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ORIGINAL PAPER

Genetic map construction and QTL mapping of resistance to blackleg (*Leptosphaeria maculans*) disease in Australian canola (*Brassica napus* L.) cultivars

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Abstract Genetic map construction and identification of quantitative trait loci (QTLs) for blackleg resistance were performed for four mapping populations derived from five different canola source cultivars. Three of the populations were generated from crosses between single genotypes from the blackleg-resistant cultivars Caiman, Camberra and ^{AV}Sapphire and the blackleg-susceptible cultivar Westar$_{10}$. The fourth population was derived from a cross between genotypes from two blackleg resistant varieties (Rainbow and ^{AV}Sapphire). Different types of DNA-based markers were designed and characterised from a collection of 20,000 EST sequences generated from multiple *Brassica* species, including a new set of 445 EST-SSR markers of high value to the international community. Multiple molecular genetic marker systems were used to construct linkage maps with locus numbers varying between 219 and 468, and coverage ranging from 1173 to 1800 cM. The proportion of polymorphic markers assigned to map locations varied from 70 to 89% across the four populations. Publicly available simple sequence repeat markers were used to

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assign linkage groups to reference nomenclature, and a sub-set of mapped markers were also screened on the Tapidor × Ningyou (T × N) reference population to assist this process. QTL analysis was performed based on percentage survival at low and high disease pressure sites. Multiple QTLs were identified across the four mapping populations, accounting for 13–33% of phenotypic variance (V_p). QTL-linked marker data are suitable for implementation in breeding for disease resistance in Australian canola cultivars. However, the likelihood of shifts in pathogen race structure across different geographical locations may have implications for the long-term durability of such associations.

Introduction

Brassica napus L. is an important commercial rapeseed crop in Australia, leading to production of canola oil. The canola industry has expanded since the 1990s due to availability of superior varieties and improved agronomic practices. Despite this progress, several factors continue to limit canola production in Australia, including susceptibility to blackleg infection (Pilet et al. 1998), seed–pod shattering (Mongkolporn et al. 2003) and ion toxicity (Kaur et al. 2006; Kaur et al. 2008).

Blackleg (stem canker), caused by the fungal species *Leptosphaeria maculans* (Desm) Ces. & De Not. is an important disease of both oilseed and vegetable brassica species, including *B. napus*. This disease has caused significant yield-losses in canola-growing regions of Canada, Australia, UK, France, Germany and many other parts of the world (Fitt et al. 2006; Rimmer 2006). Blackleg is the most serious disease of canola in Australia. The severity of blackleg has risen in recent years, due to both increased areas of cultivation and intensification of production. Although not common, seasonal losses of up to 90% have been recorded (Marcroft and Bluett 2008).

Major strategies for the control of plant disease infection include cultural practices and the deployment of resistance genes. However, cultural practices alone do not provide sufficient control, and are rarely economically feasible or reliable. The most effective strategy to limit disease impact and maximise production is the use of genetically resistant cultivars (Salisbury et al. 1995; Sprague et al. 2006). Different sources of resistance to blackleg infection have been identified and introduced into canola breeding lines (Delourme et al. 2006). Numerous studies have indicated the presence of two resistance mechanisms: qualitative resistance, which is expressed in both seedling and adult plants and is thought to be controlled by single race-specific genes; and quantitative resistance, which is expressed in the adult plant and controlled by non-race-specific genes of small individual phenotypic effect (Delourme et al. 2008). Quantitative resistance is usually considered the most desirable durable mechanism, due to effectiveness against a broad range of pathogenic isolates and lower capacity to evoke significant selection pressures on the pathogen leading to breakdown of resistance (Howlett 2004; Sprague et al. 2006). However, quantitative resistance genes do not confer such effective protection as major genes. It is hence important to identify new sources of resistance to allow pyramiding of both major and minor genes for the improvement of cultivars.

Gene-for-gene interactions between canola and the blackleg pathogen have been demonstrated through the use of segregating populations for both plant and fungus (Ferreira et al. 1995; Parkin et al. 1995; Mayerhofer et al. 1997; Ansun-Meleyah et al. 1998; Balesdent et al. 2002; Zhu and Rimmer 2003; Delourme et al. 2004). More than 10 race-specific resistance (R) genes have been genetically mapped, demonstrating that some genes are organised in region-specific clusters (Rimmer 2006). Studies of R gene evolution in *Arabidopsis* have confirmed that the majority of nucleotide binding site-leucine rich repeat (NBS-LRR) R genes are present in clusters (McDowell and Simon 2006). These clusters normally result from tandem duplications of paralogous sequences over genomic regions up to 2 Mb in size (Meyers et al. 2005; McDowell and Simon 2006).

A broad range of linkage maps have been constructed for *B. napus* using current molecular genetic marker systems of choice, such as SSRs (Piquemal et al. 2005; Qiu et al. 2006, Suwabe et al. 2006; Lombard and Delourme 2001) and various QTLs controlling disease resistance and important agronomic traits have been assigned. Delourme et al. (2004) used isolates of *L. maculans* with known avirulence genes and mapped five resistance genes (*Rlm1*, *Rlm3, Rlm4, Rlm7* and *Rlm9*) to an LG which corresponds to reference group N7 (Parkin et al. 1995) while the *Rlm2* gene was assigned to LG16 of the genetic map of Lombard and Delourme (2001). Zhu and Rimmer (2003) mapped two closely linked but distinct loci mediating resistance in the seedling and adult plants of two cultivars to N7. Other major resistance genes (*LEM1*, *LmR1, cRLKMm*, *LmFr1* and *aRLMrb*, present in Major, Shiralee, Maluka, Cresor and RB87-62, respectively), have also been mapped to N7 (Dion et al. 1995; Ferreira et al. 1995, Mayerhofer et al. 1997; Rimmer 2006). Comparison of gene locations indicated that *LmR1* is allelic to *cRLKMm*, as Shiralee and Maluka have related pedigrees and are likely to have inherited the same resistance allele by descent (Mayerhofer et al. 1997). Seedling resistance genes in Maluka (*cRLMm*) and RB87-62 (*cRLMrb*) were also found to be equivalent in function and probably in genetic provenance, based on differential interactions with a series of *L. maculans* isolates (Delourme et al. 2006). Pilet et al. (1998) identified 10 QTLs

associated with stem canker in a cross between the French winter oilseed cultivars Darmor-*bzh* and Yudal, which proved to be highly stable in the field (Pilet et al. 2001). However, some inconsistencies of QTL location were observed, due to differences of genetic backgrounds and experimental conditions. Resistance to blackleg (*L. maculans*) has also been studied in the diploid species, *B. rapa* and *B. oleracea*, the putative progenitors of allotetraploid ($2n = 4x = 38$) *B. napus*. Blackleg resistance genes have been identified in *B. rapa* and were subsequently introduced into *B. napus* (Crouch et al. 1994; Chèvre et al. 2003). Genetic studies on allelic introgression into synthetic *B. napus* cultivars indicated the presence of three genes from *B. rapa* ssp. *sylvestris*: *LepR1* on N2, and *LepR2* and *LepR3* on N10 (Yu et al. 2004, 2005).

