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Genetic analysis of loci associated with partial resistance to *Sclerotinia sclerotiorum* in rapeseed (*Brassica napus* L.)

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Abstract *Sclerotinia* stem rot is the most devastating disease of rapeseed (*Brassica napus* L.) in China. Quantitative trait loci (QTLs) involved in resistance to *Sclerotinia sclerotiorum* were detected in a rapeseed population of 128-$F_{2:3}$ families derived from a cross between the male sterility restorer line H5200 and a partial resistant line Ning RS-1. A total of 107 molecular markers including 72 RFLPs, 30 AFLPs, 3 SSRs and 2 RAPDs were employed to construct a genetic linkage map with 23 linkage groups covering 1,625.7 cM with an average space of 15.2 cM. Resistance was assessed empirically at two developmental stages: with a detached leaf inoculation at the seedling stage and in vivo stem inoculation at the mature plant stage. The observed resistance was scored for each plant as leaf resistance at the seedling stage (LRS) and stem resistance at the mature plant stage (SRM). A total of 13 loci were identified by one-way ANOVA and six QTLs were detected with MapMaker-QTL. We found that three of the six QTLs were associated with leaf resistance at the seedling stage and collectively accounted for 40.7% of the total phenotypic variation, each accounting for 23.2%, 16.6% and 13.6% respectively. Three QTLs were found corresponding to the disease resistance at the mature plant stage, explaining 49.0% of the phenotypic variation. Epistasis was observed for the resistance and the additive by additive interactions were the predominant type of epistasis. It was concluded that both single-locus QTLs and epistatic interactions played important roles in *Sclerotinia* resistance in rapeseed.

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

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Introduction

Sclerotinia sclerotiorum (Lib.) de Bary is a fungal pathogen that infects hundreds of plant species (Purdy 1979). Diseases caused by *Sclerotinia* are worldwide, and a most serious problem, since the infection can occur on leaves, stems and pods at different developmental stages. *Sclerotinia* stem rot is one of the most devastating diseases of rapeseed in China, which causes yield loss ranging from 10 to 80%, and oil quality declines as well (Oilcrop Research Institute, Chinese Academy of Sciences 1975). Chemical methods have been used to control this disease. However, due to negative environmental effects, they are not a good choice. Thus, the use of resistant cultivars may be the most economical and effective way of controlling this disease. Exploring the genetic basis of resistance to *Sclerotinia* will help improve cultivars with durable resistance, which will have both environmental and economical benefits.

Genetic studies have shown that the resistance to *Sclerotinia* can be either monogenic or polygenic, depending on the plant species and materials under investigation (Abawi et al. 1978; Baswana et al. 1991). Three quantitative trait loci (QTLs) associated with the resistance to *Sclerotinia* in soybean have been mapped (Kim and Diers 2000). Previous physiological studies on disease resistance in bean and sunflower indicated that oxalic acid was involved in pathogenesis of *Sclerotinia* (Maxwell and Lumsden 1970; Noyes and Hancock 1981; Godoy et al. 1990). Using oxalic acid as a selection indicator, several cultivars conferring partial resistance were characterized in China (Zhou et al. 1994). However, there were no rapeseed cultivars immune to *Sclerotinia*. Moreover, little is known about how many genes in rapeseed are involved in *Sclerotinia* resistance.

QTL mapping has proved to be a powerful approach to understanding the genetic basis of quantitative traits (see reviews by Tanksley 1993; Young 1996). QTLs associated with some important traits in rapeseed have been mapped, including resistance to black-leg disease (Ferreira et al. 1994, 1995a; Dion et al. 1995; Pang et al.

1996a,b; Chevre et al. 1997; Mayerhofer et al. 1997; Pilet et al. 1998a,b), resistance to club-root (Matsumoto et al. 1998), resistance to white rust (Ferreira et al. 1995b), glucosinolate content (Toroser et al. 1995; Uzunova et al. 1995) and other important agronomic traits (Teutonico and Osborn 1994; Song et al. 1995; Butruille et al. 1999; Rajcan et al. 1999). Results from mapping and genetic analysis of *Sclerotinia* resistance QTLs in rapeseed would be very useful for marker-assistant selection and durable resistance cultivar breeding.

