Identification of QTLs for resistance to Sclerotinia stem rot and *BnaC.IGMT5.a* as a candidate gene of the major resistant QTL *SRC6* in *Brassica napus*

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Introduction

Sclerotinia sclerotiorum (Lib.) de Bary is a necrotrophic and non-host-specific fungal pathogen that infects more than 400 plant species, and causes 10-30% yield losses in oilseed rape (*Brassica napus*) in China annually. Identification of gene resources are of great importance for breeding of *S. sclerotiorum* resistance in *B. napus*. The present study was conducted to map major QTLs for resistance to *S. sclerotiorum* in oilseed rape. A candidate gene for a major resistant QTL was identified through comparative mapping and homologous gene cloning.

Results

Phenotypic analysis of resistance to *S. sclerotiorum*

A doubled haploid (DH) population of 190 individual DH lines derived from microspore culture of F1 buds of the cross between Huashuang No.5 (Hua 5), a widely grown variety, and J7005, a relatively resistant pure line, was used for mapping and trait analysis. Detached leaf inoculation and stem inoculation with mycelial agar plugs were adopted for resistance evaluations. Leaf resistance (LR) at the seedling stage and stem resistance (SR) at the mature plant stage were analyzed as the target traits (Fig. 1, Fig. 2).

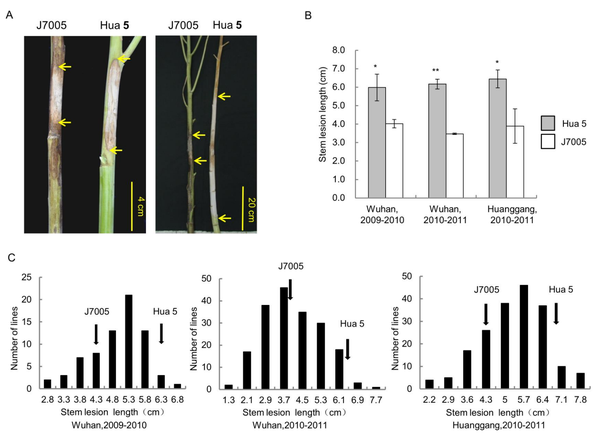

Fig. 1 Stem resistance (SR) of the two parental lines, Hua 5 and J7005, and the HJ-DH population.

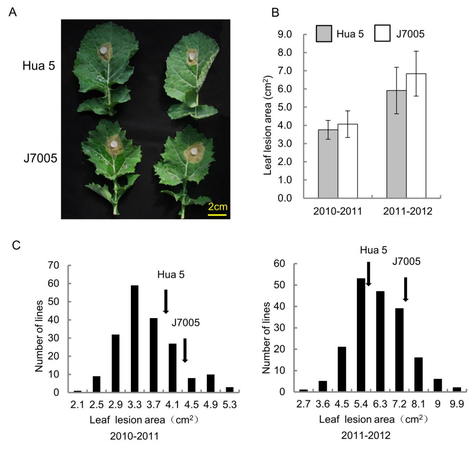

Fig. 2 Leaf resistance (LR) of the two parental lines, Hua 5 and J7005, and the HJ-DH population.

Mapping of QTLs for *Sclerotinia* resistance

Three QTLs for LR in two growing seasons, and 10 QTLs for SR in three environments were identified, respectively (Fig. 3). A major QTL for LR, *LRA9*, was identified stably across years, and explained 8.54-15.86% of the phenotypic variation. A major QTL with the largest genetic effect for SR, *SRC6* , was detected stably in all three environments, and explained 29.01%-32.61% of the phenotypic variation.