This paper describes (1) the construction of *B. napus* genetic maps based on SSR, amplified fragment length polymorphism (AFLP) and single nucleotide polymorphism (SNP) markers in four doubled haploid (DH) populations ($Caiman_3 \times Westar_{10}$, $Camberra_4 \times Westar_{10}$, $^{AV}Sapphire_5 \times Westar_{10}$ and $Rainbow_4 \times Sapphire_5$) and, (2) QTL mapping for blackleg resistance using data collected from field-based nursery screens at two locations in Victoria, Australia. Comparisons are made between results from this study and those obtained from previous QTL analyses.

Materials and methods

Plant populations

A total of four DH pair cross mapping populations were generated. Three populations were derived from F_1 crosses between a single plant selected from the $Westar_{10}$ line (blackleg susceptible, Canadian spring cultivar obtained as a result of single seed descent) and single genotypes from the French winter varieties Caiman and Camberra, and from the highly resistant (at the time of sampling) and widely cultivated Australian spring variety ^{AV}Sapphire. Bagged single plants from each of these cultivars were used in crossing and subsequently designated $Caiman_3$, $Camberra_4$ and $^{AV}Sapphire_5$, respectively. The fourth population was derived by crossing single genotypes from two blackleg resistant parental cultivars ($Rainbow_4 \times {}^{AV}Sapphire_5$), again from bagged single plants. The resistant parent varieties were all derived by open pollination between mother plants: in the case of the French winter varieties (Caiman, Camberra), individual plants were bagged in each generation, leading to higher uniformity than for the Australian varieties, which were unbagged.

Microspore culture-derived DH populations were developed by Denise Barbulescu (Department of Primary Industries, Horsham, Australia) and Pauline Fiolleau (Viterra, formally the Saskatchewan Wheat Pool). The T × N *B. napus* reference mapping population (Qiu et al. 2006) was supplied by Prof. J. Meng (National Key Laboratory of Crop Genetic Improvement, Huazhong Agricultural University, 1 Shizishan, 430070 Wuhan, China) and was used to support LG identification.

Experimental design and phenotypic evaluation

DH populations were assessed for blackleg tolerance in field nurseries located at Dahlen and Lake Bolac, Victoria, Australia in 2005. The Lake Bolac disease nursery was established at a site containing canola stubble from the previous year's commercial crop, while the Dahlen site was established after fallow with no prior history of canola cultivation, and relied on natural infection from nearby stubble. The Dahlen site was consequently considered to encounter low disease pressure, while the Lake Bolac site had high disease pressure, allowing comparison of QTL identification under the different pressure regimes.

At each nursery, individual genotypes were sown in rows (5 m long by 0.75 m apart) at 100 seeds per row/genotype, with three replicates per genotype using a nearest-neighbour completely randomised design. The field design was an alpha-lattice structure, with the intention of correcting spatial trends using appropriate models. The nurseries were carefully monitored and were managed so that plant death from factors other than blackleg infection were minimised. No other diseases or insects with potential to cause damage were noted in the nurseries. Standard agronomic growing practices for each region were employed (Salisbury and Wrattan 1999).

Total survival percentage was determined for each genotype. All plants in each row were assessed three times during the season, at emergence (open cotyledon stage, 6 weeks after sowing), then at 12 weeks after emergence, and then at maturity (28–30 weeks after sowing). Total survival was determined as the percentage of plants surviving from emergence to maturity. At maturity, plants which survived, but were infected with a basal canker and showed a lean angle of over 45° were also excluded from the analysis.

DNA extraction

Genomic DNA was extracted using the DNeasy® 96 Plant Kit (QIAGEN, Hilden, Germany) according to the manufacturers' instructions. Lyophilised leaf tissue from each parent or 1–2 seeds from each DH progeny genotype were used for each extraction and ground using liquid nitrogen or a Mixer Mill 300 (Retsch®, Haan, Germany), respectively. DNA was resuspended in 100 µl of double-distilled water to a concentration of 10 ng/µl and stored at −20°C.

SSR genotyping

A collection of c. 20,000 expressed sequence tags (EST) sequences from *B. napus*, *B. juncea* and *B. nigra* (GenBank accession numbers GT067890–GT086288 inclusive; ESM13) were generated and analysed for SSR sequence incidence. Sub-sets of these EST-based markers have previously been described (Batley et al. 2007; Hopkins et al. 2007), but a comprehensive summary of primer pair data are presented in ESM 1. In addition, previously described publicly available SSR primer pair sequences attributed to genetic maps were also obtained (Ayele et al. 2005; Burgess et al. 2006; Lowe et al. 2004; Suwabe et al. 2002, 2004; Piquemal et al. 2005; Qiu et al. 2006). *B. rapa*-derived primer pair sequences (Ling et al. 2007) were obtained from over 110,000 bacterial artificial chromosome (BAC) ends sequenced as part of the Multinational *Brassica* Genome Sequencing Project (MBGSP) (http://www.brassica.info/). BAC end and EST sequences were processed and were annotated using the Bioinformatics and Advanced Scientific Computing (BASC) database system (Erwin et al. 2007), which includes SSRPrimer (Robinson et al. 2004) and SSR Taxonomy Tree (Jewell et al. 2006), for SSR identification and PCR primer design.

