# Quantitative Trait Loci Associated with Isolate Specific and Isolate Non-Specific Partial Resistance to *Sclerotinia sclerotiorum* in Sunflower

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#### ABSTRACT

Basal stem rot caused by Sclerotinia sclerotiorum (Lib.) de Bary is one of the most important diseases of sunflower. Quantitative trait loci (QTL) implicated in partial resistance to two isolates of S. sclerotiorum (SSU107 and SSKH41) were investigated using F<sub>9</sub> recombinant inbred lines (RILs) from the cross between sunflower parental lines PAC2 and RHA266. Experiments were conducted in completely randomized design with 3-6 replications under controlled conditions. The reaction of genotypes to basal stem rot disease was evaluated by measuring the percentage of necrosis area three days after inoculation. Combined analysis of experiments showed significant interactions between sunflower genotypes and S. sclerotiorum isolates suggesting that partial resistance to S. sclerotiorum should be isolate-specific in sunflower. QTLs were mapped using an updated high-density SSR and SNP linkage map. The map consisted of 210 SSRs and 11 genederived markers placed in 17 linkage groups (LGs). The total map length was 1,653.1 cM with a mean density of 1 marker per 7.44 cM. A total of 14 QTLs were detected for partial resistance to two isolates. The phenotypic variance explained by QTLs ( $R^2$ ) ranged from 0.10 to 9.85. The sign of additive gene effects showed that favorable alleles for partial resistance to isolates came from both parents. Six QTLs were common between two isolates on LGs 1, 8 and 17, whereas the others were specific for each isolate. Colocalized QTLs on LG 1 were linked to the glutathione S-transferase gene (GST). The colocalized QTLs for partial resistance to basal stem rot isolates could be good candidates for marker assisted selection (MAS).

Keyword: Basal stem rot, *Helianthus annuus* L., isolate specificity, partial resistance, QTL mapping.

# **INTRODUCTION**

Sunflower (*Helianthus annuus* L.) is one of the most important oilseed crops with high oil content (45-50%) and quality. Basal stem rot caused by *Sclerotinia sclerotiorum* (Lib.) de Bary is one of the most important diseases of sunflower. The disease has been reported from different countries across the world, especially in temperate and humid climates. It causes severe yield losses up to 100% under favorable climatic conditions (high humidity and mild temperature) (Sackston, 1992). The fungus attacks most parts of the plants such as root, stem, capitulum, leaf, and terminal bud, at any developmental stage (Parts *et al.*, 2007). Rapid drying of the leaves and development of lesions on the tap roots and basal portion

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of the stem causes plant death within a few days after the onset of wilting (Dorrell and Huang, 1978). The fungus infects the plant by direct penetration through the cuticle and not through stomata (Boyle, 1921), and enzymatic digestion of the cuticle plays important role in the penetration process (Tariq and Jeffries, 1986).

Because of the suitability of the climate conditions for disease spread in west Azerbaijan Province, Iran, the region is a hotspot for basal stem rot epidemic. Infections of the sunflower basal stem in this region are considered a potential threat to the entire crop. Utilization of sunflower cultivars with improved partial resistance to basal stem rot in combination with appropriate crop management practices is an effective way to control the disease.

To date, sunflower genotypes with different levels of resistance to disease have been identified, but no fully resistant genotypes are available (Tourvieille et al., 1992; Degener et al., 1998, 1999; Hahn, 2002; Rönicke et al., 2004). Earlier studies found additive gene action to be more important than dominance in controlling the disease (Robert et al., 1987). Quantitative trait loci (QTL) associated with partial resistance to S. sclerotiorum in sunflower have been identified in several independent studies (Mestries et al., 1998; Bert et al., 2002; Micic et al., 2004; Davar et al., 2010). Davar et al. (2011) and Ekins et al. (2007) in two independent studies showed that S. sclerotiorum isolates differed in their aggressiveness on sunflower plants. The high genetic variability for pathogenicity in S. sclerotiorum requires simultaneous incorporation of several genes for resistance to remain effective in cultivars used over a large area. The lack of information on the interactions between resistance genes and pathogen populations can limit the effective deployment of resistance. Use of QTL approach to investigate isolate specificity of quantitative resistance has been reported in several research works (Darvishzadeh et al., 2007). The main objective of the present study was to investigate isolate specificity and isolate non-specificity of QTLs associated with partial resistance to two *S*. *sclerotiorum* isolates using a population of recombinant inbred lines (RILs) from the cross between sunflower parental lines, PAC2 and RHA266.

# MATERIALS AND METHODS

# Plant Material and Experimental Conditions

A population of F<sub>9</sub> recombinant inbred lines (RILs) developed from the cross between sunflower parental lines, PAC2 and RHA266, through single-seed descent method was used in our experiments. The RIL population was kindly provided by the National Institute of Agronomy Research (INRA)-France. The paternal line (RHA266) was obtained from the cross between H. annuus and Peredovik by USDA (United States Department of Agriculture). The maternal line (PAC2) was obtained from a cross between H. petiolaris and HA61 by INRA-France (Gentzbittel et al., 1995). Both parents have presented partial resistant to Sclerotinia disease (Poormohammad Kiani et al., 2007). However, RHA266 was more susceptible to disease than PAC2 (Poormohammad Kiani et al., 2007).