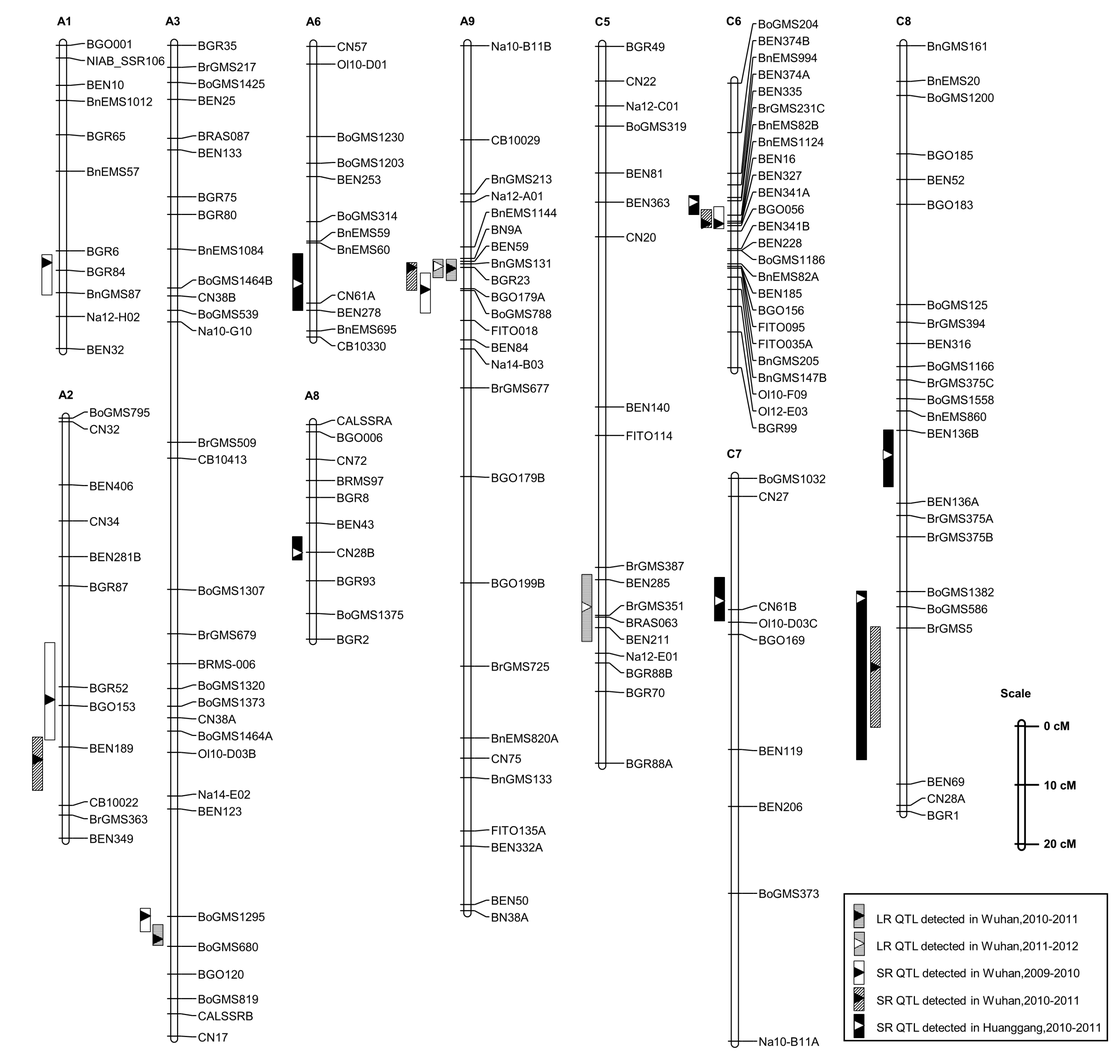

Fig.3 QTLs for LR and SR mapped on the HJ-DH genetic linkage map. The bar to the left of the LG indicates the 1-LOD confidence interval for the QTL and the triangle indicates the QTL peak position.

Comparative mapping of C6 linkage group with Arabidopsis and *B. oleracea*

Comparative analysis revealed that LG C6 was co-linear with *B. oleracea* chromosome 7 (BoC7) and Arabidopsis chromosome 1 and 3 (AtC1 and AtC3) (Fig. 4). Three Arabidopsis conserved blocks (D, E and B) were identified to correspond with the confidence interval of *SRC6* (19-24.6cM), and the peak of the *SRC6* fell in block E (Fig. 4).

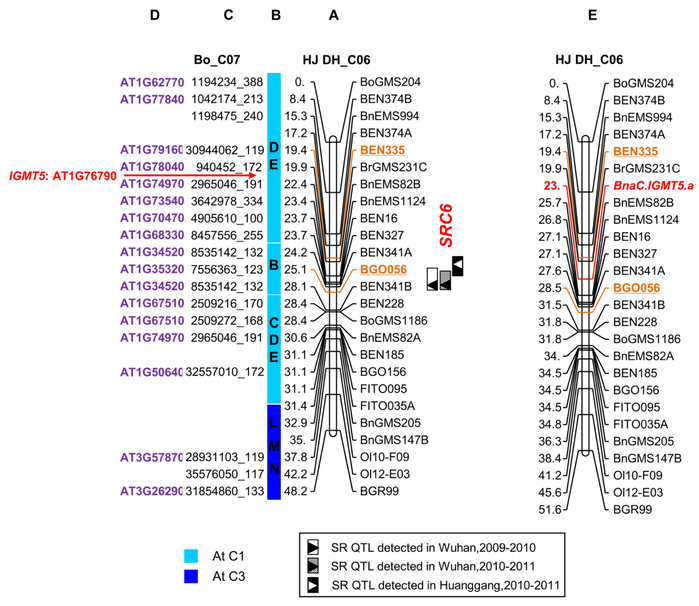

Fig. 4 Comparative map of LG C6 of *B. napus* with *B. oleracea* and Arabidopsis. Column A presents the linkage map of LG C6 of the HJ-DH population. The confidence interval of *SRC6* is shown in color. Column B is the conserved blocks identified in *B. napus*. Column C lists the homologous colinear loci in BoC07 corresponding to SSR markers in LG C6. The number designates the physical position in *B. oleracea* chromosome with the size of amplification fragments. Column D lists the genes encoding homologous loci in *A. thaliana*. Column E presents the modified LG C6 after adding *BnaC.IGMT5.a* on the map.