In this study, we investigated the genetic basis of partial resistance to *Sclerotinia* in the breeding line Ning RS-1. A segregating F_2 population of 128 individuals was generated with Ning RS-1 as male parent crossed with a male sterility restorer line H5200. We assessed the number of QTLs conferring resistance to *Sclerotinia* at two developmental stages. We also analyzed epistatic interactions contributing to the resistance.

Materials and methods

Plant materials

The breeding line of *Brassica napus*, Ning RS-1, with partial resistance to *S. sclerotiorum*, was kindly provided by Ms. Yuqing Chen, Jiangsu Academy of Agricultural Sciences, China. H5200, a Polima male sterility restorer line of *B. napus*, was obtained from Professor Tingdong Fu of Huazhong Agricultural University. H5200 was used as the female parent in this cross. One F_1 plant derived from the cross was self-pollinated to produce an F_2 population. One hundred and twenty eight F_2 individuals were selected at random and self-pollinated to generate $F_{2:3}$ families. Zhongyou 821, possessing intermediate-resistance to *Sclerotinia*, was used as a resistant control in the inoculation experiment. The pathogen isolate HY-12 was kindly provided by Dr. Guoqing Li of Huazhong Agricultural University.

DNA probes

Two sources of DNA probes were used in this study. The genomic and cDNA clones from *Brassica* were kindly provided by Professor Thomas Osborn of the University of Wisconsin, Madison. Clones with "wg" prefixed were genomic clones derived from *Pst*I fragments of *B. napus* cv Westar. Clones with "tg" prefixed were genomic DNA clones from *Eco*RI fragments of *Brassica rapa* cv. Tobin. Clones prefixed "ec" were derived from a *B. napus* cv Westar cDNA library. The EST (expressed sequence tag) clones from *Arabidopsis thaliana* were kindly provided by Professor Randy Scholl of the *Arabidopsis* Biological Resource Center, Ohio State University. These EST clones were re-named with "Pa". When more than one fragment was detected with one probe, a lower case letter, a, b or c etc., was added as a suffix to the marker name.

Field experiments

The 128 $F_{2:3}$ families, the two parents and the partial resistance control were planted using a randomized complete block design with three replicates in the field station of Huazhong Agricultural University in 1999. Each replicate was composed of 131 plots each with 20 plants in two rows. The distances were 0.3 m between rows and 0.18 m between plants within rows. Eight plants randomly selected from each plot were subjected to the disease resistance assessment.

Culture of *S. sclerotiorum*

A rapeseed isolate of *S. sclerotiorum*, HY-12, was maintained and cultured on solid Potato/Dextrose/Agar (PDA, 20% potato, 2% dextrose and 1.5% agar) medium. Mycelial agar disks of 5-mm diameter punched from the growing periphery of the 2-day old culture of *S. sclerotiorum* on PDA were used as inoculums to infect the plants (Godoy et al. 1990).

Assessment of resistance to *S. sclerotiorum*

Assessment of leaf resistance at the seedling stage (LRS): a young leaf was excised from each plant at the 9 to 12 leaf stage, 30 leaves were placed into a box bedded with wet-paper, and subsequently inoculated with two pieces of mycelial agar plug in a diameter of 5-mm cultured from this fungus separately (Godoy et al. 1990). The boxes were covered with plastic film, and then kept at 20–25 °C in dark. The lesion diameter was measured at 48 h after inoculation to evaluate the level of resistance.

Assessment of stem resistance at the mature plant stage (SRM): plants in the field were inoculated 1 month before harvest using a tooth-pick method, in which sterilized tooth-picks were co-cultured with the fungus in PDA medium for 48 h to make the mycelium grow along the tooth-pick and stopped culturing when the tooth-picks were completely covered with the mycelium. Plant stems were pierced through with the mycelium-covered tooth-picks at a height about 35 cm above the ground. The lesion length along the stems was measured 5 days after inoculation.

DNA markers and assays

Total DNA used for RFLP, AFLP, SSR and RAPD analysis was extracted from young leaves of each F_2 plant and their parents, using the SDS method described by Li et al. (1994). DNA samples were digested with restriction enzymes *Eco*RI and *Hin*dIII. Southern blotting, probe labeling and hybridization were conducted following the method of Meng et al. (1996). Post-hybridization washes varied according to the probes. Blots were washed once at low stringency (2 × SSC, 0.1% SDS, 65 °C) for 10 min, followed by two washes at high stringency (0.1 × SSC, 0.1% SDS, 65 °C) for 10 min each. For *Arabidopsis* EST clones, low stringency (2 × SSC, 0.1% SDS, 65 °C) washes were conducted once for 10 min, and then followed by two intermediate stringency (1 × SSC, 0.1% SDS, 65 °C) washes, each for 10 min.