SSR primer pairs were synthesised with the forward primer of each pair 5′-end-labelled with an appropriate fluorochrome moiety (either 6-FAM [6-carboxyfluorescein], HEX [4,7,2′,4′,5′,7′-hexachloro-6carboxyfluorescein] (Sigma-Genosys, The Woodlands, Texas, USA) NED (7′,8′benzo-5′-fluoro-2′,4,7-trichloro-3-carboxyfluorescein) or PET (Applied Biosystems, Foster City, CA, USA). PCR amplifications were performed in an 11 µl reaction volume containing 25 ng genomic DNA, 1× PCR buffer (QIAGEN), 15 pmol of each primer, 0.2 mM of each dNTP and 0.1 U HotStar Taq DNA polymerase (QIAGEN). PCR conditions included a hot start at 95°C for 15 min, followed by 35 cycles of 94°C for 60 s, 49–54°C for 60 s and 72°C for 60 s, and a final elongation step of 72°C for 10 min. PCR products were analysed using an ABI3730xl (Applied Biosystems) capillary electrophoresis platform according to the manufacturers' instructions. SSR products were combined with the ABI GeneScan LIZ500 size standard and allele sizes were determined using GeneMapper® 3.7 software package (Applied Biosystems). Data were scored and encoded as single-dose fragments (Wu et al. 1992).

AFLP genotyping

Initial *Eco*RI/*Mse*I restriction enzyme digestion and adaptor-ligation steps for AFLP reactions were performed as described in the AFLP® Core Reagent Kit (Invitrogen, Life Technologies, USA) according to the manufacturer's instructions. AFLP reactions were performed as described by Vos et al. (1995), with minor modifications. Primer pairs with single selective nucleotide (+1/+1) were used for pre-amplification. The product was then diluted 20-fold with double-distilled water. Selective amplification was performed with *Mse*I adaptor-homologous primers and *Eco*RI adaptor-homologous primers, each with three additional selective nucleotides (+3/+3). PCR products were resolved on the ABI3730xl, allele sizes were determined using GeneMapper® 3.7 software package (Applied Biosystems), and the data were scored as single-dose fragments. AFLP loci were encoded according to the nomenclature convention described by Vuylsteke et al. (1999).

SNP discovery and genotyping

A set of candidate genes was selected for in vitro SNP discovery based on amplicon design, PCR amplification, cloning and sequencing and experimental validation using the single nucleotide primer extension (SNuPe) assays using the method described in Cogan et al. (2006). Full SNuPe assay primer details and reaction conditions are presented in ESM 2.

Genetic linkage analysis

All markers were tested for Mendelian segregation ratios using Pearson's χ^2 tests for the expected 1:1 ratio (in the absence of selective pressures) of individual markers in a DH population. Markers with a χ^2 score of greater than 10 were excluded from the analysis. SSR, AFLP and SNP markers were assembled into a genetic linkage map with JOINMAP 3.0 using a log-likelihood threshold of 5 (Van Ooijen and Voorips 2001). LG assignment was based on common marker loci from *B. napus* mapping populations previously described by Parkin et al. (1995), Lowe et al. (2004), Piquemal et al. (2005) and Qiu et al. (2006), Suwabe et al. (2006) and a reference *B. rapa* map constructed from a cross between the varieties Chiifu and Kenshin (Choi et al. 2007). Genetic distances between loci were calculated using the Kosambi mapping function (Kosambi 1944).

LG nomenclature

LGs were assigned, where possible, by linking known marker position from reference linkage maps. Linkage group nomenclature has recently been proposed to be revised based on diploid progenitor identifiers, with numbering systems from A1–10 (based on synteny with *B. rapa*) and C1–9 (based on synteny with *B. oleracea*) (http://www.brassica.info/resource/maps/lg-assignments.php). In order to cross-reference unambiguously with previously published reference maps, the pre-existing nomenclature system (N1–19) has been used throughout this manuscript, in which N1–10 corresponds to A1–10, and N11–19

corresponds to C1–9. A selection of SSR markers (ESM 1) were screened across the T × N population to assist with LG attribution. For instances in which LGs could not be assigned, arbitrarily numbers from the largest to the smallest, e.g. LG1, LG2 and so on were used. LGs that could be attributed based on known marker location across the entire LG on at least one reference map, but were represented by more than one fragment, were assigned sequential numerical suffixes (e.g. N9-1 and N9-2 for N9). Alternatively, when one major group was identified based on reference maps and additional fragments were identified based on cross-comparison between all four trait-specific population maps from this study, alphabetical suffixes were applied.

In several instances, evidence was obtained for linkage group fusion, which could not be resolved through mapping analysis alone, in respect to other *B. napus* linkage maps. A composite LG nomenclature (e.g. LG N1/11) was consequently adopted.

Statistical analyses

Each replication was analysed as an un-replicated trial with replicated controls by fitting an appropriate spatial model with rows and columns (Gilmour et al. 1997). The best linear unbiased predictions (BLUPS) of genotype-specific performance were then regarded as raw data of the randomised complete block design. Genotype means from each trial site were used to construct distribution histograms.

For each trial site, multivariate analysis of variance and covariance was used to estimate variance components. Genotype and replication were also regarded as random effects. Variance components for each effect were estimated by equating observed mean squares with expected mean squares and solving the resulting system of equations.

QTL detection was conducted using simple interval mapping (SIM) and composite interval mapping (CIM) implemented in Windows QTL Cartographer version 2.5 (Wang et al. 2007). For SIM, an arbitrary LOD threshold of 2.5 was used to determine significance, while for CIM, significance levels for LOD thresholds were determined using permutations with 1,000 replications.

Results

Linkage map construction

A total of four different trait-mapping populations, derived from five different source cultivars, were generated to identify chromosomal regions involved in blackleg resistance of canola. The parents of these populations were selected on the basis of their genetic dissimilarity, and contrasting response to blackleg. Three of the four populations were derived from cross between genotypes from blackleg-resistant germplasm and a common blackleg-susceptible genotype while the fourth population was derived from cross between two blackleg-resistant sources. An individual plant from each variety was selected to generate the DH mapping populations in order to address the potential effects of intra-cultivar heterogeneity.

Sets of SSR primer pair combinations (445 EST-SSRs from the proprietary *B. napus*, *B. nigra*, *B. juncea* EST collection, 143 BAC-derived SSRs from *B. rapa*, 42 *B. oleracea* genomic-DNA derived SSRs and 206 publicly available SSRs obtained from various *Brassica* species) were tested on the parents of different DH populations to determine levels of genetic polymorphism. Primer pairs revealing variation were then screened across the full progeny sets. Up to 40 different AFLP primer pair combinations were also tested across all four trait-specific mapping populations and those detecting polymorphism were then implemented for linkage mapping. All SNP markers were screened on the parents and selected DH lines for polymorphism detection, prior to genetic mapping.

