Genetic Map Construction and Sclerotinia Resistance QTLs Identification in Rapeseed (*Brassica napus* L.)

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Abstract

Sclerotinia stem rot is one of the most devastating diseases of rapeseed in China, which causes yield loss ranging from 10 to 80%. The objective of this study was to detect the QTLs associated with this disease. A partial *sclerotinia* resistance rapeseed line M083 was crossed with a sensitive one APL01, and the BC_1F_1 derived from this cross was used as a mapping population. The 182 lines of BC_1F_2 family were inoculated with *Sclerotinia sclerotiorum* fungus to assessment the *sclerotinia* resistance at seedling stage and mature plant stage. A total of 307 markers, including 271 SRAP, 29 SSR and 7 RAPD, were employed to construct a genetic linkage map with 19 linkage groups covering 1840.36 cM with an average space of 5.99 cM. This map was aligned with the ultradense genetic map and SSR map in *Brassica napus* by using the same SRAP or SSR markers. Three QTLs, *qSRS1*, *qSRS9* and *qSRS13*, linked to *sclerotinia* resistance at seedling stage in rapeseed were identified, which located in linkage group N1, N9 and N13, and explained 5.01%, 6.43% and 5.25% phenotypic variation at seedling stage in the segregation lines of BC_1F_2 family, respectively. Three QTLs, *qSRM3*, *qSRM8* and *qSRM9*, linked to *sclerotinia* resistance at maturity stage in rapeseed were identified, which were located in linkage group N3, N8 and N9, explained 5.38%, 5.61% and 5.65% of the s*clerotinia* resistance variation at maturity stage in lines of BC_1F_2 family. All QTLs identified in this study, either at seedling or maturity stage, were minus ones, and only *qSRS9* and *qSRM9* were at the same position on linkage group N9.

Key wards: *Brsaaica napus* L.; *sclerotinia* resistance; quantitative trait loci (QTLs)

Introduction

Sclerotinia sclerotiorum (Lib.) de Bary is a soilborne pathogen capable of infecting more than 400 host plants worldwide, including canola, mustard, alfalfa, soybean, field-bean, lentil, field pea, and sunflower (Fernando et al 2004). *Sclerotinia* stem rot is one of the most devastating diseases of rapeseed in China, which causes yield loss ranging from 10% to 80% (Zhao et al 2003). There was no rapeseed cultivar immune to *sclerotinia*, but several cultivars, such as ZY821, Ning RS-1 and ZY083, conferring partial resistance to this disease, which could be used in resistance cultivar breeding (Zhou et al 1994).

QTL mapping has proved to be a powerful approach to understanding the genetic basis of quantitative traits. QTLs associated with some important traits in rapeseed have been mapped. 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 M083. A segregating BC_1F_1 population of 182 individuals was generated with M083 as male parent crossed with a sensitive line APL01. We assessed the number and effect of QTLs conferring resistance to *sclerotinia* at seedling and maturity stages in rapeseed.

Materials and Methods

Plant materials and field experiments: A partial *sclerotinia* resistance rapeseed line M083 was obtained from Institute of Oil Crops, Chinese Academy of Agricultural Sciences. A sensitive one APL01 was bred by Institute of Industrial Crops, Jiangsu Academy of Agricultural Sciences. APL01 was crossed with M083 to get F_1, and the F_1 was backcrossed with APL01 to get BC_1F_1. 182 individuals of the BC_1F_1 were used as a mapping population. The 182 lines of BC_1F_2 family were inoculated with *Sclerotinia sclerotiorum* fungus in PDA medium at seedling stage and mature plant stage. The disease indexes (DI) of *sclerotinia* resistance in the lines of BC_1F_2 family were scored according to the method introduced by Zhou (1994).

DNA marker analysis: A modified version of the CTAB method was used to extract genomic DNA. The RAPD reactions were performed in a PTC-200 Thermocycler. Amplified products were electrophoresesed in 1.4% agrose gel, and dyed by EB. The result was detected by GeneGenius Image Analyzer. The sizes of the specific bands were calculated using GeneTools software. The SSR

reactions were performed in an Eppendorf Mastercycler Gradient Thermocycler. Amplification products were resolved on 8% denaturing polyacrylamide gels and visualized by the silver staining system. The SRAP reactions were set up using the same components and amplification program reported by Li (2001). PCR products were separated with an ABI 3100 Genetic Analyzer and a five-color fluorescent dye set, including 'FAM' (blue), 'VIC' (green), 'NET' (yellow) and 'PET' (red), and 'LIZ' (orange as the standard) was used. Samples from four different color labeled primers were pooled together after running PCR reactions and 2.5 µl of the pooled samples was added to a 5.5 µl mixture of formamide and 500-LIZ size standard (ABI, California), and then denatured at 95℃ for 5 min. The plates containing the samples were then loaded into the auto sampler of the ABI 3100 Genetic Analyzer.

