. nigra* (L.) W. D. J. Koch have also been identified (Balesdent et al. 2002; Chèvre et al. 1996; Kutcher et al. 2010), but they have not been genetically mapped. Resistance to blackleg was identified in wild accessions of *B. rapa* subsp. *sylvestris* (BRS) (Mithen et al. 1987) and in *B. insularis* Moris, a species in the *B. oleracea* L. coenospecies complex (Mithen and Lewis 1988). The BRS blackleg resistance was transferred to *B. napus* using a resynthesized amphidiploid formed by interspecific hybridization between BRS and *B. oleracea* subsp. *alboglabra* (Crouch et al. 1994). Several cultivars have been derived from the resynthesized *B. napus* lines and adapted for use in Australia (van de Wouw et al. 2009). The R-genes *LepR1* and *LepR2* were identified in blackleg-resistant breeding lines originating from BRS and mapped to *B. napus* linkage groups N2 and N10, respectively (Yu et al. 2005), and *LepR3* was identified in Australian cultivars Surpass 400 and Hyola 60 and mapped to N10 (Yu et al. 2008).

Blackleg resistance conferred by single R-genes can be overcome within a few years because *L. maculans* populations evolve rapidly through genetic recombination during sexual reproduction. For example, in France, *Rlm1* was overcome by *L. maculans* within 3 years (Rouxel et al. 2003), and in Australia *LepR3* lost its effectiveness after only 2–3 growing seasons (Li et al. 2003; Marcroft et al. 2004). While resistance based on single dominant genes is unlikely to be durable, using combinations of R-genes, or pyramiding, may be an effective strategy provided that new resistance genes are discovered (Ansan-Melayah et al. 1998). Combining quantitative resistance with R-gene-mediated resistance should also be considered since quantitative resistance can increase the durability of R-genes (Brun et al. 2010). Hence, scientists worldwide are continually seeking new sources of genetic resistance to stay ahead of the erosion of blackleg resistance. In the present study, an interspecific hybridization was made between *B. napus* and BRS followed by backcrossing to *B. napus* to identify novel blackleg resistance genes and transfer them from BRS to *B. napus*. Here, we report the identification of a novel blackleg resistance locus, *LepR4*, in the progenies of *B. napus* × BRS.

Materials and methods

Plant materials

Seed of the F_1 allotriploid from a cross between *B. napus* breeding line DH12075 and BRS was kindly provided by D. L. Woods, Beaverlodge Research Farm, Agriculture and Agri-Food Canada (AAFC), Beaverlodge, Alberta, Canada. DH12075 is a doubled haploid (DH) line derived from a cross between *B. napus* cultivars Crésor and Westar (G. Séguin-Swartz, Saskatoon Research Centre, AAFC), and contains the R-gene *LmR1* from Crésor on *B. napus* linkage group N7 (Dion et al. 1995; Mayerhofer et al. 2005). Using F_1 materials derived from a susceptible *B. napus* × BRS would have simplified the project, but none were available when the project was initiated. N-o-1, a blackleg-susceptible DH line derived from Westar (Sharpe et al. 1995), was pollinated with two allotriploid plants, WT3 and WT4, to produce WT3BC_1 and WT4BC_1, respectively, the first backcross populations with *B. napus* (Yu et al. 2012). Selected plants derived from two BC_1 plants, WT3-21 (from WT3BC_1) and WT4-16 (from WT4BC_1), and selected progeny from backcrossing to N-o-1 (recurrent female parent) or selfing, were used to identify novel blackleg resistance genes and develop two blackleg-resistant lines, 61446 and 16S, respectively.

A resynthesized *B. napus* line, PSA12 (M. Beschorner and D. Lydiate, Saskatoon Research Centre, AAFC), was crossed to lines 61446 and 16S and the resulting F_1 plants were backcrossed to their respective resistant parents to produce the first backcross (BC_1). Test crosses between lines 61446 and 16S were also made. Cotyledons of the parents, F_1, F_2 and BC_1 or test cross plant populations were inoculated with isolates of *L. maculans* to determine disease reaction phenotypes. Segregation for resistance and susceptibility in the F_2 and BC_1 or test cross generations was analyzed using the Chi-square (χ^2) test for goodness-of-fit (Sokal and Rohlf 1995).