Three pairs of primers were used in SSR analysis. Primer pair SSR59A, was synthesized according to Szewc-McFadden et al. (1996). The other two, Na14 G10 and Na14G09, were downloaded from the *Brassica* database (http://www.ukcrop.net). PCR was performed in a PE-9600 thermocycler (Perkin Elmer Corp., Norwalk, Conn., USA) in a total volume of 20 µl per reaction containing 80 ng of genomic DNA, 50 ng of primers, 0.4 mM of all the four dNTPs, 1 × PCR buffer (Sangon Biotechnological Company, Shanghai, China, SBC-Shanghai), 1.5 mM of $MgCl_2$, and 1.0 U of *Taq* DNA polymerase. The PCR reaction profiles were: 94 °C for 1 min, followed by 35 cycles of 94 °C for 1 min, 61 °C for 1 min and 72 °C for 1 min, extension at 72 °C for 10 min, and then held at 4 °C. The gel was stained with silver staining kits (Promega, Madison, Wis., USA) according to the manufacturer's instructions.

Two RAPD primers, S28 with the sequence of 5′ GTGACGTAGG and S74 with 5′ TGCGTGCTTG (synthesized by SBC-Shanghai), were used in the analysis. PCR was carried out in a total volume of 20 µl per reaction, containing 50 ng of genomic DNA, 8 µM of random primers, 0.2 mM of all the four dNTPs, 1 × PCR buffer, 1.5 mM of $MgCl_2$, and 1.5 U of *Taq* DNA polymerase (all the PCR reagents were bought from SBC-Shanghai). PCR reactions were conducted following the method of Wang et al. (2000).

The AFLP analysis was conducted as described by Vos et al. (1995). Total DNA was digested simultaneously with restriction

enzymes *Eco*RI and *Mse*I. The PCR reaction was undertaken by two successive amplifications with one and three selective nucleotides at the 3′ end, respectively. Ten pairs of primers with three selective nucleotides at 3′ end were used: A53 ($E^{+AGG}M^{+CAG}$), A54 ($E^{+AGG}M^{+CTC}$), A63 ($E^{+AGT}M^{+CAG}$), A76 ($E^{+AGC}M^{+CTA}$), A78 ($E^{+AGC}M^{+CGT}$), A45 ($E^{+ACT}M^{+CTG}$), A55 ($E^{+AGG}M^{+CTG}$), A14 ($E^{+AAC}M^{+CTC}$), A72 ($E^{+AGC}M^{+CAC}$) and A74 ($E^{+AGC}M^{+CTC}$). Markers generated by the AFLP technique were prefixed with the letter A and suffixed by the molecular weight of the marker fragment.

Statistical analysis

The genotype of F_2 individuals was scored as co-dominant and dominant markers. For co-dominant markers, alleles from NingRS-1 were scored as A, alleles from H5200 as B and the heterozygote as H. For dominant markers, the bands present in NingRS-1 were scored D and those derived from H5200 as C.

The association of marker loci with trait values was assessed by one-way analysis of variance (ANOVA), using a significance level of $p < 0.01$. Digenic interactions were screened among all possible two-locus combinations of the co-dominant markers with two-way ANOVA based on unweighted cell means (Snedecor and Cochran 1980; Yu et al. 1997). The significance of the digenic interactions on resistance was assessed at the level of $p < 0.05$. The significant digenic interactions were further partitioned into four components: additive (first locus) × additive (second locus) (AA), additive × dominance (AD), dominance × additive (DA) and dominance × dominance (DD).

