Euphytica (2010) 173:25–35
DOI 10.1007/s10681-009-0095-1

Mapping of QTLs detected in a *Brassica napus* DH population for resistance to *Sclerotinia sclerotiorum* in multiple environments

Xiangrui Yin · Bin Yi · Wei Chen · Weijie Zhang · Jinxing Tu · W. G. Dilantha Fernando · Tingdong Fu

Received: 17 September 2008 / Accepted: 1 December 2009 / Published online: 24 December 2009

Abstract Sclerotinia stem rot, caused by the fungus *Sclerotinia sclerotiorum*, is one of the most devastating diseases of rapeseed (*Brassica napus* L.) in China. The two major factors limiting the development of disease resistance are (1) the absence of accessions with complete resistance and (2) the lack of a single method that can be widely applied to assess tolerance—even though accessions with differential tolerance to *S. sclerotiorum* have been identified in China. In the study reported here, we have used one doubled haploid (DH) population consisting of 72 lines, which was derived from the F_1 generation of a cross between a partially resistant line (DH821) and a susceptible line (DHBao604), to identify quantitative trait loci (QTLs) involved in the resistance to *S. sclerotiorum*. Three inoculation methods, namely, mycelial toothpick inoculation (MTI), mycelial plug inoculation (MPI), and infected petal inoculation (IPI), were used to assess resistance at the adult plant stage. A genetic linkage map with 20 linkage groups covering 1746.5 cM, with an average space of 6.93 cM, was constructed using a total of 252 molecular markers, including 91 simple sequence repeats, 72 randomly amplified polymorphic DNA, 86 sequence-related amplified polymorphisms, two restriction fragment length polymorphisms, and one expressed sequence tag. Composite interval mapping identified ten, one and ten QTLs using MTI, MPI and IPI methods, respectively, at a LOD > 2.5. One QTL was detected in linkage group N12 by MTI in 2004 and 2005 and by IPI in 2005. Another QTL was detected in linkage group N3 and N4 by MPI in 2006 and 2007. There was one common QTL detected by MTI in 2005 and by MPI in 2006. These results provide information on the genetic control of resistance to *S. sclerotiorum* in oilseed rape.

Keywords *Brassica napus* · Partial disease resistance · QTL mapping · Resistance identification method · *Sclerotinia sclerotiorum*

Introduction

Sclerotinia stem rot is caused by the necrotrophic fungus *Sclerotinia sclerotiorum*, and more than 400 plant species are hosts to this pathogen (Boland and Hall 1994). It is one of the most devastating diseases of canola in the Yangtze River basin in China. Yield losses due to this disease can be significant, 10–30%,

Electronic supplementary material The online version of this article (doi:10.1007/s10681-009-0095-1) contains supplementary material, which is available to authorized users.

X. Yin · B. Yi · W. Chen · W. Zhang · J. Tu (✉) · T. Fu
Huazhong Agricultural University, Wuhan 430070, Hubei, China
e-mail: tujx@mail.hzau.edu.cn

W. G. D. Fernando
Department of Plant Science, University of Manitoba, 15 Gillson Street, Winnipeg MB R3T, Canada

with yield losses of up to 80% recorded in some years. The disease had also reduced the quality of canola oil and meal (McCartney et al. 1999).

The disease severity of Sclerotinia causing stem rot in *Brassica* crops is variable and depends on the time of infection, climatic conditions, and microenvironment in the plant canopy. The symptoms of Sclerotinia infection usually appear several weeks after flowering begins, with the development of pale-grey to white lesions on the stem and upper branches and pods of the plant. Temperatures of between 20 and 25°C and a relative humidity of more than 80% are required for optimal plant infection and disease development (Heran et al. 1999). In a study conducted in Canada, significant variations in disease incidence could be explained by plant morphology (Jurke and Fernando 2008). Thus, one approach is to develop varieties with a plant canopy that is able to withstand Sclerotinia infection. Zhongshuang No. 9, a variety with an increased upright growth habit, lodging resistance, and narrow canopy, is one example of such a variety. Some varieties with certain morphological traits are better able to avoid Sclerotinia infection, including petal-less varieties (apetalous varieties), which escape Sclerotinia infection to a relatively larger extent (Fu et al. 1990).

There are no known varieties with complete resistance to Sclerotinia. The highest available level of field resistance (i.e., tolerance) in rapeseed (and recently some canola material) was attained with Zhongyou 821 and Zhongshuang No. 9 in China (Li et al. 1999; Wang et al. 2004). One other confounding factor is that there is no single, widely accepted method for evaluating resistance to *S. sclerotiorum* in rapeseed. Over the years, different research groups have employed different methods to evaluate the resistance of rapeseed genotypes. Some varieties have been identified as resistant or tolerant sources on the basis of long-term field-based observation. However, even in those varieties, when they are inoculated with different methods, such as mycelial plug onto detached leaf, oxalic acid assays in the growth-chamber, mycelial tooth-pick, plug, infested petal inoculations, and spraying of the whole plant with mycelia suspension in the field, the disease severity index may not be sufficiently uniform to be able to correlate each method and arrive at a reliable evaluation of resistance of the different sources. Bradley et al. (2006) compared four inoculation methods, including field screening, petiole inoculation technique, detached leaf assay, and the oxalic acid assay. Although the petiole inoculation technique could differentiate cultivars, it could not correlate the reaction of the cultivars to *S. sclerotiorum* in the field. Li et al. (2007) screened 53 genotypes from Australia, India, and China using three inoculation methods. They found that the use of the appropriate inoculation and assessment methods could significantly reduce variability in the responses commonly observed in screening for resistance against the pathogen in the host. However, compared with their previous test (Li et al. 2006), there were a number of exceptions in terms of the relative resistance rankings. These researchers suggested that variations in genotype performance under different environmental conditions highlights the challenges facing plant breeders in determining resistant genotypes as well as the need for continued assessments of genotypes under different environmental conditions. To resolve the problems mentioned above, it is therefore necessary to identify the resistance with differential methods over a long period and to use molecular markers to improve the accuracy of these methods.