Two DH *B. napus* lines, AD9 and AD49, which carry blackleg resistance genes *LepR1* and *LepR2*, respectively (Yu et al. 2005), were kindly provided by Advanta Canada Inc. (Winnipeg, Manitoba, Canada) and used for resistant checks.

Plant growth conditions, preparation of *Leptosphaeria maculans* isolates and plant inoculations

Plant growth conditions, preparation of *L. maculans* isolates and plant inoculations followed protocols described previously (Yu et al. 2005). All isolates of *L. maculans* were from the collection at the Saskatoon Research Centre, AAFC. Isolates pl87-41 and WA51 were obtained from *B. napus* plants collected in the USA and Australia, respectively, and were chosen based on their reaction on plants carrying the R-genes *LepR1*, *LepR2*, *LepR3* and *LmR1* (Yu et al. 2005, 2008, 2012). Disease reaction on cotyledons was rated 10–15 days post-inoculation (dpi) using the 0–9 scale described by Williams (1985). Disease ratings of 0–4 and 5–9 were considered as resistant and susceptible interactions, respectively.

Evaluation of blackleg resistance under field conditions

Blackleg resistance was evaluated in two field nurseries in Australia (Wagga Wagga, New South Wales and Horsham, Victoria) from 2003–2005, and in one field nursery in Canada (Saskatoon, Saskatchewan) in 2004 and 2005. A randomized complete block design with three and four replicates was used in Australia and Canada, respectively. Plots consisted of single rows of each test entry and check, and inoculum consisted of ascospores released from naturally occurring canola stubble infested with *L. maculans*. At Saskatoon, 25 plants from each row were uprooted and rated using a 0–5 scale (0, no symptoms; 5, plant dead) previously described by van den Berg et al. (1993). At Wagga Wagga and Horsham, plant numbers were counted soon after emergence and again at maturity to determine percent plant survival. Blackleg-resistant *B. napus* cultivars were used as checks in the field trials.

Microsatellite analysis

DNA extraction, PCR amplification and microsatellite marker analysis followed protocols described previously (Yu et al. 2008). Microsatellite markers were kindly provided by D. J. Lydiate and A. G. Sharpe (Saskatoon Research Centre, AAFC). Microsatellite alleles were scored as "+" (BRS) and "−" (non-BRS). Genetic distances between marker loci in centimorgans (cM) were determined with JoinMap 4 (Stam 1993).

Results

Identification of plants carrying novel blackleg resistance genes from the progenies of *Brassica napus* × BRS

Plants carrying novel blackleg resistance genes, i.e., genes other than *LepR1*, *LepR2* or *LepR3*, which were previously introgressed from BRS and identified in *B. napus* (Yu et al. 2005, 2008), were identified by inoculating cotyledons of BC_1 plants with two *L. maculans* isolates, WA51 and pl87-41. Plants carrying only *LepR1* are resistant to isolate WA51 and susceptible to isolate pl87-41, and plants carrying only *LepR2* or *LepR3* (or both) are resistant to isolate pl87-41 and susceptible to isolate WA51. Plants carrying only *LmR1* (from DH12075) are susceptible to both isolates (Yu et al. 2012). The BC_1 plant WT3-21 was resistant to both isolates, indicating that it potentially carried a novel blackleg resistance gene(s) or that it carried *LepR1* and either *LepR2* or *LepR3* or both. Nineteen polymorphic microsatellite markers between cultivar Westar and line DH12075 from nine C-genome chromosomes, N11–N19, were previously described (Yu et al. 2012). Twelve of these markers from six C-genome chromosomes, N12–N17, were identified in plant WT3-21 (Supplementary Table 1). Successive backcrosses to line N-o-1 were performed and plants that were resistant to both isolates were selected in each generation. To determine whether cotyledon resistance to the differential isolates in the BC_2 population was associated with *LepR1*, *LepR2* or *LepR3*, we analyzed 45 plants with microsatellite markers that are closely associated with these R-genes, which are located on *B. napus* linkage groups N2 and N10. As shown in Fig. 1, cotyledon resistance to the differential isolates is not associated with the microsatellite markers in the BC_2 population derived from plant WT3-21. Further analysis was performed using microsatellite markers sR2028d, sN3888Fa and CB10524a, which are closely linked to *LepR1*, *LepR2* and *LepR3*, respectively (F. Yu and S. R. Rimmer, unpublished data). Plants were grouped into resistant and susceptible classes. For each marker and within each class, the numbers of individuals that carried alleles from BRS and non-BRS plants were counted. The ratio of the numbers of individuals for each genotype was compared with the expected ratio 1:1, assuming a random assortment of alleles, using the χ^2 test. No deviation from the expected 1:1 ratio in both resistant and susceptible classes was found in the population (Table 1), indicating that no association between resistance to isolate WA51 or pl87-41 and blackleg resistance genes *LepR1*, *LepR2* or *LepR3* was present.