Linkage map construction was performed using the program Mapmaker/EXP 3.0 (Lander et al. 1987, 1989). A LOD threshold of 3.0 and a maximum recombination distance of 37.2 was used to group loci. A combination of three-point and multipoint analysis was applied to find the most probable locus order within each linkage group. Recombination frequencies were transformed to centiMorgans (cM) using the Kosambi mapping function (Kosambi 1944). Linkage groups with one or two markers that appeared in previously published maps of *B. napus* were assumed to be in the same linkage groups as previously designated (Butruille et al. 1999). While linkage groups without reference markers were named randomly. QTLs were identified with the program Mapmaker/QTL 1.1 (Lincoln et al. 1992), which uses the interval-mapping procedure. A LOD score of 2.0 was applied to indicate putative QTLs, and a LOD score of above 3.0 was used to indicate QTLs, which had a significant effect on the phenotype. A multiple QTL analysis was used to determine the total phenotype variance.

Results

Segregation pattern of the resistance

Both LRS and SRM measurements of the resistance to *Sclerotinia* segregated within the $F_{2:3}$ population. For LRS, the average lesion diameter on leaves of individual plants ranged from 2.05 to 3.77 cm. For SRM, the lesion length ranged from 2.33 to 5.82 cm. The continuous distribution at both developmental stages suggested a polygenic nature of the resistance (Fig. 1). The correlation coefficient between LRS and SRM was very low (0.15, $p < 0.01$), implying that different loci might be involved at different developmental stages.

Identifying the resistance loci

One-way ANOVA was used to detect loci associated with the two measures of resistance. A total of two AFLPs and nine RFLPs, four of which were generated by *Brassica* genomic clones and five by *Arabidopsis* EST clones, were found to be associated with the two measures of resistance. Nine markers were associated with LRS, and the marker A54.400 explained the largest amount of variation (9.39%). Two RFLP markers, Wg6c1 and Wg4d5, were detected as associated with both LRS and SRM (Table 1).

Data for 113 segregating loci were used to construct a linkage map. Mapmaker analysis at a LOD threshold of 3.0 placed 107 of these loci into 23 linkage groups span-

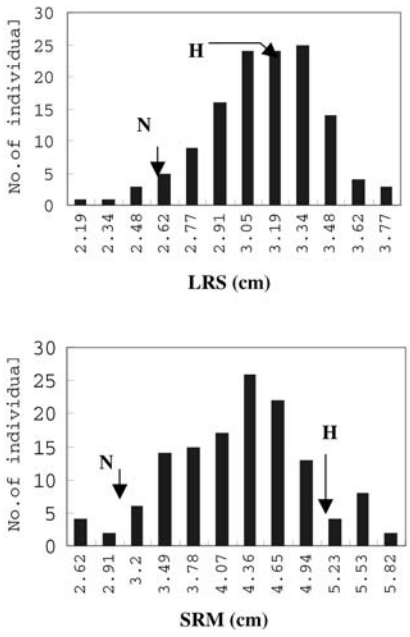

Fig. 1 Frequency distribution of individual plants with various degrees of resistance to *S. sclerotiorumin* in the $F_{2:3}$ population derived from the cross between H5200 and Ning RS-1. The resistance was estimated by the lesion length (cm) on leaf or stem. The mean value of the parent was shown by *arrows*. LRS: leaf resistance at the seedling stage; SRM: stem resistance at the mature plant stage. *N* refers to Ning RS-1, and *H* refers to H5200

Table 1 Loci associated with *Sclerotinia* resistance based on one-way ANOVA ($p < 0.01$)

Trait	Locus	*P* value	R^2 (%)
LRS	A54.400	0.000	9.39
	Pa65	0.000	7.33
	Pa126	0.000	5.98
	A54.90	0.000	5.23
	Pa27	0.002	5.06
	Wg6c1	0.002	4.89
	Pa10 (35F2T7)	0.001	4.60
	Wg4d5	0.005	4.22
	Wg6h1c	0.003	3.37
SRM	Wg6c1	0.004	4.43
	Wg4d5	0.005	4.16
	Pa111	0.008	3.87
	Wg6b10	0.01	2.64

Table 2 Putative QTLs for resistance to *Sclerotinia* detected by Mapmaker/QTL analysis in the $F_{2:3}$ population

Traits	QTLs	LG	Marker interval	Pos.[a]	Peak LOD	R^2 (%)[b]	Add[c]
LRS	*qLRS1*	17	Pa10-Wg6c1	2.0	3.12	13.6	–0.157
	qLRS2	3	Wg2d5a-Pa145	22	2.03	23.2	0.006
	qLRS3	12	Pa65-Pa21	12	2.34	16.6	0.073
						40.7	
SRM	*qSRM1*	15	A75.260-A55.350	18	3.20	39.8	–0.349
	qSRM2	10	Wg2d5d-A54.380	16	2.54	17.4	–0.177
	qSRM3	7	A76.270-A45.210	12	2.20	30.4	–0.415
						49.0	