Data analysis: Each polymorphic band was treated as a separate character that was scored as a dominant marker and recorded as one for presence and zero for absence. Linkage analysis was performed on the segregation data of all markers in 182 BC_1F_1 plants using the software package JoinMap version 3.0. The recombination frequencies were converted into centiMorgans (cM) using the Kosambi function, and the linkage map was drawed using the software of MapDraw version 2.1 (Liu et al, 2003). QTLs were detected by the software package Windows QTL Cartographer Version 2.5 (Statistical Genetics, North Carolaina State University, USA). The minimum LOD value, which meaned QTL existed, was 2.5.

Results and Discussion

Frequency distribution of *sclerotinia* resistance in lines of BC_1F_2 family

The DI of *sclerotinia* resistance in BC_1F_2 lines derived from the cross APL01 and M083 were tested by inoculation at seedling stage and maturity stage, respectively. Results showed that the DI of *sclerotinia* resistance in the partial resistant parent M083 was 9.41 at the seedling stage, while the sensitive one APL01 was 32.86. The DI ranged from 1.67 to 67.5 in lines of BC_1F_2 family. So transgressive segregation existed in DI of *sclerotinia* resistance in BC_1F_2. The frequency distribution for DI of *sclerotinia* resistance in BC_1F_2 lines was showed in figure 1. At maturity stage, the DI of M083 and APL01 were 7.50 and 20.00, respectively. The DI in lines of BC_1F_2 family ranged from 0 to 68.18, and the frequency distribution for DI in BC_1F_2 was also showed in figure 1.

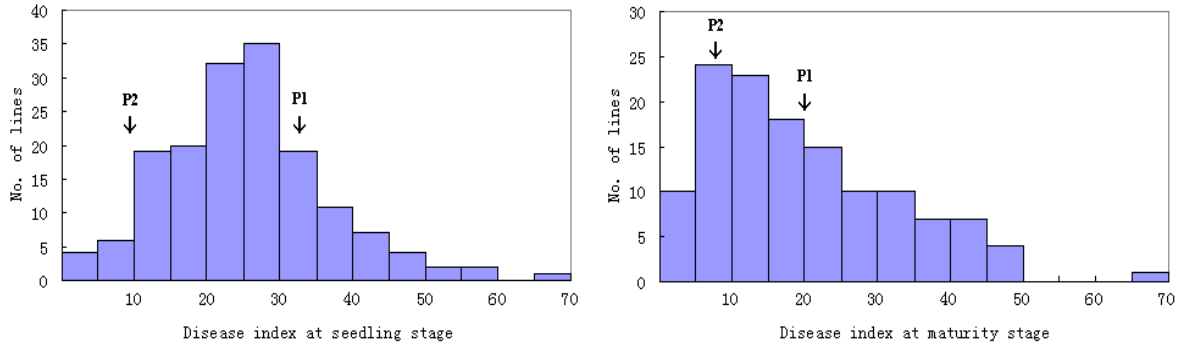

Fig. 1 The frequency distribution for disease index of *sclerotinia* resistance in lines of BC_1F_2 family at seedling and maturity stage

Genetic map in rapeseed (*Brassica napus* L.)

307 molecular markers were used to construct the genetic map in rapeseed (*Brassica napus* L.), in which 271 markers were SRAP, 29 were SSR and 7 were RAPD markers. The genetic map contained 19 linkage groups, N1 – N19, and the genetic distance was totally 1840.36 cM. The average distance between two markers was 5.99cM. The alignments of the 19 linkage groups and the ultradense genetic recombination map for *Brassica napus* were done. The linkage groups N1 – N19 in this paper were corresponded with the linkage groups N1 – N19 in the ultradense genetic recombination map for *Brassica napus* (Sun et al, 2007), and also corresponded with the linkage groups N1 – N19 of SSR map (Piquemal et al, 2005), respectively.

QTLs linked to *sclerotinia* resistance in rapeseed (*Brassica napus* L.)

After running Windows QTL Cartgrapher Version 2.5, three QTLs, *qSRS1*, *qSRS9* and *qSRS13*, linked to *sclerotinia* resistance at seedling stage in rapeseed were identified. *qSRS1* was located in the region of A0216Ya209 - A0216Gb501 on linkage group N1, which could explain 5.01% of the *sclerotinia* resistance variation at seedling stage in the segregation population BC_1F_1. *qSRS9* was mapped in the region of A0224Bb316 - A0216Ra244 on linkage group N9, and accounted for 6.43% phenotypic variation. *qSRS13* was located between Na12-G05a - A0228Rb412 on linkage group N13 and explained 5.25% phenotypic variation (Table 1 and Fig 2).

Three QTLs, *qSRM3*, *qSRM8* and *qSRM9*, linked to *sclerotinia* resistance at maturity stage in rapeseed were identified. *qSRM3* was located in the region of m11e40 - m10e44a on linkage group N3, which could explain 5.38% of the *sclerotinia* resistance variation at maturity stage in the segregation population BC_1F_1. *qSRM8* was located between m5e42 - m11e37b on linkage group N8 and explained 5.61% phenotypic variation. *qSRM9* was mapped in the region of A0224Bb316 - A0216Ra244 on N9, and accounted for 5.65% phenotypic variation (Table 1 and Fig 2).