Four BC_2 plants resistant to both isolates were backcrossed again to line N-o-1 to produce the BC_3 generation. Four phenotypes, namely resistant to isolate WA51, resistant to isolate pl87-41, resistant to both isolates and susceptible to both isolates, were observed in BC_3 population 6-14; no resistant plants were found in the other three BC_3 populations (Supplementary Table 2). Three plants (7.1 %) were resistant to both isolates; of these, plants 6-14-4 and 6-14-5 were self-pollinated to produce BC_3S_1 populations

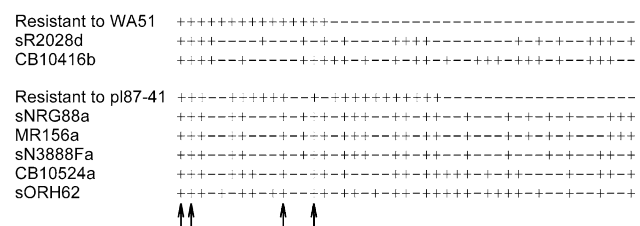


Fig. 1 Microsatellite allele and cotyledon disease reaction phenotype scoring data from 45 plants in BC_2 population N-o-1 × WT3-21. Cotyledons of BC_2 plants were inoculated with *Leptosphaeria maculans* isolates WA51 and pl87-41. +, allele from WT3-21 or resistant phenotype; −, allele from N-o-1 or susceptible phenotype. *Arrows* indicate plants selected for backcrossing to N-o-1 to produce BC_3 populations

Table 1 Association between cotyledon disease reaction phenotype and microsatellite marker score in the BC_2 population derived from plant WT3-21

Isolate/marker[a]	Phenotype	Number of plants with markers[b]			χ^2	P[c]
		+	−	Total		
WA51/sR2028d	R	7	8	15	0.07	0.796
	S	12	18	30	1.20	0.273
pl87-41/sN3888Fa	R	14	7	21	2.33	0.127
	S	10	14	24	0.67	0.414
pl87-41/CB10524a	R	13	8	21	1.19	0.275
	S	11	13	24	0.17	0.683

[a] *Leptosphaeria maculans* isolates WA51 and pl87-41 give differential reactions on cotyledons of plants carrying *LepR1* and *LepR2*, but cannot distinguish between *LepR2* and *LepR3*. Microsatellite markers sR2028d, sN3888Fa and CB10524a are closely linked to *LepR1*, *LepR2* and *LepR3*, respectively

[b] +, allele from *Brassica rapa* subsp. *sylvestris* (BRS); −, allele from non-BRS

[c] Expected segregation ratio was 1:1

consisting of 44 and 36 plants, respectively. Ten (22.7 %) and three (8.3 %) plants were resistant to both isolates in populations 6-14-4 and 6-14-5, respectively. Six resistant plants from population 6-14-4 were self-pollinated to produce the BC_3S_2 generation. All of the plants tested in BC_3S_2 population 6-14-4-6, herein designated as 61446, were resistant to both isolates (Supplementary Table 2).

The BC_1 plant WT4-16 was also resistant to both isolates and carried microsatellite markers from eight C-genome chromosomes, N12–N19 (Supplementary Table 1). Four of the 31 plants in the BC_1S_1 generation were resistant to both isolates, and two resistant plants produced sufficient seed to evaluate resistance to *L. maculans* in the BC_1S_2 generation. All of the plants tested in BC_1S_2 population 16s-2, herein designated as 16S, were resistant to both isolates (Supplementary Table 2).

The lines 61446 (BC_3S_2) and 16S (BC_1S_2) were nonsegregating for resistance to *L. maculans*. However, their parental lines, 6-14-4 and WT4-16, segregated for resistance and susceptibility, with more than 50 % of plants susceptible to both isolates (Supplementary Table 2). This suggested that the gene(s) responsible for resistance in these lines was recessive.