Molecular markers and quantitative trait loci (QTLs) mapping have been used for identifying loci related to resistance in *Brassica napus*. Zhao and Meng (2003) detected three QTLs involved in leaf resistance at the seedling stage and three different QTLs for stem resistance at the adult plant stage in an $F_{2:3}$ population derived from a cross between a partially resistant Chinese semi-winter line (NingRS-1) and a semi-winter male sterile restorer line. Zhao et al. (2006) identified the QTLs involved in the resistance to *S. sclerotiorum* in two segregating populations of doubled haploid (DH) lines, the HUA population and the MS population, using a petiole inoculation technique and two scoring methods, namely, days to wilt (DW) and stem lesion length (SLL), to assess the resistance. A total of eight genomic regions affecting resistance were detected in the HUA population, with four of these regions affecting both measures of resistance. Only one region affecting both measurements was detected in the MS population. Individual QTLs explained 6–22% of the variance. In five of the QTLs from both populations, alleles from the resistant parent contributed to the resistance. The QTL on N2 from the HUA population had the highest LOD score and

R^2 value and was detected for SLL in the first evaluation.

Segregating populations of DH lines have been used extensively in QTL mapping studies because they are unique populations and can be used in replicated trials in different years and locations. Several molecular map constructions and QTL studies have been performed with DH populations (Ferreira et al. 1995a, b; Toroser et al. 1995; Thormann et al. 1996; Kole et al. 2002; Quijada 2003; Udall et al. 2005). In the study reported here, we employed one DH population to identify the QTL related to *S. sclerotiorum* resistance using 4 years of data on resistance assessments performed using three inoculation methods.

Materials and methods

Plant materials

In a previous screening study, we identified *B. napus* line DH821, derived from Zhongyou 821, as partially resistant to *S. sclerotiorum*, and DHBao604 as susceptible. Seventy-two DH lines were derived by microspore culture from the F_1 generation of a cross between DH821 and DHBao604. A linkage map comprising 91 simple sequence repeats (SSRs), 72 randomly amplified polymorphic DNA (RAPDs), 86 sequence-related amplified polymorphisms (SRAPs), two restriction fragment length polymorphisms (RFLPs), and one expressed sequence tag (EST), covering a total length of 1746 cM of the rapeseed genome, was used for QTL mapping (Yi et al. 2006).

Experimental design and resistance tests

All DH lines and the two parents were sown by hand each September and identified for resistance in April of each year from 2004 to 2007 in the nursery at Huazhong Agricultural University, Wuhan, China. The field planting followed a randomized complete block design with two replications (four replications in 2004). Each DH line in a plot was sown in two 1.5-m-long rows, with a row spacing of 33 cm. Twelve individual plants in each line were inoculated, and the lesion size was measured. The highest and lowest values in each DH line were deleted from the data set prior to the data being processed. The nursery (tent) was covered with two layers of white mesh to ensure a moist and warm environment for disease development from the flowering to maturing stages.

Inoculum source

Sclerotia of *S. sclerotiorum* were obtained from the stems of rapeseed in the nursery at Huazhong Agricultural University. The isolate was kept at 4°C and cultured on potato dextrose agar (PDA) at 22°C in darkness. The daughter sclerotia were kept at 4°C for the following year's inoculation, and mycelium was sub-cultured on PDA for inoculation.

Mycelial toothpick inoculation (MTI)

The inoculation of mycelial toothpicks was prepared following the procedure described by Zhao and Meng (2003). Ten plants in the middle rows were inoculated at the end of flowering by first cutting a 2.0-mm-diameter opening on the stem near the second or third no-petiole leaf. One mycelial toothpick was placed into each opening. The lesion length on the stem was measured once every 2 days from the third to ninth dpi (days post-inoculation).

Mycelial plug inoculation (MPI)

At the end of flowering, ten plants in the middle of each row were selected for inoculation. Each plant was inoculated by placing a single 8-mm-diameter *S. sclerotiorum* mycelial plug cut from the actively growing margin of a 48-h culture grown on PDA at the second or third clasped leaves lacking petiole attachment. The plug was fixed to the stem surface with Parafilm and remained in contact with the stem surface. The lesion length on the main stem was measured every 2 days from the third to the seventh dpi.