The number of progeny for each sib-ship, final marker loci number and composition, number of mapped loci, map length, number of LGs and average marker density for each of the trait-specific population maps were determined (Table 1), along with full data-scoring matrices for all mapping populations (ESMs 3–6). The proportion of marker loci amenable to mapping, which coalesced into LGs, ranged from 70 to 89%. In the $Caiman_3 \times Westar_{10}$ ($C_3 \times W_{10}$) genetic linkage map, a total of 436 AFLP and SSR markers were attributed to 17 well-supported LGs covering 1611.2 cM. The numbers of markers (AFLP, SSR and SNP) on the $Camberra_4 \times Westar_{10}$ ($C_4 \times W_{10}$), $^{AV}Sapphire_5 \times Westar_{10}$ ($S_5 \times W_{10}$) and $Rainbow_4 \times {}^{AV}Sapphire_5$ ($R_4 \times S_5$) maps were 468, 404 and 219, with map coverage values of 1800, 1718 and 1173 cM, respectively (ESMs 7–10). The $C_4 \times W_{10}$ map contained the largest number of mapped markers and highest average marker density (1 locus per 3.8 cM). The genetic map spanned 19 LGs, matching the karyotypic number for this species. All four maps contained variable number of additional smaller groups, consisting of up to four markers.

Screening of 33 selected EST and BAC-derived SSR markers from the trait-specific population maps across the T × N population (ESM 11) increased LG attribution by 10–20%. In several instances, LGs were present as multiple fragments: for example, N2, N7, N9 and N13 of the $C_3 \times W_{10}$ map were fragmented and could hence not be coalesced through use of the available marker resources. Similar situations were observed for other genetic maps ($C_4 \times W_{10}$ LGs N9, N13 and N17; $S_5 \times W_{10}$ LGs N2, N4 and N17; $R_4 \times S_5$ LGs N7 and N9). In several instances, LGs were conjoined through terminal genetic markers. This was observed for both non-homologous and homoeologous

Table 1 Summary statistics for molecular genetic marker assay number and type and for cumulative genetic map and average marker distance, for linkage maps constructed from four *B. napus* trait-specific mapping populations

Population	No. of individuals	Total no. of marker loci generated	No. of mapped marker loci	Map length	No. of linkage groups		Average marker density (cM)
					Assigned	Unassigned	
$Caiman_3 \times Westar_{10}$	91	540 (271 SSR; 269 AFLP)	436 (241 SSR; 195 AFLP)	1611.2 cM	14	3	3.7
$Camberra_4 \times Westar_{10}$	76	527 (232 SSR; 263 AFLP; 32 SNP)	468 (216 SSR; 220 AFLP; 32 SNP)	1800.6 cM	16	3	3.8
$^{AV}Sapphire_5 \times Westar_{10}$	133	457 (220 SSR; 227 AFLP; 10 SNP)	404 (195 SSR; 193 AFLP; 16 SNP)	1717.9 cM	12	4	4.2
$Rainbow_4 \times {}^{AV}Sapphire_5$	91	311 (112 SSR; 191 AFLP; 8 SNP)	219 (86 SSR; 126 AFLP; 7 SNP)	1173.0 cM	12	4	5.3

LGs (e.g. $C_3 \times W_{10}$ N5/N15; $C_4 \times W_{10}$ N1/11 and N2/12; and $S_5 \times W_{10}$ N4/13, N7/10 and N9/18).

Genetic analysis

Significant differences were observed in the survival percentage of the genotypes for each cross at both sites. In all four populations, variation was observed for disease resistance at both the Dahlen and the Lake Bolac trial sites. Narrow sense heritabilities were calculated for all four populations at both trial sites and a best-fitted segregation model was calculated based on the frequency distribution (Table 2; ESM12). $Westar_{10}$ was used as a susceptible control for both trial sites. For each population analysis, more than 10 replicates of W_{10} were used and mean percentage survival across all replicates was 1.15 for Dahlen and 0.23 for Lake Bolac.

Identification of QTL regions affecting disease resistance was performed (Table 3). In $C_3 \times W10$, SIM detected two QTLs on N10 and N15 with LOD scores of 3.0 and 4.4 for the Dahlen (low disease pressure) site, which in combination explained an estimated 57% of V_p (Figs. 3, 4). SIM also identified a QTL on N1 with LOD score of 2.9 accounting for 20% of V_p for the high disease pressure site at Lake Bolac (Fig. 1). CIM identified a QTL on N15 with a LOD score of 5.6 (Fig. 4) at Dahlen, explaining 19% of V_p, but the two other regions identified by SIM failed to exceed the threshold value for significance.

In the $C_4 \times W_{10}$ mapping population, a total of three QTLs for the Lake Bolac trial data were only identified as significant by SIM on N5, N10, and LG2 with LOD scores 2.6, 2.7, and 2.8, respectively (Figs. 3, 4). Two QTL on N1/11 and N17-2 were identified as significant using both SIM and CIM for the Dahlen site trial data (Figs 1, 2), accounting for a total of 17–18% and 24–13% of V_p, respectively (Table 3). The QTL on N17-2 was detected at exactly the threshold limit using CIM, but has been declared significant and hence included in the analysis.

A single QTL on N2 with a LOD score of 3.8 was detected as significant using SIM, for the Lake Bolac trial site data obtained for the $S_5 \times W_{10}$ population (Fig. 4). CIM detected three QTLs on N1, N11 and LG1, which accounted for a total of 33% of V_p (Figs. 1, 2, 4). For the $R_4 \times S_5$ population, a single QTL was detected on N9-2 using CIM for Lake Bolac-derived data, with a LOD score of 3.7 and accounting for 13% of V_p (Fig. 4).

Discussion

Genetic map construction

All of the trait-specific population maps in this study were based on combinations of SSR, AFLP and SNP markers.