[a] The position of the Peak LOD was calculated in cM from the left marker
[b] R^2 is the percentage of phenotypic variation explained by the detected QTL
[c] The negative value of the additive effect represents the alleles from the resistant parent Ning RS-1 has a positive effect on the trait

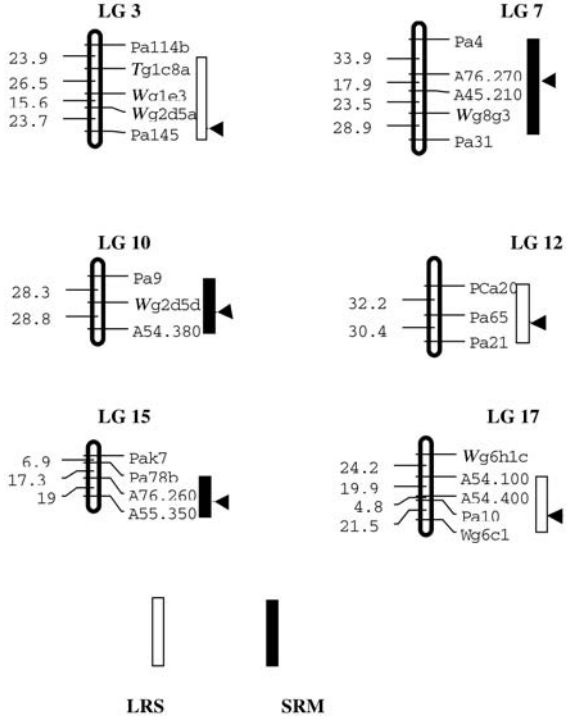

Fig. 2 Locations of putative QTLs contributing to *Sclerotinia* resistance at the seedling stage and at the mature plant stage respectively in the $F_{2:3}$ population derived from a cross between H5200 and Ning RS-1. The *numbers* of the linkage groups (LG) are shown at the top (only linkage groups containing the resistant QTLs are shown). The one-LOD confidence interval for each QTL was indicated by *different bars* on the right, and *cMs* on the left. The position of the peak LOD was shown by a *small black triangle*

ning a total of 1,625.7 cM with an average marker distance of 15.2 cM. Thirteen linkage groups, LG1 to LG 8, LG10, LG13, LG14, LG15 and LG17, had one or two markers that appeared in previously published maps of *B. napus* (Butruille et al. 1999) and the other ten linkage groups were randomly named. The 107 markers on the 23 linkage groups were used for QTL mapping. Thirty-two co-dominant markers identified from the 107 markers were used for two-locus interaction analysis.

Table 3 Two-locus interactions affecting *Sclerotinia* resistance at the seedling stage detected at $p < 0.05$ by two-way ANOVA

Locus 1	Locus 2	Type	V_{1-2} (%)[a]	*P* value
Wg1g4a	Pa31	AA	5.95	0.008
		AD	5.19	0.013
Ec4d11	Wg9f2	AA	8.71	0.006
		DA	5.10	0.035
Wg9f2	SSR59A	AA	4.38	0.043
		AD	7.68	0.008
SSR59A	Wg6c1	AA	4.10	0.013
		DD	7.56	0.001
Wg1g4a	Wg1e3	AA	4.45	0.018
Pa31	Pa114b	AA	6.31	0.007
Wg9f2	Tg1c8a	AA	7.56	0.009
Wg1e3	Wg1a6	AA	5.02	0.014
		DD	3.41	0.042
Wg6c1	Wg9d5	AA	5.47	0.007

[a] V_{1-2} indicates the phenotypic variation explained by the specific type of two-locus interaction