Infected petal inoculation (IPI)

Mycelial plugs were transferred to potato dextrose broth (PDB) medium and incubated for 2 days at the stationary phase. While the 9-cm petri plate was kept covered, the mycelial suspension was mixed evenly and subcultured in 100-ml PDA. After a 36-h shaking incubation (250 rpm), the mycelia suspension was again mixed evenly. Petals of rapeseed flowers were mixed with the mycelium suspension and incubated for 2 h at 22°C. Ten plants in the middle of each row

were selected for inoculation. Each plant was inoculated by placing four infected petals at the second or third clasped leaves lacking petiole attachment with a wetted cotton pad to ensure moisture. The lesion length on the main stem was measured every 2 days from the 9th to 15th dpi.

Genetic map construction

DNA extraction

Total DNA was extracted individually from young leaves using the CTAB method (Doyle and Doyle 1990). DNA concentration and purity was measured by a Beckman spectrophotometer (Beckman Coulter, Fullerton, CA) at a wavelength of 260 versus 280 nm. The final DNA concentration was 25 ng/μl in TE buffer (10 mM Tris, 1 mM EDTA, pH 8.0).

Three classes of molecular markers, SSR, SRAP, and RFLP, were used for assaying the polymorphisms of the DH population. SSR analysis followed the methods of Piquemal et al. (2005). Most of the SSR primer pair sequences were obtained from public sources: http://ukcrop.net/perl/ace/search/BrassicaDB (Lowe et al. 2002) and http://www.brassica.info/ssr/SSRinfo.htm (prefixed by Ra, Ol, Na, BN, MB, BRMS- and MR); http://osbornlab.agronomy.wisc.edu/research/maps/ssrs.html (prefixed by FITO). Primer pairs prefixed "BRAS" and "CB" were from the electronic supplementary material of Piquemal et al. (2005), those prefixed "s" were obtained from Agriculture and Agri-Food Canada (http://www.brassica.agr.gc.ca/index_e.shtml), and those prefixed "P" were from private sources. The RFLP analysis was carried out as described by Thormann et al. (1994). Two genomic DNA probes were kindly supplied by Prof. T. C. Osborn, University of Wisconsin, Madison, WI, USA. Using the sequence of EST from the supression subtractive hybridization (SSH) library, we developed an EST–PCR marker. SRAP analysis followed the methods described in Li and Quiros (2001). Ten forward primers and 17 reverse primers were employed, which resulted in 170 primer combinations. Polymorphic pairs were named by combining the name of forward and reverse primers.

When a primer pair generated more than one polymorphic locus, the name of the pair was eventually followed by a letter to distinguish the different loci. For example, primer pair Ra2-F11 corresponds to three genetic loci, Ra2-F11A, Ra2-F11B, and Ra2-F11C, respectively. Linkage analysis was performed using Mapmaker Exp/3.0b (Lander et al. 1987). A minimum LOD score of 5.0 and a maximum distance of 30 cM were used to group loci into the linkage groups. The 'three point' command was utilized for multi-point analysis. For each linkage group, the order command with a LOD of 3.0 and five specified parameters were used to choose a seed order (order with the highest log-likelihood ratio) of highly informative markers. The remaining markers from each linkage group were manually added in sequence to the seed order using the 'try' command. During interaction, a marker with the most likely position was placed, and the map order was re-tested using the 'ripple' command. The recombination fraction frequencies were converted to map distances using the Kosambi mapping function by employing the 'centiMorgan' function command.

Statistical analysis and QTL detection

The three evaluation methods were analyzed separately. QTLs were identified using the CIM procedure within the QTL Cartographer suite of computer programs (QTLcart V2.5; Basten et al. 2002). The likelihood of a QTL and its corresponding effect at every 2-cM interval was estimated. Four to nine cofactors for each trait were selected by SRmapqtl using a forward–backward regression algorithm (cofactor in for $P < 0.01$; cofactor out for $P > 0.05$). Cofactors within a 10-cM interval on either side of the QTL test site were not included in the Zmapqtl QTL model (Model 6) to avoid the inclusion of putative tail effects of the tested chromosome position. A LOD threshold of 2.5 was used for significance in all trials.

Results

Phenotypic data and segregation in the populations

DH821 showed a higher level of resistance than DHBao604 in each year based on MTI, MPI and IPI evaluations (Table 1). All DH lines showed higher levels of resistance than the susceptible parent (DHBao604), and some had higher levels of

Table 1 Descriptive statistics of lesion length (cm) caused by *Sclerotinia sclerotiorum* evaluated using different inoculation methods on the parents and their DH population

Year	Method	Parent (mean)		DH population			
		DH821	DHBao 604	Mean ± standard deviation	Range	Skew	Kurt
2004	Toothpick	2.065	4. 85	2.59 ± 0.687	1.53–4.38	0.463	−0.581
2005	Toothpick	1.375	2.92	1.85 ± 0.454	0.71–2.77	−0.317	0.066
	Petal	2.66	6.62	3.45 ± 1.131	1.25–6.44	0.354	0.037
2006	Mycelial plug	3.08	5.01	2.54 ± 0.579	1.87–4.21	0.0621	−0.926
2007	Mycelial plug	3.95	5.38	2.91 ± 0.604	1.81–4.24	0.103	−0.897

DH, Doubled haploid

resistance than DH821. Lesions on the susceptible lines started appearing by day 1 post-inoculation, and all plants had lesions by day 3 post-inoculation. This result indicates that the resistance to *S. sclerotiorum* exhibited in both parents was partial and that the resistance mechanism may involve effecting a delay in the disease progress. The DH population showed a continuous distribution and fitted to a normal distribution for all inoculation methods (Table 1).