Table 2 Estimated narrow-sense heritability values for phenotypic data generated from trials performed at the Dahlen and Lake Bolac nurseries, including best-fit segregation models

Population	Dahlen (DAH) site			Lake Bolac (LBO) site		
	V_g	V_e	H^2	V_g	V_e	H^2
$Caiman_3 \times Westar_{10}$	510.2	109.3	0.81 ± 0.04	319.8	73.6	0.82 ± 0.03
$Camberra_4 \times Westar_{10}$	271.9	237.2	0.39 ± 0.10	82.6	131.1	0.53 ± 0.08
$^{AV}Sapphire_5 \times Westar_{10}$	283.4	203.1	0.79 ± 0.03	418.9	110.2	0.58 ± 0.06
$Rainbow_4 \times {}^{AV}Sapphire_5$	95.2	139.9	0.66 ± 0.05	222.1	116.6	0.40 ± 0.08

Table 3 Summary data for disease resistance QTL analysis from trials performed at the Dahlen and Lake Bolac nurseries for all genetic mapping populations

Population	*B. napus* chromosome (N) and trial site	SIM				CIM (1000 permutations)				
		Max LOD score	Position	a	% variance	LOD threshold	Max LOD score	Position	a	% variance
$Caiman_3 \times Westar_{10}$	N1 (Lake Bolac)	2.9	33.4	−12.6	20	*3.8*	*3.5*	*36.0*	*−14.0*	*22.7*
	N10 (Dahlen)	3.0	47.5	−19.9	34	*3.8*	*1.4*	*56.0*	*8.2*	*5*
	N15 (Dahlen)	4.4	136.5	−16.6	23	3.8	5.6	136.5	−15.4	19
$Camberra_4 \times Westar_{10}$	N5 (Lake Bolac)	2.6	67.9	−11.2	33	*3.7*	*0.5*	*70.3*	*−2.7*	*1.5*
	N1/11 (Dahlen)	2.9	64.8	3.8	17	3.6	5.1	64.8	4.0	18
	N10 (Lake Bolac)	2.7	13	10.7	31	*3.7*	*0.3*	*13.0*	*2.9*	*2*
	N17-2 (Lake Bolac)	2.7	17.8	9.6	24	3.7	3.7	15.8	6.9	13
	LG2 (Lake Bolac)	2.8	2.0	−10.2	28	*3.7*	*2.1*	*6.0*	*−7.7*	*14*
$^{AV}Sapphire_5 \times Westar_{10}$	N2 (Lake Bolac)	3.8	12.7	11.2	26	*2.5*	*1.3*	*16.7*	*4.6*	*4*
	LG1 (Lake Bolac)	*1.0*	*36.2*	*−4.3*	*4*	2.5	3.6	36.2	−7.0	10
	N1 (Lake Bolac)	2.5	44.6	−35.6	14	2.5	5.6	39.6	9.2	15
	N11 (Dahlen)	*0.8*	*11.4*	*2.0*	*3*	2.5	2.9	11.4	3.2	8
$Rainbow_4 \times {}^{AV}Sapphire_5$	N9-2 (Lake Bolac)	*N/A*				3.2	3.7	44.6	−5.1	13

QTLs that are not significantly identified using either analytical method are italicised

SSR markers are highly abundant, multi-allelic and co-dominant in nature, making them ideal markers for genetic mapping and for characterisation of germplasm (Saal et al. 2001). SSRs have been obtained from sample genome sequencing efforts for the diploid taxa *B. oleracea* and *B. rapa*. However, the majority of SSR primer pairs used in this study were originally identified from *Brassica* genic sequences, which are anticipated to show higher levels of amplification efficiency and especially cross-species transfer than for loci located in non-coding DNA. Cross-species amplification by *B. juncea*- and *B. nigra*-derived EST-SSRs in *B. napus* has been reported to be highly efficient (Hopkins et al. 2007), consistent with this hypothesis. The use of SSR markers from a range of closely related *Brassica* species is particularly effective for comparative genetic map alignment.

In the current study, a total of 206 public SSRs were used to screen the trait mapping populations and were critical for assignment of LGs to *B. napus* reference maps (Lowe et al. 2004; Suwabe et al. 2002, 2004; Piquemal et al. 2005; Qiu et al. 2006). In those cases in which no public markers were represented on a given LG, the attribution could generally be inferred through comparison of incidence and order of common loci assigned to other *B. napus* maps obtained in this study.

AFLP markers are not as informative as SSRs for genetic map construction, as they are both dominant and non-locus-specific in nature, and hence not generally readily transferred between genetic maps. However, AFLP markers were useful for generation of a large number of polymorphic loci per primer pair combination, to fill gaps in SSR marker coverage. AFLPs have been routinely used to construct linkage maps in different *Brassica* species and to effectively increase the density of genetic linkage maps (Ke et al. 2004; Voorrips et al. 1997; Negi et al. 2000; Sebastian et al. 2000; Zhao et al. 2005). In three of the four genetic linkage maps ($C_4 \times W_{10}$, $S_5 \times W_{10}$, $R_4 \times W_{10}$), SNP markers were also used for mapping analysis. SNPs are highly informative functionally associated genetic markers (Andersen and Lübberstedt 2003; Forster et al. 2008). SNP markers used in this study were discovered through resequencing of amplicons generated from proprietary EST templates. *B. napus* SNP markers have been derived from coding sequences of the A and C sub-genomes,

