

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

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).

BnEMS82B

F BnEMS1124

BEN16

BEN327

BGO056

- BEN341B

~ BEN228 - BoGMS11

- BnEMS82A

FITO035A

- BnGMS205

BnGMS147E

L OI10-F09

L OI12-E03

L BGR99

BEN185

BGO156 FITO095

BEN341A

	D	С	В		Α			E	-
	Bo_	C07		HJ	DH_	C06	HJ	DH_C06	
AT10	62770 1194	234_388		0. ר		ر BoGMS204	0. ר	г BoGMS204	
AT10	77840 1042	174_213		ר 8.4		/ _L BEN374B	8.4	Γ BEN374B	
	1198	475_240		٦ 15.3	\ /	BnEMS994	15.3	// _Γ BnEMS994	F
				17.2	$\left \right $	BEN374A	۲7.2 ا		1.
AT10	79160 30944	062_119) D	19.4	Π	F <u>BEN335</u>	19.4	BEN335	L.
5: AT1G76790 AT10	78040 9404	452_172	E	ן 19.9		BrGMS231C	<mark>- 19.9</mark>	BrGMS231C	

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

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.**



BnaC.IGMT5. 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).

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.

A1	A3	A6	A9	C5	C6 _{CB0GMS204}	C8
	SSR106 BrGMS217	OI10-D01	Na10-B11B	BGR49	BEN374B	BnGMS161
BEN10	BoGMS142	5		CN22	THE BEN374A	BnEMS20
BnEMS	S1012 H-BEN25			Na12-C01	BLINGO	BoGMS1200
	BRAS087			BoGMS319	BnEMS82B	
	BEN133	B0GW61250	CB10029		BnEMS1124	BGO185
BnEMS	657	BOGMS1203	-BnGMS213	BEN81	FBEN327	BEN52
	BGR75		Na12-A01		BEN341A	BEN02
	BGR80	BoGMS314	BnEMS1144	DEIN303		BGO183
		BnEMS59		CN20	BEN341B	
BGR6	BnEMS1084	4 BnEMS60			BoGMS1186	
BGR84	BoGMS1464	4В			BnEMS82A	
BnGMS			BGO179A		BEN185	BoGMS125
++Na12-F	H02 BoGMS539	BnEMS695			FITO095	BrGMS394
BEN32		UCB10330			FITO035A	BEN316
-			Na14-B03			BoGMS1166
A2		A 8	BrGMS677		OI10-F09	BrGMS375C
BoGM	S795			BEN140	-OI12-E03	BnEMS860
		BG0006		FITO114	LBGR99	BEN136B
	CB10413				C7	
		BRMS97	BGO179B		<mark>Ĥ</mark> ──BoGMS1032	
		BGR8			CN27	BEN136A
		BEN43				BrGMS375A
						BrGMS375B
H-BEN28	31B			BrGMS387		
H-BGR8	7 H-BoGMS130	7 BGR93	H-BGO199B	BEN285		BoGMS1382



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.





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.**

1.60 1.62 1.73 1.73 5.81 5.89 5.90 5.96 6.07 6.10 6.10 6.16 6.24 7.03

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.

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