Correlation coefficients between the different resistant assessments

Correlation analysis was based on the 4-year data set of resistant assessments (Table 2). In general, the most significant ($P \leq 0.01$) correlation coefficients were found for between-year data from the same inoculation method, but a few significant correlations existed in data from the different inoculation methods. For example, stem lesion length by the MTI at 3 dpi was significantly and positively correlated with that at 7 dpi in 2004 ($r = 0.5789$, $P < 0.01$), and the same relationship was found between this variable by the MTI at 3 dpi in 2005 and at 7 dpi in 2007. Stem lesion length by the MPI at 3 dpi was significantly and positively correlated with that at 7 dpi in 2006 ($r = 0.9071$, $P < 0.01$), and the same relationship was found between that at 3 or 7 dpi in 2006 and that at 3 or 7 dpi in 2007. The increase in stem lesion length by MPI from 3 to 7 dpi in 2006 was significantly and positively correlated with the stem lesion length at 3 and 7 dpi, and also with the increase in stem lesion length from 3 to 7 dpi in 2007 ($r = 0.7154$, $P < 0.01$). However, a correlation of stem lesion length between different inoculation methods did not exist in most cases. Even when the r value was higher than the significant level, the correlation still had a low value.

Identification of QTLs for *S. sclerotiorum* resistance

Mycelial toothpick inoculation

Data from 3 dpi (3d) were used as an index for infection of *S. sclerotiorum*, and data from 7 dpi (7d) and 7d-3d (difference in lesion length between the two time points, 3 and 7 dpi) were used as the index of lesion expansion for *S. sclerotiorum*. Two QTLs for infection were identified using the MTI method. The QTLs on N11 were both detected in 2004 and 2005, and alleles from DH821 increased resistance. Six QTL were identified for expandability witth the MTI method. The QTL on N7 was repeatedly detected when using data from 7d and data of 7d-3d. Two QTLs were detected on N10, and one of these was also significant for infection with MTI. The QTL on N12 was significant in both years, and alleles from DHBao604 enhanced resistance. The QTL located on N7 explained the largest proportion of the variance and had the highest LOD score in this study. However, it was only significant in 2004 with the MTI method.

Infected petal inoculation

Data from 10 dpi (10d) were used as the index for infection of *S. sclerotiorum*, and data from 15 dpi (15d) and 15d-10d (difference in lesion length between the two time points, 10 and 15 dpi) were used as the index of expandability for *S. sclerotiorum.*

Table 2 Correlation coefficients between different resistance assessments

Year/resistance assessments[a]	MTI 2004			MTI and IPI in 2005				MPI in 2006		
	Tp3d	Tp7d	Tp7-3d	Tp3d	Tp7d	Tp7-3d	Petal-10d	Mp3d	Mp7d	Mp7-3d
2004										
Tp7d	0.57869**									
Tp7-3d	0.01444	0.82389**								
2005										
Tp3d	0.28174*	0.39312**	0.24714*							
Tp7d	0.20998	0.34434*	0.24258**	0.9090**						
Tp7-3d	0.05339	0.17756	0.16402	0.5318**	0.8364**					
Petal-10d	−0.2840*	−0.2419**	−0.0699	−0.0361	−0.0425	−0.0391				
2006										
Mp3d	−0.05431	0.07208	0.12349	0.2519	0.2511	0.18169	0.12504			
Mp7d	−0.04804	0.11777	0.1727	0.2757*	0.2826*	0.21506	0.10613	0.9071**		
Mp7-3d	−0.0214	0.14187	0.18205	0.20677	0.22127	0.18085	0.0424	0.4582	0.7897**	
2007										
Mp3d	0.00033	0.14649	0.17192	0.2825*	0.3068*	0.25641*	0.08891	0.8673**	0.9373**	0.71548**
Mp7d	−0.07158	0.15645	0.23495*	0.2740*	0.31886	0.29335*	0.08891	0.8086**	0.9349**	0.79581**
Mp7-3d	−0.15398	0.13077	0.26363*	0.19177	0.25164	0.2651*	0.07724	0.5278**	0.7029**	0.71538**

*, **Significant at 0.05 and 0.01 probability level, respectively

MTI, Mycelial toothpick inoculation; IPI, infected petal inoculation; MPI, mycelial plug inoculation

[a] Tp3d and Tp7d indicate 3 and 7 days post-inoculation (dpi), respectively, with the toothpick inoculation method; Mp3d and Mp7d, 3 and 7 dpi, respectively, with the mycelial plug method; Petal-10d, 10 dpi with infected petals; Tp7-3d, the difference in lesion size between two measurements with MTI; Mp7-3d, the difference in lesion size between two measurements with mycelial plug inoculation

Only one QTL on N12 was identified for infection through the IPI method and this QTL was also significant for MTI. No QTL was identified for expandability of IPI.