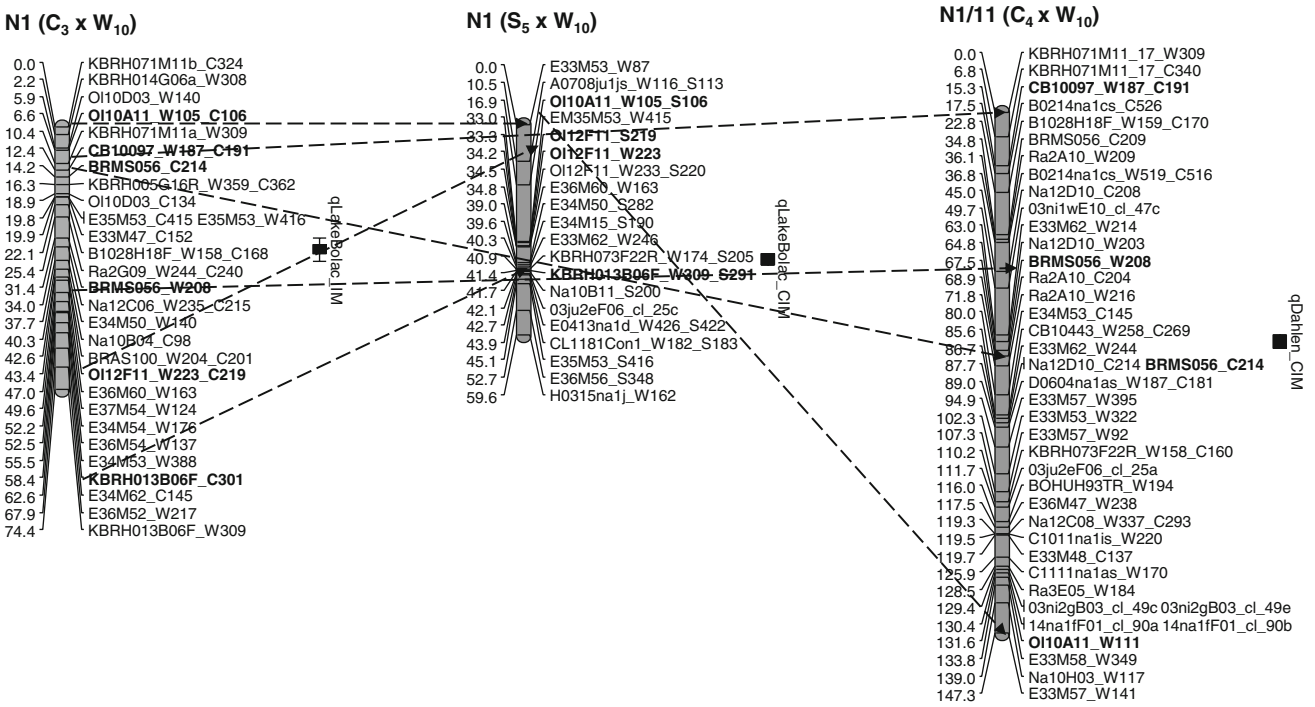

Fig. 1 QTLs identified on N1 of the $C_3 \times W_{10}$, $C_4 \times W_{10}$ and $S_5 \times W_{10}$ genetic maps, with locations of bridging markers indicated by *dotted lines*. Marker nomenclature is as described in ESMs 1 and 2, and AFLP nomenclature is as described by Vuylsteke et al. (1999)

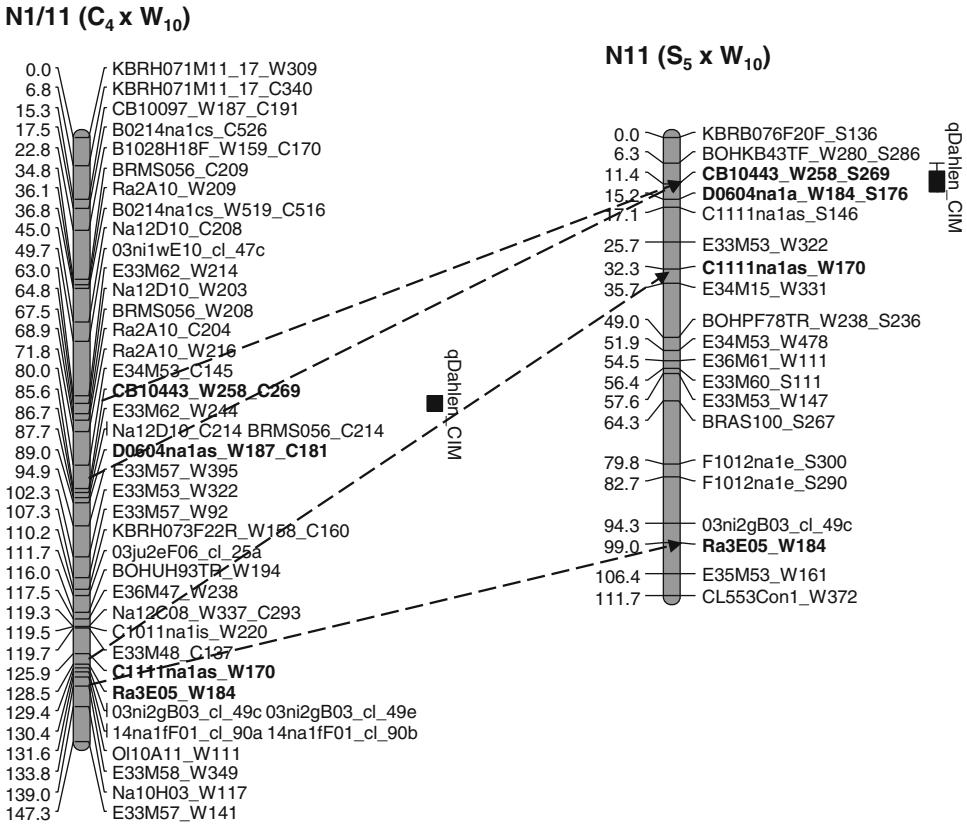

Fig. 2 QTLs identified on N11 of the $C_4 \times W_{10}$ and $S_5 \times W_{10}$ genetic maps. Details are as provided in the legend for Fig. 1