A single locus controls cotyledon resistance in lines 61446 and 16S

Resistant lines 61446 and 16S were used to develop new populations to study the inheritance of cotyledon resistance. Crosses were made between the susceptible line PSA12 and the resistant lines, and between lines 61446 and 16S. Cotyledon disease reaction phenotype was then determined in the F_1, F_2 and BC_1 populations, and in test cross progenies after inoculation with isolate WA51.

Average disease ratings (scale: 0–9; Williams 1985) on cotyledons of susceptible (PSA12) and resistant (61446 and 16S) lines were 8.5, 2.3 and 1.5, respectively. F_1 plants from the crosses PSA12 × 61446 and PSA12 × 16S were all susceptible with mean disease ratings of 8.2 and 8.0, respectively, similar to the susceptible parent PSA12. Analysis of segregation for resistance and susceptibility fitted ratios of 1:3 and 1:1 in the F_2 and BC_1 populations, respectively (Table 2), confirming that a single recessive allele was associated with the resistance in both lines 61446 and 16S.

Cotyledons of 207 F_1 plants from a test cross between lines 61446 and 16S were inoculated with *L. maculans* isolate WA51. All F_1 progenies were resistant (Table 2), indicating that the recessive resistant alleles in the two lines are allelic.

Cotyledon resistance to additional isolates of *Leptosphaeria maculans*

Cotyledon resistance in lines 61446 and 16S was further characterized with six additional isolates of *L. maculans* collected from Canada and Europe and derived from pathogenicity groups (PG) 2, 3, 4 and T (Table 3). Isolates pl87-41 and WA51 were also included for comparison (Table 3). Line 16S was resistant to all of the isolates tested with disease ratings ranging from 1.0–3.0. Line 61446 was resistant to six of the eight isolates tested with disease ratings ranging from 1.0–2.7, and susceptible to isolates 04-51 and 165 with disease ratings of 7.1 and 7.3, respectively (Table 3). Based on these observations, lines 16S and 61446 likely carry different alleles at the *LepR4* locus; the resistance alleles were designated as *LepR4a* in line 16S and *LepR4b* in line 61446.

Table 2 Genetic analysis of resistance to *Leptosphaeria maculans* isolate WA51 in lines 61446 and 16S based on cotyledon inoculation tests

Parents and crosses	Type	Number of plants			Expected R:S	χ^2	*P*
		R	S	Total			
61446	R parent	12	0	12			
16S	R parent	12	0	12			
PSA12	S parent	0	12	12			
PSA12 × 61446	F_1	0	12	12			
PSA12 × 61446	F_2	26	61	87	1:3	1.11	0.292
61446 × (PSA12 × 61446)	BC_1	51	45	96	1:1	0.38	0.538
PSA12 × 16S	F_1	0	12	12			
PSA12 × 16S	F_2	43	141	184	1:3	0.26	0.610
16S × (PSA12 × 16S)	BC_1	17	28	45	1:1	2.69	0.101
61446 × 16S	Test cross (F_1)	207	0	207			

Cotyledons were rated 10–15 dpi using a 0–9 scale (Williams 1985). Disease ratings of 0–4 and 5–9 were considered as resistant (R) and susceptible (S), respectively

Table 3 Disease reaction of lines carrying resistance genes based on cotyledon inoculation tests with eight isolates of *Leptosphaeria maculans*

Isolate	PG[a]	Origin	Plant genotype (R-gene present)					
			Westar (control)	16S (*LepR4a*)	61446 (*LepR4b*)	AD9 (*LepR1*)	AD49 (*LepR2*)	Surpass 400 (*LepR3*)
pl87-41	2	USA	7.2 ± 0.13	2.1 ± 0.37	2.0 ± 0.17	8.2 ± 0.12	2.5 ± 0.38	2.3 ± 0.14
04-53	2	Canada	9.0 ± 0.00	1.1 ± 0.09	2.7 ± 0.65	1.6 ± 0.23	4.8 ± 0.83	2.6 ± 0.16
2354	3	Canada	7.0 ± 0.00	1.0 ± 0.00	1.1 ± 0.08	1.0 ± 0.00	3.3 ± 0.36	1.2 ± 0.11
04-51	3	Canada	8.0 ± 0.00	3.0 ± 0.33	7.1 ± 0.14	1.3 ± 0.14	2.4 ± 0.18	2.4 ± 0.15
WA51	4	Australia	8.4 ± 0.24	1.2 ± 0.11	1.3 ± 0.16	1.1 ± 0.09	5.6 ± 0.40	4.2 ± 0.24
IBCN66	4	Europe	7.1 ± 0.48	1.0 ± 0.00	1.2 ± 0.17	1.0 ± 0.00	3.0 ± 0.00	4.5 ± 0.28
165	T	Canada	9.0 ± 0.00	1.2 ± 0.11	7.3 ± 0.16	1.8 ± 0.21	3.2 ± 0.31	3.6 ± 0.19
05-08	T	Canada	8.7 ± 0.19	1.0 ± 0.00	1.0 ± 0.00	1.0 ± 0.00	4.4 ± 0.22	4.6 ± 0.29

