QTL mapping of tolerance to Sclerotinia stem rot in *Brassica napus* L

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INTRODUCTION

Sclerotinia Stem rot (SSR) is caused by a non-host specific, necrotrophic and ubiquitous fungus *Sclerotinia sclerotiorum*
SSR is a threat in all major Brassica growing nations
Average annual disease incidence is 13 %
Percent yield losses can go upto 100 %
No effective control measure
Very limited genetic-based resistance to SSR
Resistance is quantitative and highly influenced by environment
No standard screening method

MATERIAL AND METHOD

Four screening methods; 1) Detached leaf assay (DLA), 2) Cotyledon assay (CA), 3) Mycelial stem inoculation (MSI) and 4) Petiole inoculation technique (PIT) were tested for reliability and suitability for large scale screening (LSS)
Three doubled Haploid populations (H1, H2 and H3) sharing the same resistant parent (Zhongyou 821) were used in this study
Phenotyping was done using Petiole inoculation technique on 12 plants per line and repeated three times
Data were recorded on days to wilting (DW) every day for two weeks
Genetic maps of H1 and H2 were developed using SRAP marker technique (Li and Quiros, 2001). Genetic map of H3 has already been published (Sun *et al.* 2007)
QTL analyses were carried out using the composite interval mapping on WinQTLCart 2.5 at LOD value of 2.5

RESULTS

DLA showed significant replicate effects, CA did not show significant genotype differences therefore were not consider reliable
MSI and PIT showed significant genotypic difference and no replicate effect thus were considered reliable. PIT is suitable for LSS
Frequency distribution graphs of DW showed continuous variation in all three populations
Disease symptoms appeared within a day after inoculation (DAI) as water soaked lesions and susceptible plants showed complete stem girdling in 2 to 4 DAI
DW showed significant difference among replicates in the H1 and H2 populations
In the H1 population, 508 polymorphic markers were allocated to 19 linkage groups with total genetic distance of 1526.5 cM
In the H2 population, 478 polymorphic markers were assigned to 19 linkage groups with total genetic distance of 1247.7 cM
QTL detected in three populations have been listed in table 1, 2 and 3

Figure 1. Disease reaction on some checks 3 DAI using PIT

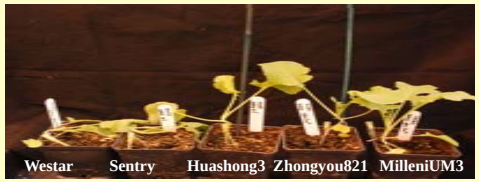

Table 1. Putative QTL identified for resistance to SSR in the DH population H1

Evaluation	QTL	LOD	LG	Flanking Markers	R^2	Additive effect*
R1	*H1R1-1*	4.1	N07	FC1PM5-273 SA7BG35-381	10.6	0.66
	H1R1-2	2.8	N16	BG23PM32-375 BG23PM32-134	7.1	0.55
	H1R1-3	2.8	N19	BG23BG44-384 BG23BG62-206	7.0	0.47
	H1R1-4	2.8	LG04	PM88BG73-149 SA7PM16-447	6.9	0.45
R2						
	H1R2-1	4.5	N07	SA12PM17-320 SA7BG35-381	12.1	0.65
	H1R2-2	2.7	N16	ODD3PM32-309	8.8	-0.49
	H1R2-3	2.5	N16	SA7BG62-357 SA7PM5-144	6.5	-0.45
	H1R2-4	2.5	LG02	SA7BG53-194 FC1BG4-232	8.2	0.53
R3						
	H1R3-1	7.8	N07	ODD3PM32-303 SA7BG35-381	22.1	0.83
	H1R3-2	2.6	N16	SA7PM5-116 SA7BG68-314	7.0	0.46
	H1R3-3	2.7	N16	BG23PM32-375 BG23PM32-134	7.4	0.47
	H1R3-4	4.3	N16	H1ME2BG63-503 ODD3PM4-388	11.3	0.46
	H1R3-5	3.1	N16	SA7BG62-357 SA7PM5-144	8.7	0.50
	H1R3-6	2.6	N17	BG23BG69-249 SA7BG39-351	22.7	0.79
Average						
	H1AV-1	11.0	N07	ODD3PM32-303 SA7BG35-381	28.5	0.85
	H1AV-2	2.7	N16	ODD3PM32-309	5.7	-0.36
	H1AV-3	6.6	N16	BG23BF37-153 BG23PM1-279	16.8	0.63
	H1AV-4	3.5	N16	ME2BG11-544 SA7PM5-144	10.0	-0.46
	H1AV-5	3.6	LG04	PM88BG10-248	7.8	0.40