Mycelial plug inoculation

Data from 3d were used as the index for infection of *S. sclerotiorum*, and data from 7d and 7d-3d (difference in lesion length between the two time points, 3 and 7 dpi) were used as the index of expandability for *S. sclerotiorum*. Two QTLs were identified for infection through the MPI method. The QTL on N4 was only detected in 2006, and it was also significant for expandability by MTI in 2005. The QTLs on N3 were detected in 2006 and 2007, and the alleles from DH821 enhanced resistance. Five QTLs were identified for expandability by the MPI method. The QTL on LG11 was repeatedly detected when 7d and 7d-3d data were used. The QTL on N17 was significant in both years, and alleles from DH821 increased resistance. The QTL on N3 was also significant for infection by the MPI method.

A total of 21 distinct QTLs were identified by the MTI, MPI, and IPI methods during the 4 years of testing the DH population. In each year, the number of QTLs identified varied from four to six, with each QTL explaining 10.21–36.06% of the phenotypic variation (Table 3; Fig. 1). For 14 QTLs, DH821 (the resistant parent), which contributed the resistance allele, accounted for 11.63–36.06% of the phenotypic variation. For the remaining seven QTLs, DHBao604 (the susceptible parent) contributed the resistance allele and accounted for 10.38–17.33% of the

Table 3 Putative QTLs for resistance to *S. sclerotiorum* detected in the DH population

Inoculation method	Resistance assessment[a]	Linkage group	Flanking marker	Max LOD[b]	Var%[c]	A[d]	Year
MTI	3d	N10	Na10G08-S223	3.29	14.6	−0.22	2004
		N11	S1113-O10F1a	3.69	21.5	−0.27	2004
			S1113-Na12C8b	4.61	23.3	−0.20	2005
	7d	N7	Na12A02-S450	3.54	14.6	−0.74	2004
		N10	S286-Na10D07	3.51	12.8	−0.36	2004
		N10	M8E12-Na10G08	6.7	36.1	−0.40	2005
	3d-7d	N4	Na12E05-M8E11	2.59	12.1	−0.10	2005
		N7	SSR-23-S209	4.17	31.6	−0.48	2004
		N12	SSR-57-S370	3.16	13.6	0.28	2004
			SSR-57-M8E5	2.61	10.2	0.32	2005
IPI	10d	N12	S1042-SSR-57	3.31	17.3	0.53	2005
MPI	3d	N4	S1049-Ol10B01	2.58	12.9	−0.17	2006
		N3	M8E12-S1095	2.64	12.5	−0.17	2006
			M8E12-S1095	2.79	16.4	−0.36	2007
	7d	N3	M8E12-S1095	2.66	11.6	−0.26	2006
		LG11	M2E13-Na12C01	3.34	14.8	0.26	2007
		N17	SSR-19-Ra2G10a	2.57	15.1	−0.30	2006
			SSR-19-Ra2G10a	2.66	11.6	−0.26	2007
	3d-7d	N6	S1226-S1339	3.34	14.9	0.14	2006
		N1	M10E5-SSR-84	3.18	12.2	0.12	2006
		LG11	M2E13-Na12C01	2.79	10.4	0.16	2007

QTL, Quantitative trait locus

[a] See footnote to Table 2 for definition

[c] Peak effect of the QTL (LOD, limit of detection)

[c] Proportion of the phenotypic variation explained by the QTL

[d] Negative values indicate alleles from the resistant parent increase resistance, positive values indicate alleles from the susceptible parent enhance resistance. Stem lesion length was measured in centimeters and days to wilt were measured each day post-inoculation

phenotypic variation. There were no common QTLs detected during the 4 years of the study. However, common QTLs were detected between 2 years or between two different inoculation methods. For example, on the N10, N12, N11 linkage groups, overlap of QTL regions were detected by the MTI method in 2004 and 2005. Similarly, on the N3 and N17 linkage groups, overlap of QTL regions were detected by the MPI method. On N4, one QTL region was detected between MTI (in 2005) and MPI (in 2006), and on the N12, a QTL region was found to be associated with both MTI and IPI methods.

Discussion

It is essential to use an appropriate identification method for genetic studies of *S. sclerotiorum* resistance in canola/rapeseed. In our study, we used three inoculation methods to assess resistance to *S. sclerotiorum* during four growing seasons under field conditions. A total of 21 QTLs were detected, of which ten were detected by the MTI method in 2004 and 2005, one was detected by the IPI method in 2005, and ten were detected by the MPI method in 2006 and 2007. Each method detected a number of QTLs related to the resistance for each growing season. Some of these were located in the same region, but some were not. Based on the correlation analysis and QTL mapping, in most cases some common QTL could be detected by the same inoculation method. For example, the QTL regions on N3 and N11 linkage groups were detected by MPI in 2006 and by MTI in 2004 and 2005, respectively. Our results show that the interaction between the host and pathogen to be complex and that inoculation methods could possibly simultaneously activate many resistance responses. Inoculation with the mycelial toothpick may bypass the inherent resistance of the fungus to infect the host because the pathogen was placed inside the wounded stem. The aim of the infected petal method is to imitate natural infection in the field; consequently, a plastic pocket enveloping the infected petal is used to maintain a state of persistent humidity. The resistance assessments based on this method may include both infection and expansion of the disease. The MPI method may activate two types of resistance, but the pathogen concentration is higher than that in the IPI method, and this would be the most artificial method of the three methods used.