Cotyledons were rated 10–15 dpi using a 0–9 scale (Williams 1985). The values represent the average rating of 10–12 plants

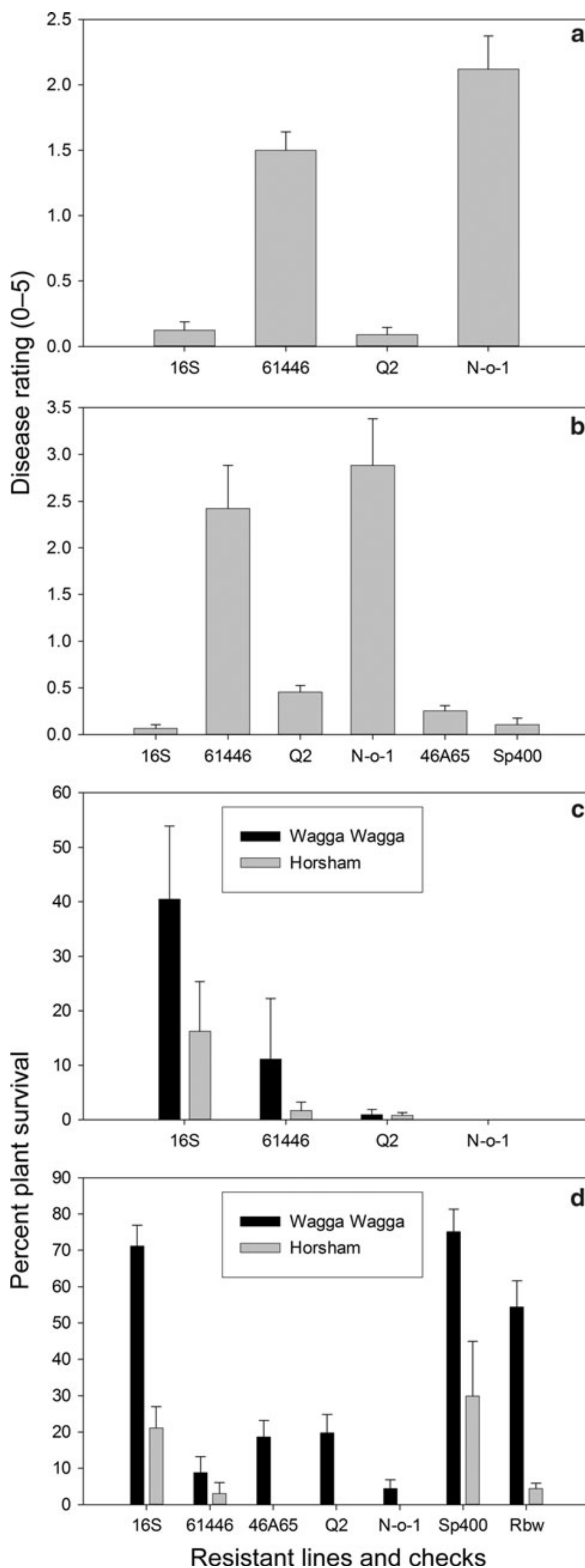
[a] Pathogenicity group. See Rimmer (2006) for a discussion of pathogenic variability and PGs