A total of six QTLs were identified for the two measures of resistance using Mapmaker/QTL. Half of the positive loci detected by one-way ANOVA were located closely to the QTL intervals (Table 2, Fig. 2). However, two loci, Pa126 and A54.90, identified by one way ANOVA as showing association with Sclerotinia resistance, did not have correspondence with the QTLs, as they were not placed in the linkage group. Three QTLs were detected to determine LRS and located in three different genomic regions. The major QTL, *qLRS1*, located on LG17, explained 13.6% of the phenotypic variation with a LOD score of 3.12. The other two putative QTLs had lower LOD scores. These three QTLs jointly accounted for 40.7% of the total phenotypic variation. Another three QTLs, identified to be associated with SRM, were also located in three different genomic regions. The major QTL, *qSRM1*, being mapped on linkage group 15, explained 39.8% of the total phenotypic variation. Two AFLP markers flanked this region and were linked at a distance of 19.0 cM. The other two QTLs, however, had lower LOD scores. The three QTLs together explained 49.0% of the total phenotypic varia-

tion. None of the QTLs detected were associated with both measures of resistance.

Digenic interactions for the resistance

Data from 32 co-dominant loci were employed for interaction analysis. Nine digenic combinations from a total of 375 two-locus combination tests showed significant ($p < 0.05$) interactions for LRS resistance (Table 3). Most of the digenic interactions involved the loci of which one member either located on one of the QTL intervals or had a significant effect on the disease resistance when one-way ANOVA was performed. The predominant type of digenic interactions was additive × additive (AA). Dominance × dominance (DD) and DA or AD digenic interaction was much in deficiency.

Discussion

Based on this segregating population, 113 loci were assembled into 23 linkage groups and five unlinked loci with a LOD threshold 3.0. The number of linkage groups is greater than the number of chromosomes in the haploid genome of *B. napus*. This is mostly due to the limited number of markers used in the analysis. Furthermore, some of the linkage groups were designated on the basis of only one or two markers that were common with Butruille et al. (1999).

We detected 13 marker-locus associations (by ANOVA) and six QTLs (using Mapmaker/QTL) for the two measures of resistance to *Sclerotinia*, LRS and SRM, in *B. napus*. In this experiment, LRS was assessed at the seedling stage using a detached leaf inoculation method, indicating that the LRS QTLs may be leaf-specific or seedling-specific resistant. For SRM, the assessment was conducted at the mature plant stage and the pathogen was inoculated in vivo into the stem. Therefore, the resistance measured as SRM may reflect a form of stem resistance and these QTLs may be stem-specific or mature plant-specific. It suggests that different resistance loci might be involved at different developmental stages or in different organs. Based on one-way ANOVA, two RFLP markers, Wg6c1 and Wg4d5, were associated with both measures of resistance. Thus, these may represent common QTLs for the two measures of resistance.

In this population, the total phenotypic variations explained by the QTLs were 40.7% for LRS and 49.0% for SRM respectively. Other unexplained variations may be contributed by epistasis, by undetected QTLs or environmental errors. Alleles from the partial resistant parent gave a larger contribution to the resistance phenotype, while the susceptible parent contributed alleles with small effect on the resistance. New genotypes that combine alleles from both parents may arise in this population and could be used in a breeding program.

One genomic region on linkage group 17 was shown to be associated with LRS by QTL mapping. Markers flanking this region were also identified by one-way ANOVA as significantly associated with resistance. One of the EST clones of *Arabidopsis*, Pa10, coding for catalase, has a homologous segment in this region. It has been reported that oxalate played an important role in the pathogenesis of *Sclerotinia* (Maxwell and Lumsden 1970; Noyes and Hancock 1981; Godoy et al. 1990; Cessna et al. 2000). It might be interesting to explore whether the Pa10 analogue in this QTL region of *B. napus* has a function for oxalate degeneration and *Sclerotinia* resistance as well.

Epistasis has been regarded as an important genetic basis of heterosis in rice (Yu et al. 1997). It was also shown to control a large part of resistance in a pepper-*Phytophthora capsici* interaction (Lefebvre et al. 1996). A set of epistasis loci contributing to the *Sclerotinia* resistance was identified in our study. Nine pairs of significant interactions were detected for LRS from the 375 pairs of interactions between 32 co-dominant loci. With $\alpha = 0.05$ for the individual test, the expected numbers of spurious interactions (false positives) would be less than five for LRS. The number we detected is beyond the expected false positive, indicating that real two-locus interactions existed in this population. Interestingly, in most cases, two interaction loci were located in different linkage groups and one of the loci had a significant main effect or was located on the QTL interval. Our data showed that the AA type of interactions were much more frequent than the DD and AD/DA types, which is similar to the results of the previous analysis of digenic interaction in rice heterosis (Yu et al. 1997). It seems that epistasis, especially the interactions between additive loci, were a universal force to increase different biochemical functions in plants. It might be deduced that QTLs played an important role with a manner of additive interactions with other loci on disease resistance. Considering that only a very small number of co-dominant loci had been involved in the experiment, and much more QTLs should exist except for the six detected ones, the loci interactively and independently influencing disease resistance would be great in quantity and complex in function.