* Negative sign denotes that the resistance allele were donated by Bao 64; no sign donates alleles were donated by Zhongyou 821

DISCUSSION AND CONCLUSION

In this study PIT has been identified as reliable method has been reported as a reliable method in previous studies (Zhao et al., 2004; Bradley et al., 2006)
SSR is highly influenced by environmental changes that might be the reason for replicate effects of H1 and H2
Precise comparison about the locations of QTL is difficult as there were very few common polymorphic markers among the populations
Few QTL were identified in only one replicate, these QTL may be active during specific environmental condition
QTL which were identified in more than one replicate are probably constitutive to disease response and were less influenced by environment
Linkage groups N07, N12 and N16 has been identified in more than one replicate and more than one population. These linkage groups have been reported to be involved in resistance to SSR in previous studies (Zhao *et al.* 2004; Yin *et al.* 2010)
A number of QTL explaining high phenotypic variance have been identified. Markers linked to these QTL can be used to introduce QTL quickly into breeding lines

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Table 2. Putative QTL identified in the DH population H2

Evaluation	QTL	LOD	LG	Flanking Markers	R^2	Additive effect*
R1	*H2R1-1*	3.0	N13	BG23BG4-301 FC1BG43-364	10.2	0.41
	H2R1-2	3.7	N13	PM88PM34-149 PM88BG10-140	15.4	-0.52
	H2R1-3	3.1	LG06	PM88BG68-327	13.9	-0.48
R2						
	H2R2-1	2.9	N03	PM88BG73-478 SA7PM4-377	8.6	-0.37
	H2R2-2	3.7	N09	BG23PM32-469 SA7BG1-264	10.7	-0.41
	H2R2-3	3.7	N09	PM88BG68-92 SA7PM4-295	10.8	0.42
	H2R2-4	2.6	LG05	SA7PM5-95	7.6	-0.36
	H2R2-5	2.7	LG06	SA7BG39-348	8.1	0.39
	H2R2-6	2.7	LG06	SA12PM16-383 ODD3PM16-383	7.6	0.36
R3						
	H2R3-1	4.0	N05	BG23BG69-456	17.0	-0.55
	H2R3-2	3.1	N07	BG23BG4-235 SA7PM16-295	9.0	-0.4
	H2R3-3	4.4	LG06	SA7BG39-348 PM88BG68-327	14.9	0.53
Average						
	H2AV-1	2.8	N03	PM88BG73-478 SA7PM4-377	8.9	-0.34
	H2AV-2	4.5	N09	PM88BG68-92 SA7PM4-295	14.0	0.41
	H2AV-3	4.1	N09	SA7PM17-263	13.3	-0.41

* Negative sign denotes that the resistance allele were donated by Japanese breeding line; no sign donates alleles were donated by Zhongyou 821

Table 3. Putative QTL identified in the DH population H3

Evaluation	QTL	LOD	LG	Flanking Markers	R^2	Additive effect*
R1	*H3R1-1*	3.2	N06	*830Fg168 *901Fy239	11.8	0.59
	H3R1-2	4.3	N12	*1129Db500 *1203Ey229	19.4	0.79
	H3R1-3	5.2	N16	*0128Dg214	20.5	0.59
R2						
	H3R2-1	4.0	N07	*815Br300 *815Cy167 *817Bg474	13.1	0.72
	H3R2-2	3.2	N12	*1231Cg186	9.9	0.57
	H3R2-3	3.4	N14	*0505Ab171	10.0	0.75
	H3R2-4	3.1	N16	*812By442 *819Fg134	10.8	-0.67
	H3R2-5	4.5	N16	*1215Fg232 *815Cb361	14.9	-0.97
	H3R2-6	6.0	N16	*718Dg129	22.8	0.63
R3						
	H3R3-1	3.8	N03	*0210Ay250 *817Cg465 *0129Bg182	13.5	0.62
	H3R3-2	3.6	N03	*1202Eb214 *0119cr164	12.9	0.47
	H3R3-3	4.2	N09	*0213Dy305 *718Fg174	15.2	0.51
	H3R3-4	4.9	N15	*901Br462	18.2	0.55
Average						
	H3AV1-1	3.7	N12	*819Dg375 *1129Db500 *822Ab378	13.1	0.55
	H3AV1-2	7.7	N16	*718Dg129	35.2	0.58

* Negative sign denotes that the resistance allele were donated by Westar and no sign donates alleles were donated by Zhongyou 821

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