Stem canker resistance in blackleg nurseries

Field evaluation of stem canker resistance for lines 61446 and 16S was initially conducted in two blackleg nurseries in Australia in 2003. At Wagga Wagga, New South Wales, line 16S (carrying *LepR4a*) demonstrated strong blackleg resistance with all plants surviving until the end of the growing season; however, only 20 % of line 61446 plants (carrying *LepR4b*) survived (Supplementary Table 3). At Horsham, Victoria, more than 40 % of line 16S plants survived, demonstrating stem canker resistance comparable to that of some of the resistant Australian cultivars; however, only 7 % of line 61446 plants survived (Supplementary Table 3). Lines 16S and 61446 were tested further in blackleg nurseries in both Canada and Australia in 2004 and 2005. At Saskatoon, Saskatchewan, Canada, stem canker resistance in line 16S was as strong as that of Canadian resistant cultivar Q2 in 2004 (Fig. 2a) and Canadian resistant cultivars Q2, 46A65 and Australian resistant cultivar Surpass 400 in 2005 (Fig. 2b). Line 61446 demonstrated a higher level of resistance than the susceptible line N-o-1 and much weaker resistance than line 16S. Q2 was highly resistant to stem canker in Canada, but highly susceptible in Australia. Stem canker severity was higher overall in Australia in 2004 and 2005 than in 2003, but the same general pattern was observed in all years. Higher levels of blackleg resistance in line 16S were observed at Wagga Wagga than at Horsham in 2004 (Fig. 2c) and 2005 (Fig. 2d). Line 61446 was susceptible in both blackleg nurseries in Australia in 2004 and 2005.

Genetic mapping of *LepR4a* and *LepR4b*

A BC_1 population consisting of 30 plants (12 resistant and 18 susceptible) from the cross 61446 × (PSA12 × 61446) was analyzed with microsatellite markers to map the resistance gene *LepR4b*. Cotyledons of BC_1 plants were inoculated with isolate WA51 and disease reaction



◀ **Fig. 2** Stem canker resistance in blackleg nurseries. A randomized block design with four replicates at Saskatoon, Saskatchewan, Canada and three replicates at two sites in Australia (Wagga Wagga, New South Wales and Horsham, Victoria) was used. At Saskatoon, 25 plants from each plot were uprooted and rated on a scale of 0 (no symptoms) to 5 (plant dead) (van den Berg et al. 1993) to determine mean disease rating in 2004 (**a**) and 2005 (**b**). In Australia, plant numbers were counted soon after emergence and again at maturity to determine percent plant survival in 2004 (**c**) and 2005 (**d**). *Sp400* Surpass 400; *Rbw* Rainbow

phenotypes were further confirmed in the BC_1S_1. Microsatellite markers were chosen from A-genome linkage groups N1–N10. Of 293 markers, 124 were polymorphic between lines PSA12 and 61446. The BC_1 population was analyzed with 1–5 robust polymorphic markers on each A-genome linkage group. Results (Supplementary Fig. 1a) indicated that cotyledon resistance to isolate WA51 in this population was associated with markers sR7492a and sN8593a on *B. napus* linkage group N6. Polymorphic markers covering the entire N6 linkage group were further analyzed in the population. The most probable position of *LepR4b* on N6 is between microsatellite markers sN2189b and sR9571a (Supplementary Fig. 1b).

We focused on mapping *LepR4a* in line 16S since this line demonstrated cotyledon resistance to a wide spectrum of *L. maculans* isolates and strong stem canker resistance. A BC_1 population consisting of 117 plants (50 resistant and 67 susceptible) from the cross 16S × (PSA12 × 16S) was analyzed with microsatellite markers. Previous results had indicated that the resistance alleles in lines 61446 and 16S were allelic; thus, microsatellite markers on linkage group N6 were screened for polymorphism between the resistant parental line 16S and the susceptible parent PSA12. Nineteen of 50 markers showed polymorphism between the parents. As expected, the resistance allele at the *LepR4a* locus was mapped to the same location as that in line 61446. It was positioned in the interval flanked by markers sN2189b and sORH72a, 4.9 cM from sN2189b and 5.6 cM from sORH72a (Fig. 3).

DNA sequences from *B. napus* clones for microsatellite markers sN2189b and sORH72a were used to search similar genomic DNA sequences in *B. rapa* at http://brassicadb.org/brad/. They hit *B. rapa* genes Bra017959 (starting from location 9.07 Mb) and Bra024309 (starting from location 14.85 Mb) on *B. rapa* linkage group A6, respectively, in an interval of 5.78 Mb (Fig. 4). Thus, the genetic distance of 1 cM in this region is equivalent to 550 Kb. In this region, 659 genes were annotated as shown at http://brassicadb.org/brad/. The genes Bra018037, Bra018057, Bra018198 and Bra019483 (Fig. 4) encode putative nucleotide binding site (NBS)-leucine-rich repeat (LRR) class disease resistance proteins.