The QTLs detected in different years and experiments could be used in marker-assisted selection. In our study, we found an effect on QTL region located on N17, which was detected in 2 different years with the MPI method. On linkage group N12, we found that an effect was detected in 2004 and 2005 and that both effects were associated with the MTI and IPI methods. On linkage group N3, we detected one region in 2006 and 2007 that was associated with the MPI method. Earlier studies also detected the QTLs on N3, N12, and N17. Zhao et al. (2006) used a petiole inoculation technique and two scoring methods, days to wilt (DW) and stem lesion length (SLL) to assess resistance. They identified four significant QTLs for DW in the three evaluations of the HUA population. The QTL *DW12* had inherited the resistant allele from RV289, which was a single plant selected from variety Hua dbl2, with Zhongyou 821 being the other parent of the variety. One significant region was detected on N3 in the MS population that affected both DW and SLL. Zhao and Meng (2003) detected an effect on N17 and a weak effect in the HUA population (one evaluation, LOD score of 2.5). Because the map constructions used different marker systems, we cannot determine whether the QTL on the same linkage groups were in the same region or not. It is not surprising that even a susceptible line, such as DHbao604, was still showing some form of resistant loci. The fact that the remaining QTLs were specific for each population suggests that the resistance in different parents is due, at least in part, to alleles at different QTLs. Combining the different resistant alleles characterized in these studies may ultimately provide high levels of resistance that could be useful for breeding sclerotinia-resistant canola/rapeseed cultivars. Additional studies are needed to determine the individual and combined effects of these QTL alleles in different genetic backgrounds. The recurrent selection population procedure with the aim of improving resistance is a good strategy as this approach may result in the accumulation of multiple loci into one line from different parents.

Perhaps the greatest benefit of using ESTs as genetic markers is the ability to combine QTL mapping with available resistant mechanical, physiological, and phenotypic data, thereby forming a bridge between genetic variation and functional