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References

Abawi GS, Provuidenti R, Crosier DC, Hunter JE (1978) Inheritance of resistance to white mold disease in Phaseolus coccineus. J Hered 69:200–202

Baswana KS, Rastogi KB, Sharma PP (1991) Inheritance of stalk rot resistance in cauliflower (Brassica oleracea var. Botrytis L.). Euphytica 57:93–96

Butruille DV, Guries RP, Osborn TC (1999) Linkage analysis of molecular markers and quantitative trait loci in populations of inbred backcross lines of Brassica napus. Genetics 133:950–964

Cessna SG, Sears VE, Dickman MB, Low PS (2000) Oxalic acid, a pathogenicity factor for Sclerotinia sclerotiorum, suppresses the oxidative burst of the host plant. Plant Cell 12:2191–2200

Chevre AM, Barret P, Eber F, Dupuy P, Brun H, Tanguy X, Renard M (1997) Selection of stable Brassica napus-B. juncea recombinant lines resistant to blackleg (Leptosphaeria maculans). I. Identification of molecular markers, chromosomal and genomic origin of the introgression. Theor Appl Genet 95:1104–1111

Dion Y, Gugel RK, Rakow GFW, Seguin-Swartz G, Landry BS (1995) RFLP mapping of resistance to the blackleg disease [causal agent, Leptosphaeria maculans (Desm.) Ces. et de Not.] in canola (Brassica napus L.). Theor Appl Genet 91:1190–1194

Ferreira ME, Williams PH, Osborn TC (1994) RFLP mapping of Brassica napus using doubled haploid lines. Theor Appl Genet 89:615–621

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, Williams PH, Osborn TC (1995b) Mapping of a locus controlling resistance to Albugo candida in Brassica napus using molecular markers. Phytopathology 85:218–220

Godoy G, Steadman JR, Dickman MB, Dam R (1990) Use of mutants to demonstrate the role of oxalic acid in pathogenicity of Sclerotinia sclerotiorum on Phaseolus vulgaris. Physiol Mol Plant Pathol 37:179–191

Kim HS, Diers BW (2000) Inheritance of partial resistance to Sclerotinia stem rot in soybean. Crop Sci 40:55–61

Kosambi DD (1994) The estimation of map distance from recombination values. Ann Eugen 12:172–175

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

Lander ES, Botstein D (1989) Mapping Mendelian factors underlying quantitative traits using RFLP linkage maps. Genetics 121:185–199

Lefebvre V, Palloix A (1996) Both epistatic and additive effects of QTLs are involved in polygenic induced resistance to disease: a case study, the interaction of pepper-Phytophthora capsici Leonian. Theor Appl Genet 93:503–511

Li J, Shen BZ, Han JX (1994) An effective way of total DNA extraction from Rapeseed. J Huazhong Agric Univ 13:521–523

Lincoln S, Daly M, Lander E (1992) Mapping genes controlling quantitative traits with MAPMAKER/QTL. Whitehead Institute Tech Rep, Cambridge, Massachusetts

Matsumoto E, Yasui C, Ohi M, Tsukada M (1998) Linkage analysis of RFLP markers for clubroot resistance and pigmentation in Chinese cabbage (Brassica rapa ssp. Pekinensis). Euphytica 104:79–86

Maxwell DP, Lumsden RD (1970) Oxalic acid production by Sclerotinia sclerotiorum in infected bean and in culture. Phytopathology 60:1395–1398

Mayerhofer R, Bansal VK, Thiagarajah MR, Stringam GR, Good AG (1997) Molecular mapping of resistance to Leptosphaeria maculans in Australian cultivars of Brassica napus. Genome 40:294–301