Identification of *BnaC.IGMT5.a* as a candidate gene for *SRC6*

Searching for previous microarray data showed that *BnIGMT5* was induced up to 31.1-fold at 72 hpi in ZhongYou 821, a resistant cultivar, but the gene expression in susceptible Westar had no significant difference after inoculated (Zhao et al., 2009).

Through homologous cloning, genomic sequences of *BnIGMT5* in *B. napus* genome were isolated and compared (Fig. 5A). Comparative mapping showed that *BnaC.IGMT5.a* was in the confidence intervals of *SRC6*, and just at the peak of *SRC6* detected in Huanggang, 2010-2011(Fig. 4).

Based on the alignment of sequences from two parental lines, we found that *BnaC.IGMT5.a* may have been deleted or inserted with a great fragment in J7005 and the 10 most susceptible lines, while the gene could be amplified and had complete sequence in Hua 5 and the 10 most resistant lines (Fig. 5C). Expression of *BnaC.IGMT5.a* in Hua 5, the donor parent for resistant allele, had a dramatic increase at 24, 48, 72, 96 hpi compared with mock-inoculated control (Fig. 5B).

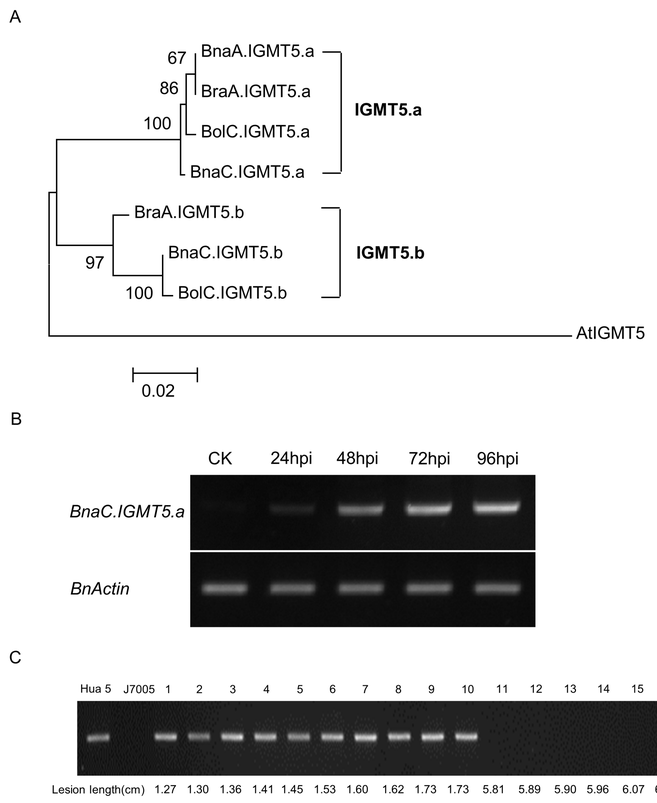

Fig.5 *Molecular* cloning of IGMT5 genes in three Brassica species and induced expression of *BnaC.IGMT5.a* after inoculation with *Scelrotinia* pathogen. (A) Phylogenetic analysis of IGMT5 genes *in B. rapa*, *B. oleracea* and *B. napus*. (B) *BnaC.IGMT5.a* expression is induced after inoculation with *sclerotinia* pathogen. RT-PCR analysis was conducted with RNAs from pooled tissues after inoculations at each time point. *BnActin* was used as an internal control. (C) *BnaC.IGMT5.a* is associated with resistant phenotype. PCR products amplified from the copy-specific marker of *BnaC.IGMT5.a* are presented. Lane 1-10 are the samples from most resistant lines and lane 11-20 the most susceptible lines from the HJ-DH population.

Conclusions:

1. The resistance to *S. sclerotiorum* in *B. napus* is mainly controlled by multiple quantitative genes with additive effects.

2. Two major QTLs, *LRA9* and *SRC6*, were stably identified across years and environments.

3. *BnaC.IGMT5.a* was identified as the candidate genes for *SRC6 and* may be involved in the defense against *S. sclerotiorum* in oilseed rape.

Acknowledgement

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