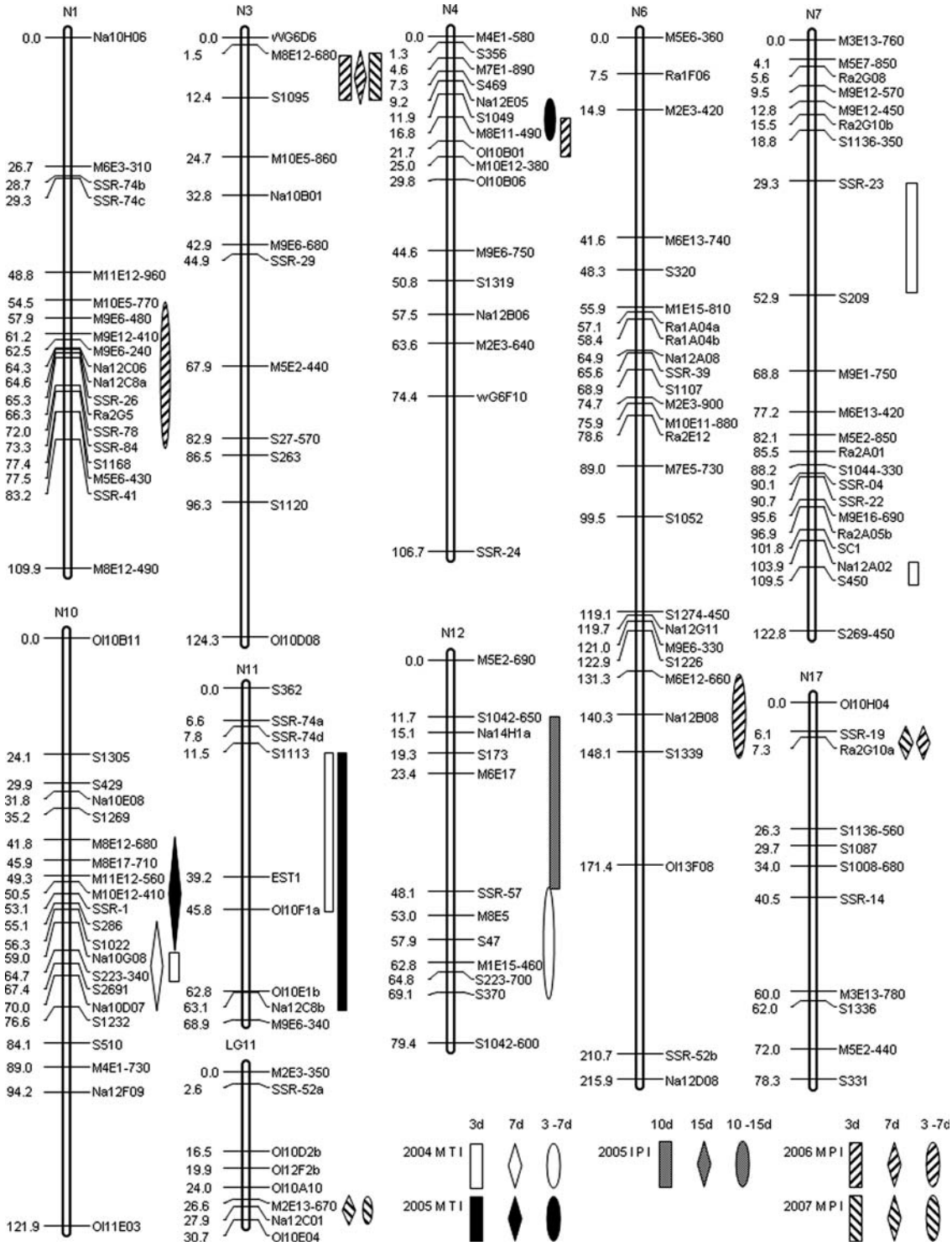

Fig. 1 Quantitative trait loci (QTLs) for *Sclerotinia sclerotiorum* resistance as measured by stem lesion length detected in the doubled haploid population with the mycelial toothpick inoculation (*MTI*), infected petal inoculation (*IPI*), and mycelial plug inoculation (*MPI*) methods. Only the linkage groups with significant QTLs are illustrated. *3d*, *7d*, *10d*, *15d* 3, 7, 10, and 15 days post-inoculation, respectively, *3-7d*, *10-15d* difference in lesion length between the two time points, respectively

variation at the molecular level. Interestingly, one EST marker selected from the SSH library was mapped in the region of N11, which was detected twice in two seasons by the toothpick inoculation method and explained more than 20% of the phenotype variance. The SSH library was constructed from leaves sampled from plants at 0.5–28.5 h post-inoculation and comprised a forward library containing 1920 clones and a backward library containing 1008 clones. Fifty unrepeated resistance-related ESTs were identified. To confirm the reliability of the reverse northern blot result and analyze the expressive mode of different kinds of genes, we made northern blots with cDNA probes from leaves sampled at different times post-inoculation. Three clones were selected from the northern blot, but only one was successfully mapped to the linkage groups. The EST accounted for one type of disease defense which was similar to the thioredoxin protein related to defense against stress (data not shown). Zhao et al. (2007) also investigated gene expression changes between *S. sclerotiorum* infection in partially resistant and susceptible genotypes of oilseed *B. napus* by microarray from *Arabidopsis*. A total of 686 and 1547 genes were found to be differentially expressed after infection in the resistant and susceptible lines, respectively. These researchers inferred that some candidate defense genes were identified by integration of the early response genes in the partially resistant line with the previously mapped QTLs in the linkage group of N14 (Zhao et al. 2006). These candidate genes should be investigated at the molecular level in future investigations.

Acknowledgements The authors wish to thank Dr. F. Yu and the late Dr. S. R. Rimmer for developing the DH population. The authors also wish to acknowledge Ms. Paula Parks for critically reviewing the manuscript for grammatical errors. The project was supported by the program of nyhyzx07-054 and the High-tech program "863" (2006AA10A112).

References

Basten CJ, Weir BS, Zeng ZB (2002) QTL cartographer, version 1.16. Department of Statistics, North Carolina State University, Raleigh

Boland GJ, Hall R (1994) Index of plant hosts of *Sclerotinia sclerotiorum*. Can J Plant Pathol 16:93–108

Bradley CA, Henson RA, Porter PM, LeGare DG, del Rio LE, Khot SD (2006) Response of canola cultivars to *Sclerotinia sclerotiorum* in controlled and field environments. Plant Dis 90:215–219

Doyle JJ, Doyle JL (1990) Isolation of plant DNA from fresh tissue. Focus 12:13–15

Ferreira ME, Rimmer SR, Williams PH, Osborn TC (1995a) Mapping loci controlling *Brassica napus* resistance to *Leptosphaeria maculans* under different screening conditions. Phytopathology 85:213–217

Ferreira ME, Satagopan J, Yandell BS, Williams PH, Osborn TC (1995b) Mapping loci controlling vernalization requirement and flowering time in *Brassica napus*. Theor Appl Genet 90:727–732

Fu S, Lu Z, Chen Y (1990) Inheritance of apetalous character in rape (*Brassica napus* L.) and its potential in breeding. In: Chu Q, Fang G (eds) Proc Symp China Int Rapeseed Sci. Shanghai Scientific and Technical Publ, Shanghai, pp 7–8

Heran A, McCartney HA, Li Q (1999) The effect of petal characteristics, inoculum density and environmental factors on infection of oilseed rape by *Sclerotinia sclerotiorum*. In: Wratten N, Salisbury PA (eds) Proc 10th Int Rapeseed Congress: New Horizons for an Old Crop. T.R.I Publ, Canberra

Jurke CJ, Fernando WGD (2008) Comparison of growth room screening techniques for the determination of physiological resistance to sclerotinia stem rot in *Brassica napus*. Arch Phytopathol Plant Prot 41:157–174