The QTLs linked to *sclerotinia* resistance in rapeseed, either at seedling or maturity stage, were all minus ones, which were difficult to use in *sclerotinia* resistance rapeseed breeding. Among these six QTLs, *qSRS1*, *qSRS9*, *qSRS13*, *qSRM3* and *qSRM9* had positive effect to *sclerotinia* resistance, while *qSRM8* had negative effect. Only *qSRS9* at seedling stage and *qSRM9* at maturity stage were at the same position on linkage group N9.

Table 1 Positions, intervals, effects and variety of *sclerotinia* resistance QTLs on different linkage groups in rapeseed (*Brassica napus* L.)

QTL	Linkage group	Flanking markers	Interval (cM)	QTL position (cM)	Max LOD	Effect	(Var.%)
qSRS1	N1	A0216Ya209-A0216Gb501	14.39	2.01	2.70	5.14	5.01
qSRS9	N9	A0224Bb316-A0216Ra244	22.67	6.01	3.79	5.80	6.43
qSRS13	N13	Na12-G05a-A0228Rb412	5.04	29.04	2.48	5.26	5.25
qSRM3	N3	M11e40-m10e44a	6.25	44.14	2.99	6.15	5.38
qSRM8	N8	M5e42-m11e37b	10.45	59.67	2.91	-6.29	5.61
qSRM9	N9	A0224Bb316-A0216Ra244	22.67	6.01	2.69	6.42	5.65

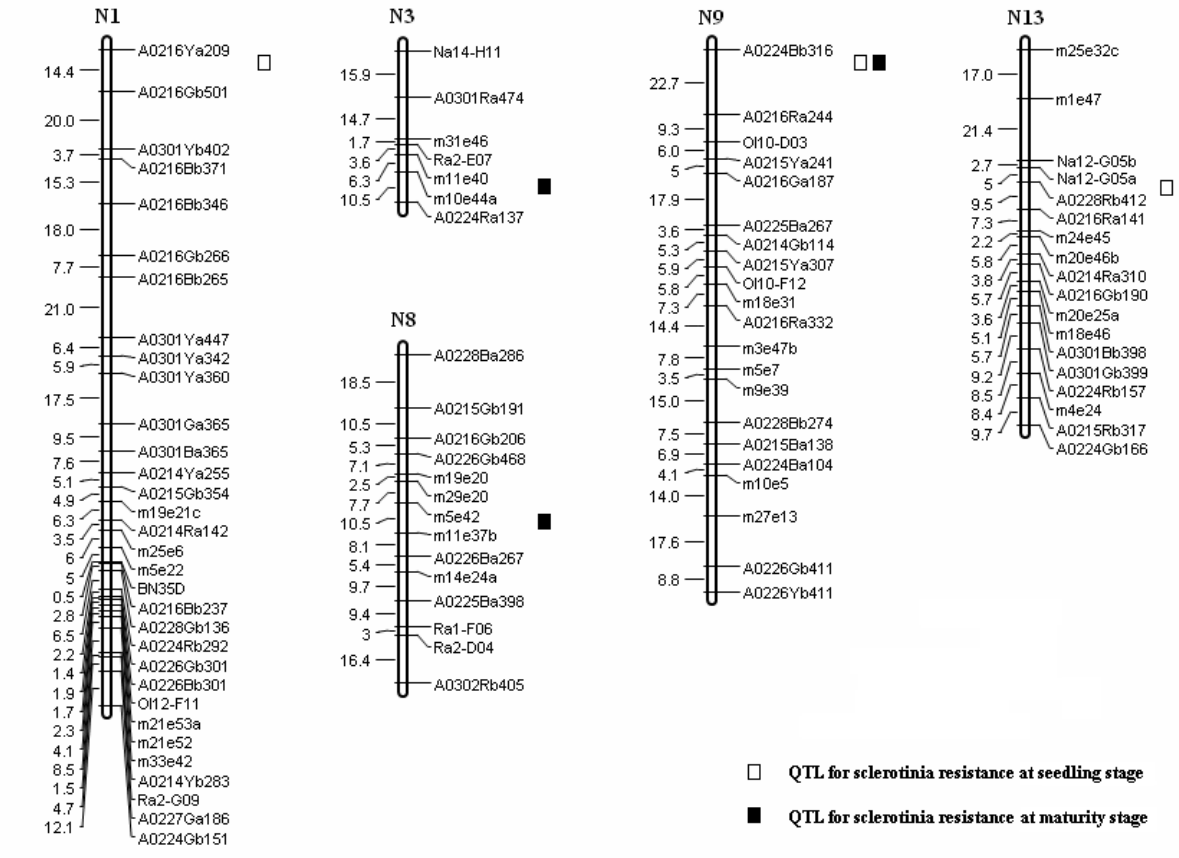

Fig. 2 QTLs linked to sclerotinia resistance in rapeseed (*Brassica napus* L.)

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