Meng J, Sharp A, Bowman C, Tian Z, Qian X, Lydiate D (1996) Genetic diversity of Brassica napus accessions mainly from China detected with RFLP markers. Chinese J Genet 23:221–232

Noyes RD, Hancock JG (1981) Role of oxalic acid in the Sclerotinia wilt of sunflower. Pysiol Plant Pathol 18:123–132

Oilcrop Research Institute, Chinese Academy of Sciences (1975) Sclerotinia disease of oilseed crops. Agriculture Press, Beijing

Pang ECK, Halloran GM (1996a) The genetics of blackleg [Leptosphaeria maculans (Desm.) Ces. et de Not.] resistance in rapeseed (Brassica napus L.). I. Adult-plant resistance in F2 and first backcross populations.Theor Appl Genet 93:932–940

Pang ECK, Halloran GM (1996b) The genetics of blackleg [Leptosphaeria maculans (Desm.) Ces. et de Not.] resistance in rapeseed (Brassica napus L.). II. Seedling and adult-plant resistance as quantitative traits. Theor Appl Genet 93:941–949

Pilet ML, Delourme R, Foisset N, Renard M (1998a) Identification of loci contributing to quantitative field resistance to blackleg disease, causal agent Leptosphaeria maculans (Desm.) Ces. Et de Not., in winter rapeseed (Brassica napus L.). Theor Appl Genet 96:23–30

Pilet ML, Delourme R, Foisset N, Renard M (1998b) Identification of QTLs involved in field resistance to light leaf spot (Pyrenopeziza brassicae) and blackleg resistance (Leptosphaeria maculans) in winter rapeseed (Brassica napus L.). Theor Appl Genet 97:398–406

Purdy LH (1979) Sclerotinia sclerotiorum: history, diseases and symptomatology, host range, geographic distribution and impact. Phytopathology 69:875–890

Rajcan I, Kasha KJ, Kott LS, Beversdorf WD (1999) Detection of molecular markers associated with linolenic and erucic acid levels in spring rapeseed (Brassica napus L.). Euphytica 105:173–181

Snedecor GW, Cochran WG (1980) Statistical methods. Iowa state University Press, Ames, Iowa

Song K, Slocum MK, Osborn TC (1995) Molecular marker analysis of genes controlling morphological variation in Brassica rapa (syn. campestris). Theor Appl Genet 90:1–10

Szewc-McFadden AK, Kresovich S, Bliek SM, Mitchell SE, McFerson JR (1996) Identification of polymorphic, conserved simple sequence repeats (SSRs) in cultivated Brassica species. Theor Appl Genet 93:534–538

Tanksley SD (1993) Mapping polygenes. Annu Rev Genet 27: 205–233

Teutonico RA, Osborn TC (1994) Mapping of RFLP and qualitative trait loci in Brassica rapa and comparison to the linkage maps ofB. napus, B. oleracea, and Arabidopsis thaliana. Theor Appl Genet 89:885–894

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

Uzunova M, Ecke W, Weissleder K, Robbelen G (1995) Mapping the genome of rapeseed (Brassica napus L.). I. Construction of an RFLP linkage map and localization of QTLs for seed glucosinolate content.Theor Appl Genet 90:194–204

Vos P, Hogers R, Bleeker M, Reijans M, Theo van de Lee, Hornes M, Frijters A, Pot J, Pelemen J, Kuiper M, Zabeau M (1995) AFLP: a new technique for DNA fingerprinting. Nucleic Acids Research 23:4407–4414

Wang J, Yang G, Fu T, Meng J (2000) Development of PCR-based markers linked to the fertility restore gene for the Polima cytoplasmic male sterility in rapeseed (Brassica napus L.) Acta Genet Sinica 27:1012–1017

Young ND (1996) QTL mapping and quantitative disease resistance in plants. Annu Rev Phytopathol 34:479–501

Yu SB, Li JX, Xu CG, Tan YF, Gao YJ, Li XH, Zhang QF, Saghai Maroof M (1997) Importance of epistasis as the genetic basis of heterosis in an elite rice hybrid. Proc Natl Acad Sci USA 94:9226–9231

Zhou BW, Yu Q, Liu SY, Zhou LC, Chen DY (1994) Identification and screening of oilseed rape resistance to Sclerotinia sclerotiorum and virus. Oil Crops of China (supplement) 4:57–61