Kole C, Thormann CE, Karlsson BH, Palta JP, Gaffney P, Yandell B, Osborn TC (2002) Comparative mapping of loci controlling winter survival and related traits in oilseed *Brassica rapa* and *B. napus*. Mol Breed 9:201–210

Lander ES, Green P, Abrahamson J, Barlow A, Daly MJ, Lincoln SE, Newburg I (1987) MAPMAKER: an interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. Genomics 1:174–181

Li G, Quiros CF (2001) Sequence-related Amplified Polymorphism (SRAP) a new marker system based on a simple, PCR reaction: its application to mapping, and gene tagging in *Brassica*. Theor Appl Genet 103:455–461

Li Y, Chen J, Bennett R, Kiddle G, Wallsgrove R, Huang Y, He Y (1999) Breeding, inheritance, and biochemical studies on *Brassica napus* CV Zhongyou 821: tolerance to *Sclerotinia sclerotiorum* (stem rot). In: Wratten N, Salisbury PA (eds) Proc 10th Int Rapeseed Congress: New Horizons for an Old Crop. T.R.I. Publ, Canberra

Li CX, Li H, Sivasithamparam K, Fu TD, Li YC, Liu SY, Barbetti MJ (2006) Expression of field resistance under Western Australian conditions to *Sclerotinia sclerotiorum* in Chinese and Australian *Brassica napus* and *Brassica juncea* germplasm and its relation with stem diameter. Aust J Agric Res 57:1131–1135

Li CX, Li H, Siddique AB, Sivasithamparam K, Salisbury P, Banga SS, Banga S, Chattopadhyay C, Kumar A, Singh R, Singh D, Agnihotri A, Liu SY, Li YC, Tu J, Fu TD, Wang YF, Barbetti MJ (2007) The importance of the type and time of inoculation and assessment in the determination of resistance in *Brassica napus* and *B. juncea* to *Sclerotinia sclerotiorum*. Aust J Agric Res 58:1198–1203

Lowe AJ, Jones AE, Raybould AF, Trick M, Moule CJ, Edwards KJ (2002) Transferability and genome specificity

of a new set of microsatellite primers among *Brassica* species of the U triangle. Mol Ecol Notes 2:7–11

McCartney HA, Doughty KJ, Norton G, Booth EJ, Kightley SPJ, Landon G, West G, Walker KC, Thomas JE (1999) A study of the effect of disease on seed quality parameters of oilseed rape. In: Wratten N, Salisbury PA (eds) Proc 10th Int Rapeseed Congress: New Horizons for an Old Crop. T.R.I. Publ, Canberra

Piquemal J, Cinquin E, Couton F, Rondeau C, Seignoret E, Doucet I, Perret D, Villeger MJ, Vincourt P, Blanchard P (2005) Construction of an oilseed rape (*Brassica napus* L.) genetic map with SSR markers. Theor Appl Genet 111:1514–1523

Quijada PA (2003) Introgression of germplasm from winter into spring *Brassica napus*: detection and confirmation of quantitative trait loci. PhD thesis. University of Wisconsin, Madison

Thormann CE, Ferreira ME, Camargo LEA, Tivang JG, Osborn TC (1994) Comparison of RFLP and RAPD markers to estimating genetic relationships within and among cruciferous species. Theor Appl Genet 88:973–980

Thormann CE, Romero J, Mantet J, Osborn TC (1996) Mapping loci controlling the concentrations of erucic and linolenic acids in seed oil of *Brassica napus* L. Theor Appl Genet 93:282–286

Toroser D, Thormann CE, Osborn TC, Mithen R (1995) RFLP mapping of quantitative trait loci controlling seed aliphatic glucosinolate content in oilseed rape (*Brassica napus* L.). Theor Appl Genet 91:802–808

Udall JA, Quijada PA, Osborn TC (2005) Detection of chromosomal rearrangements derived from homoeologous recombination in four mapping populations of *Brassica napus* L. Genetics 169:967–979

Wang HZ, Liu GH, Zheng YB, Wang XF, Yang Q (2004) Breeding of the *Brassica napus* cultivar Zhongshuang 9 with high-resistance to *Sclerotinia sclerotiorum* and dynamics of its important defense enzyme activity. Sci Agric Sin 37(1):23–28

Yi B, Chen W, Ma CZ, Fu TD, Tu JX (2006) Mapping quantitative trait loci for yield and yield components in *Brassica napus* L. Acta Agron Sin 32:676–682

Zhao J, Meng J (2003) Genetic analysis of loci associated with partial resistance to *Sclerotinia sclerotiorum* in rapeseed (*Brassica napus* L.). Theor Appl Genet 106:759–764

Zhao J, Udall JA, Quijada PA, Grau CR, Meng J, Osborn TC (2006) Quantitative trait loci for resistance to *Sclerotinia sclerotiorum* and its association with a homeologous non-reciprocal transposition in *Brassica napus* L. Theor Appl Genet 112:509–516

Zhao J, Wang J, An L, Doerge RW, Chen ZJ, Grau CR, Meng J, Osborn TC (2007) Analysis of gene expression profiles in response to *Sclerotinia sclerotiorum* in *Brassica napus*. Planta 227(1):13–24