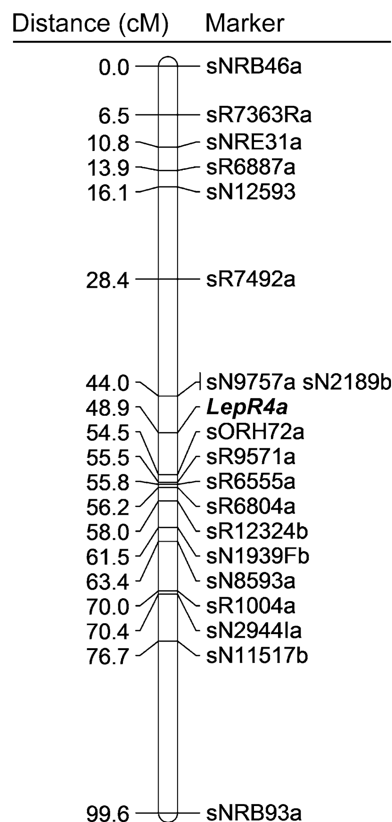


Fig. 3 *LepR4a* map location on *Brassica napus* linkage group N6. Genetic distances (cM) are shown on the *left*. The map was drawn using MapChart 2.2 (Voorrips 2002) based on mapping data from 117 BC_1 plants from the cross 16S × (PSA12 × 16S)

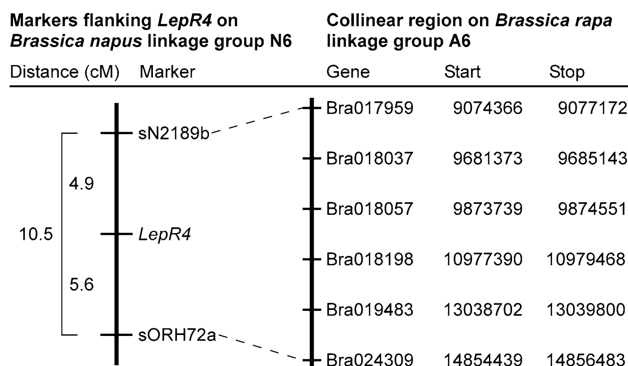


Fig. 4 Collinearity between a 10.5-cM region containing *LepR4* on *Brassica napus* linkage group N6 and a 5.8-Mb segment of *B. rapa* linkage group A6. *Broken lines* drawn between the *B. napus* clones and *B. rapa* genes indicate that they have a homologous genomic sequence. Locations in bp for each gene from beginning (Start) to end (Stop) on *B. rapa* linkage group A6 are shown on the *right*

Discussion

A small proportion of plants resistant to both *L. maculans* isolates WA51 and pl87-41 were found in the progenies of *B. napus* × BRS (Yu et al. 2012). In the current study, microsatellite marker analysis in combination with conventional breeding and pathology screening resulted in the selection of two lines, 61446 and 16S, in which 100 % of the plants tested were resistant to both of these isolates. Genetic analysis, molecular mapping and pathology testing provided strong evidence that the resistance in these two lines was recessive and conditioned by different alleles at the *LepR4* locus.

Blackleg resistance genes previously mapped in *B. napus* are of the dominant resistance type and are positioned on *B. napus* linkage groups N2 (*LepR1*), N7 (*Rlm1*, *Rlm3*, *Rlm4*, *Rlm7* and *Rlm9*) and N10 (*Rlm2*, *LepR2* and *LepR3*) (Delourme et al. 2004; Yu et al. 2005, 2008). Both *LepR4a* and *LepR4b* are recessive, and are located on linkage group N6. This is the first report of the genomic location of a recessive blackleg resistance gene derived from the A-genome. Other recessive blackleg resistance genes have been reported from the B-genome. For example, recessive gene $r_j lm2$ was identified from *B. napus* lines carrying B-genome chromosome additions derived from *B. juncea* (Saal et al. 2004), although its map location is yet to be determined, and *LMJR2* was provisionally mapped on linkage group J18 in *B. juncea* (Christianson et al. 2006).