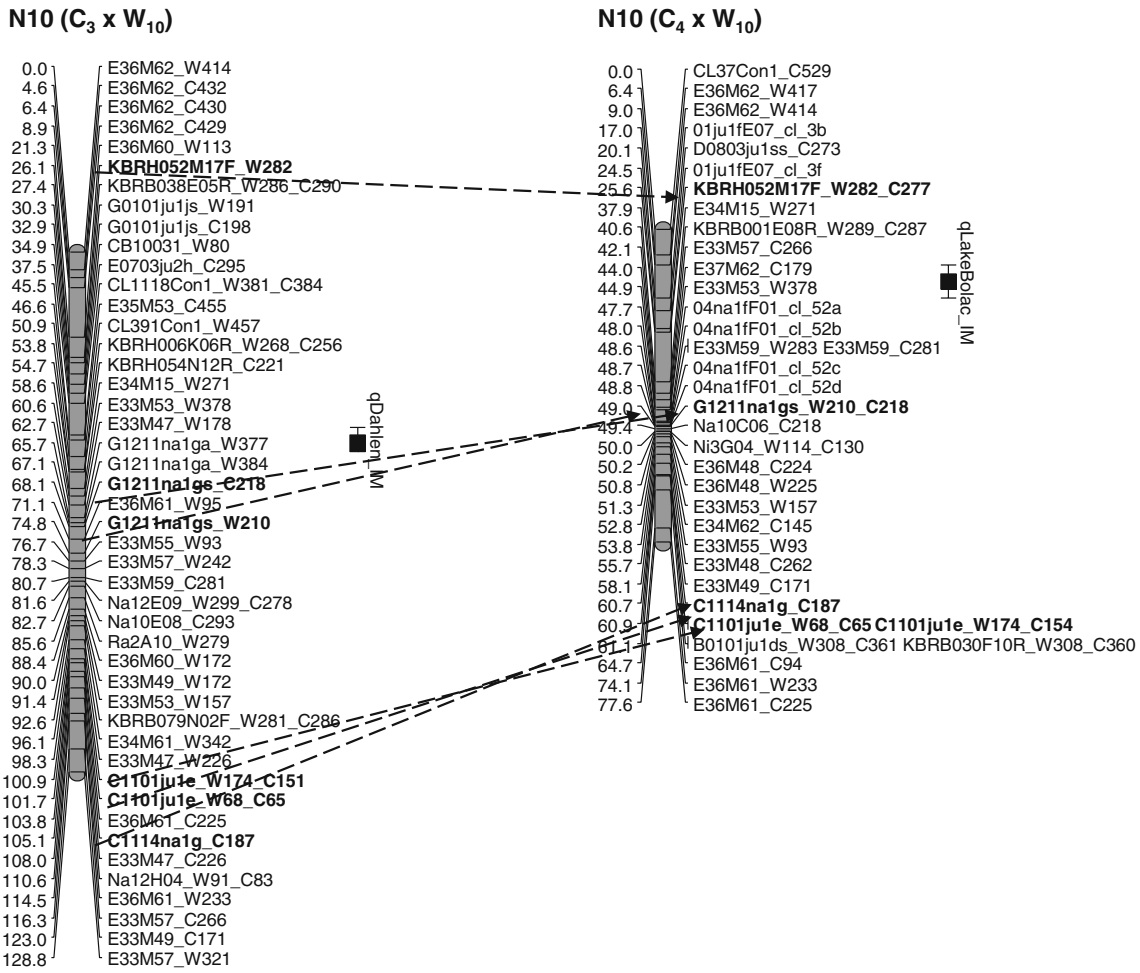

Fig. 3 QTLs identified on N10 of the $C_3 \times W10$ and $C_4 \times W_{10}$ genetic maps. Details are as provided in the legend for Fig. 1

and proved useful for assignment of LGs (Trick et al. 2009). SNP detection and use in genetic mapping has become a common tool for the International *Brassica* research community (Mukhlesur et al. 2007; http://informatics.plantsciences.ucdavis.edu/haggard/brassica.html; http://urgi.versailles.inra.fr/projects/CT_Colza/GOP-T4/PRESENTATION_GOP-T4.htm).

Although the current study was not specifically designed to address issues of homoeology, evidence was obtained that many SSR primer pairs amplified single loci from both the A or C sub-genomes, as previously observed (Saal et al. 2001; Mayerhofer et al. 2005; Piquemal et al. 2005). For example, putative homoeoloci were detected by the markers KBRH071M11b on N1 and N11 and KBRB073E07 on N7 and N17 of $C_3 \times W_{10}$, and by CB10229 on N5 and N15 of $C_4 \times W_{10}$. In some other cases, specific LGs were fused: for example, N5 and N15 of $C_3 \times W_{10}$, N1 and N11 of $C_4 \times W_{10}$, and N2 and N12 of $C_4 \times W_{10}$. N2/N12 and N1/N11 are known to represent homoeologous pairs (Parkin et al. 1995, 2003; Sharpe et al. 1995; Mayerhofer et al. 2005). A possible explanation for the fused LGs is the contribution of marker alleles with commonality of amplification product sizes between homoeoloci. Scoring of such features in a single-dose dominant manner may generate segregation distortion effects and the observed terminal LG coalescence. Moreover, some LGs could not be assigned due to an absence of cross-population reference markers. The relatively small population sizes used in the current study may have contributed to this problem, due to limited sampling of recombination events required for accurate genetic map construction.

Evidence for locus duplication within sub-genomes was also obtained. The region on the $S_5 \times W_{10}$ map harbouring duplicated loci from Ol12F11 lies under a blackleg resistance QTL on N1. Extensive intrachromosomal tandem duplication has also been observed for the *LmR1* disease resistance gene locus on N7 (Mayerhofer et al. 2005). Assignment of blackleg resistance genes to duplicated genomic regions may complicate fine mapping efforts (Mayerhofer et al. 2005). Amplification of loci derived from the same SSR primer pair was also observed for non-homoeologous LGs. For example, SSR primer pairs

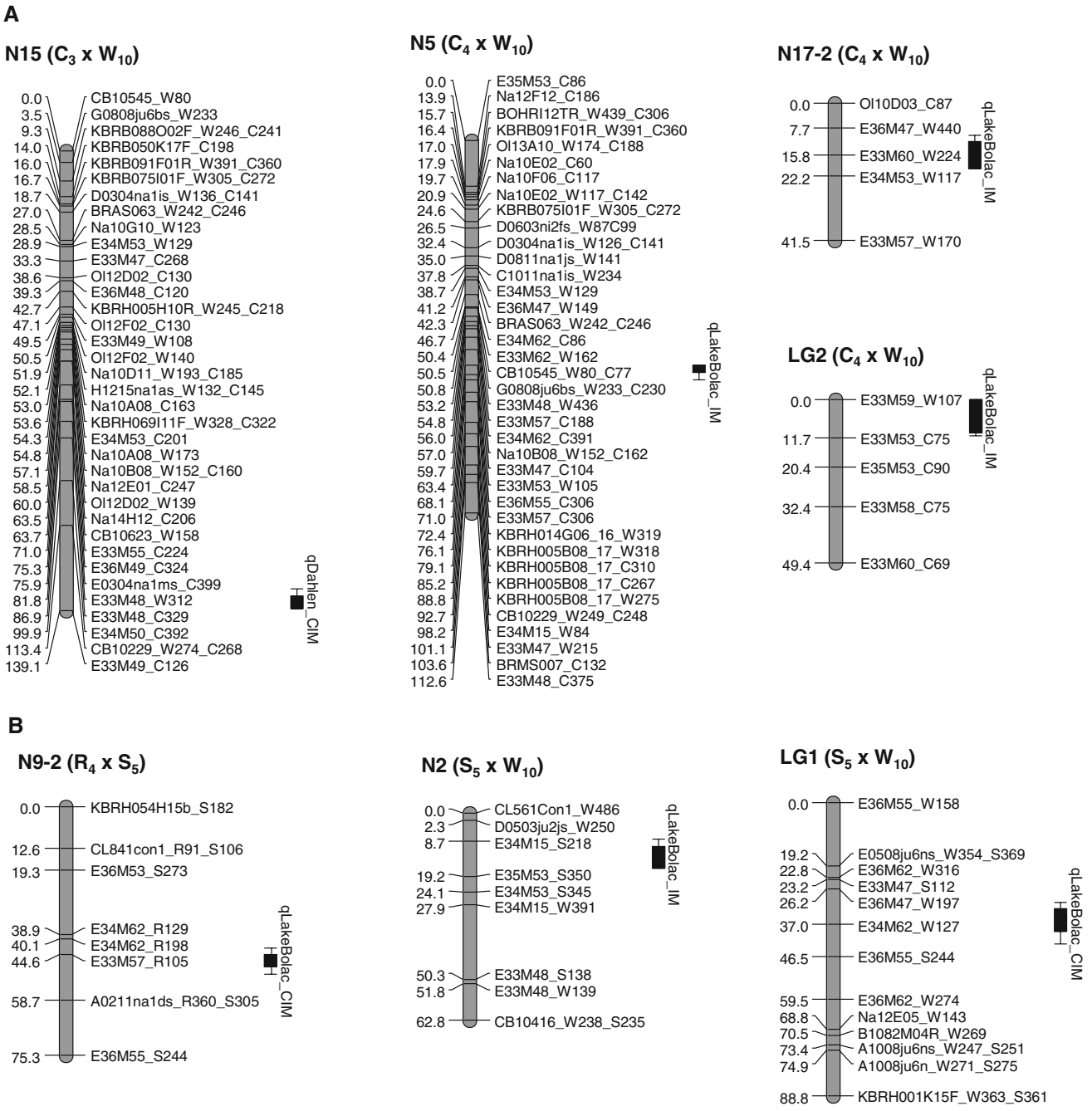

Fig. 4 Individual significant QTLs identified on LGs from analysis of (**a**) $C_3 \times W_{10}$ and $C_4 \times W_{10}$ mapping populations, (**b**) $R_4 \times S_5$ and $S_5 \times W_{10}$ mapping populations. Details are as provided in the legend for Fig. 1

KBRH052E24a and BOHWU84TR obtained duplicated loci between LGs N2-A and N7 of the $C_3 \times W_{10}$ map. Similarly, loci derived from primer pairs Ol12F11, F1012na1e and C1011na1is were duplicated between LGs N1, N11 and N14 of the $S_5 \times W_{10}$ map.

Trait-dissection for blackleg resistance

Composite interval mapping identified QTLs on $C_3 \times W_{10}$ N15 for the Dahlen trial site, explaining 19% of V_P. In $C_4 \times W_{10}$, a single QTL was detected on N1/N11 for the Dahlen-derived data, which contributed 18% of V_p. Of the four trait-specific populations used in this study, multiple QTLs (on N1, N11 and LG1) were detected with CIM only for $S_5 \times W_{10}$, collectively accounting for 33% of V_p. CIM failed to detect any significant QTLs for the $R_4 \times S_5$ mapping population.

Simple interval mapping identified additional QTLs on N10 of both $C_3 \times W_{10}$ and $C_4 \times W_{10}$, which may correspond to the location of the *LepR2* and *LepR3* genes in

B. rapa ssp. *sylvestris* (Rimmer 2006). In similar fashion, a QTL was detected on $S_5 \times W_{10}$ N2 using SIM which may correspond to the previously identified *B. rapa LepR1* blackleg resistance gene (Yu et al. 2008). Other major resistance genes for blackleg (*Rlm1*, *Rlm3*, *Rlm4, Rlm7* and *Rlm9*) have been assigned to N7 of *B. napus* (Delourme et al. 2004). The resistance genes *LEM1* (from cv. Major), *LmFr1* (from cv. Crésor), *LmR1* (from cv. Shiralee), *cRLMm* (from cv. Maluka) and *cRLMrb* (from cv. RB87-62) have also been assigned to N7 (Dion et al. 1995; Ferreira et al. 1995; Mayerhofer et al. 1997; Rimmer 2006). *LmR1*, *cRLMm*, *cRLMrb* and *LEM1* have been proposed to correspond to *Rlm4* (Ferreira et al. 1995; Mayerhofer et al. 1997; Rimmer 2006). Despite information on genetic map location, little is understood of the relationship between these genes and corresponding roles in the *B. napus*–*L. maculans* defense response interaction (Mayerhofer et al. 2005).

Although the majority of single dominant genes controlling the blackleg resistance trait have been located to N7 (Mayerhofer et al. 2005), the current study failed to declare significant QTLs on this LG. This anomaly may be due to stable differences in race structure between geographical regions of the Northern and Southern hemispheres, or to dynamic shifts in pathotype composition arising from migration or recombination events in the sexual fungal pathogen. The detection of novel QTLs that have not been observed in previous studies is also suggestive of pathotype variation, and (subject to validation) may provide valuable sources of resistance.

A total of 1–4 QTL were detected for each mapping population as significant for each trial site with either SIM or CIM. Size of sib-ship ranged in value from 76 to 133. On this basis, the number of QTLs and accounted proportions of V_p are individually and collectively close to expectation from comparison with other studies (Kearsey and Farquhar 1998). The majority of the declared QTLs derived from the Lake Bolac trial site data, in which the increased selection pressure may have enhanced power of detection. Alternatively, the use of percentage survival data may have been more effective at higher compared to low disease pressure sites.

None of the QTLs was found to be common between datasets obtained at the two trial sites for a given population. However, in some instances, similar QTLs were detected at a specific location between different populations [e.g. N1 (Lake Bolac) in $C_3 \times W_{10}$ and $S_5 \times W_{10}$ and N11 (Dahlen) in $C_4 \times W_{10}$ and $S_5 \times W_{10}$]. This may reflect the presence of different blackleg fungus pathotypes at the two different trial sites during the time of phenotypic analysis.

The development of durable blackleg disease resistance in superior elite cultivar lines without the use of transgenic technology will rely on the introgression of multiple race-specific resistance genes and QTLs for non-specific resistance from a broad range of *Brassica* genetic backgrounds. Genetic markers flanking the QTL-containing regions identified in this study are capable of further validation in a diverse set of *B. napus* germplasm, enabling MAS for breeding blackleg resistance in canola. Moreover, additional candidate gene assessment in the delimited regions of interest may be performed using the whole genome sequencing approach. This will be further useful for diagnostic SNP marker development in applied breeding programs.

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