Plants carrying recessive resistance genes $r_j lm2$ in *B. napus* (Saal et al. 2004), *mlo* in barley (Büschges et al. 1997) and *edr1* (Frye and Innes 1998) and *RRSR-1* (Deslandes et al. 2002) in *Arabidopsis* all demonstrated a broad spectrum of resistance to their respective pathogens. Similarly, *LepR4a* was effective against a wide range of *L. maculans* isolates in cotyledon inoculation tests (Table 3), suggesting that *LepR4a* is a broad spectrum resistance gene controlling blackleg resistance. However, at the same locus, *LepR4b* only conferred resistance to some of the isolates. Results from several years of field trials in Australia and Canada showed that line 16S (carrying *LepR4a*) had moderate to high levels of stem canker resistance, while line 61446 (carrying *LepR4b*) was not effectively resistant to stem canker (Supplementary Table 3; Fig. 2). Accordingly, line 16S is much more valuable than line 61446 to plant breeders developing blackleg-resistant canola cultivars. The reason for different resistance specificity from the recessive resistance alleles remains elusive. Future work should focus on assessing the mechanism(s) of resistance conferred by *LepR4a* and *LepR4b*.

Two types of resistance, race non-specific and race-specific, have been widely used in *B. napus* breeding programs. Race non-specific resistance is a quantitative adult plant stage resistance that is durable and mediated by many genes with small individual effects, while race-specific resistance is qualitative, under the control of single R-genes and effective from the seedling to the adult plant stage (Delourme et al. 2006). *LepR4a* is a recessive gene

conferring a wide spectrum of resistance against *L. maculans* at the cotyledon stage and strong stem canker resistance at the adult plant stage, which could represent a new type of blackleg resistance in *B. napus*. Thus, the identification of *LepR4a* in this study enables new opportunities for developing durable blackleg-resistant canola cultivars. The recessive gene *mlo* has been successfully used to control powdery mildew (*Blumeria graminis* f. sp. *hordei*) in barley; the resistance is apparently durable in the field despite extensive cultivation in Europe (Jørgensen 1992). While the durability of blackleg resistance conferred by *LepR4a* alone is yet to be determined, evidence that quantitative resistance can increase the durability of race-specific blackleg resistance genes (Brun et al. 2010), coupled with the known additive effects of some R-genes when in combination (Yu et al. 2005), suggests that the recessive gene *LepR4a* has the potential to enhance both race-specific and race non-specific blackleg resistance in *B. napus*.

Host defence responses against specific isolates of a pathogen can be triggered by the presence of corresponding (usually dominant) race-specific resistance genes. The largest class of "gene-for-gene" type resistance genes in plants cloned to date is represented by a family of proteins containing NBS and LRR domains (Chisholm et al. 2006; Dangl and Jones 2001); LRRs represent those components having a role in recognition specificity through protein–protein interactions (Chisholm et al. 2006; Gururani et al. 2012). Some recessive resistance genes also function in a gene-for-gene manner (Deslandes et al. 2002; Iyer-Pascuzzi and McCouch 2007) while others do not appear to be involved in recognition processes as described in Flor's (1971) gene-for-gene model, and instead confer resistance of a more broad-spectrum nature in several plant species by negatively regulating host defense responses (Büschges et al. 1997; Frye et al. 2001; Fukuoka et al. 2009). Four genes encoding the NBS-LRR class of disease resistance protein have been found in a region of *B. rapa* linkage group A6 that shares sequence homology with the *LepR4* region of *B. napus* linkage group N6 (Fig. 4). No information is yet available on the function of the *LepR4* gene, but further development of large mapping populations for *LepR4* should allow the localization of all these genes at a high enough resolution to make map-based cloning possible.

Line 16S (carrying *LepR4a*) was highly resistant to a wide range of *L. maculans* isolates at the cotyledon stage, and demonstrated moderate to high levels of stem canker resistance at the adult plant stage. However, the line was developed through only one generation of backcrossing with triploid *B. napus* × BRS materials using *B. napus* as the recurrent parent. Theoretically, line 16S contained 25 % of unwanted genetic background from BRS. Late maturity was usually observed in both the greenhouse and the field (F. Yu and S. R. Rimmer, unpublished data), and analysis of microsatellite markers indicated that one of the C-genome chromosomes, N11, was missing (Supplementary Table 1). Development of *B. napus* materials carrying *LepR4a*, limited BRS genetic background and a full set of C-genome chromosomes is in progress.

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