The response of 88 RILs and 2 parental lines was evaluated against SSU107 and SSKH41 isolates of S. sclerotiorum in two experiments Institute separate at of Biotechnology, Urmia, Iran in 2010-2011. The experiments conducted were in completely randomized design under controlled conditions (12 hours photoperiod and 25±2°C/18±2°C light/dark temperature, with a light intensity of 200  $\mu \text{Em}^{-2} \text{ s}^{-1}$ , and relative humidity). In the first 65% experiment, parental lines and RILs were inoculated with SSU107 isolate and, in the second one, parental lines and RILs were inoculated with SSKH41 isolate. Six and 3 replications were considered for the first and second experiments, respectively. Each replication consisted of six seedlings. The plants were inoculated in growth stage of V6-V8 (sunflower plant with at least six to eight leaves) (Schneiter and Miller, 1981).

The selected isolates 'SSU107' and 'SSKH41' are moderately aggressive (Davar et al., 2010) and were derived from the samples collected from northwest regions of Iran where sunflower is cultivated. For these isolates, the first two letters refer to S. sclerotiorum Lib. de Bary and the third and fourth letters show the abbreviated name of the locations where the isolates were collected (U: Urmia, KH: Khoy). Fungal isolates were cultured on potato dextrose agar (PDA 42 g L<sup>-1</sup>, pH 6) medium and grown in the dark at room temperature (25±1°C). Disk mycelia of isolates (3 mm diameter) were cut from the growing edge of the colonies (2 days old on PDA) and were placed on the basal stem of plants and covered by Parafilm for 48 hours (Davar et al., 2010). Parafilm retains moisture for fungal growth. Three days after inoculation, the percentage of necrotic area on 1 cm of the stem base (where the mycelia were placed) was measured visually, giving the percent between 0 (no symptoms) and 100 (region completely necrotic).

## **Statistical Analysis**

Individual and combined analysis of variance (ANOVA) on disease severity data were performed using the general linear model (GLM) procedure in the SAS software version 9.1 (SAS Institute Inc.).

## **SSR Mapping**

The genomic DNA of RILs and their parents (PAC2, RHA266) were extracted according to Porebski *et al.* (1997). We used Picogreen fluorescent staining (Quanti- $iT^{TM}$  Picogreen®, Invitrogen) to quantify DNA concentration with the BioTek FL600 Fluorescence Microplate Reader. One hundred and fourteen SSRs were studied. All SSR markers are public and can be

provided upon request (Poormohammad Kiani et al., 2007b; Paniego et al., 2002; Tang et al., 2002). We used a multiplex PCR method, in which several SSR markers were simultaneously amplified in the same reaction. Four SSR markers were used in the same reaction combining markers with different sizes of amplification products. PCR was done according to Schuelke (2000). PCR products were diluted with ultrapure water (2 µL of each PCR product in 20  $\mu$ L water) and 2  $\mu$ L of diluted PCR products mixed with 7.94 µL Formamide HiDi<sup>TM</sup> and 0.06  $\mu$ L GS<sup>TM</sup> 500 LIZ<sup>TM</sup> size standard (Applied Biosystems). After denaturing at 94°C for 5 minutes, samples were loaded on a ABI3730 sequencer and fragments were sized using the GeneMapper® software version 4 (Applied were Biosystems). Chi-square tests performed to test for segregation distortion of each locus. All new SSRs were mapped to our previous map (Poormohammad Kiani et al., 2007b) by Carthagène (De Givry et al., 2005) and Mapmaker (Lander et al., 1987).

#### **Candidate Genes Mapping**

Some important tocopherol and phytosterol pathway-related genes, enzymatic antioxidant-related genes, drought-responsive genes and Arabidopsis Sec14 homologue genes were selected for genetic mapping (Table 1). The respective sequence data for these candidate genes (CGs) were obtained from The Arabidopsis Information Resource (www.arabidopsis.org). In order to seek the Helianthus homolog sequences to the Arabidopsis genes, we used the Compositae expressed sequence tag (EST) assembly clusters, available at the Helianthus-devoted portal bioinformatics Heliagene (www.heliagene.org). The Helianthus EST clusters presenting the reciprocal blast with the highest score and lowest E value with regard to the original Arabidopsis genes were chosen for our study. All primers were designed by MATLAB. Forward primers were tailed at 5' with M13-Fwd tail

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Table 1. Primer used for PCR (polymerase chain reaction), HRM (high resolution melting), CAPS (cleaved amplified polymorphic sequences) and InDel (short insertions and deletions) makers.<sup>a</sup>

(Same

Toward conco	Accession		Sequence of primer (5'-3')	
I arget gene	AGI-Arabidobsis	Homologue with Heliagene cluster	5' M13F-Fwd 3'	5' M13R-Rev 3'
Tocopherol 1	pathway-related genes			
VTE4	AT1G64970	HuCL02246C001	TGAATCTGACGGTTTAGAAC ATCCGTATGATTGAACAAGC	AAACTCCGTTCAGAAAGCG ATGTGCTCTCCACTCTCCATTG
VTE2	AT2G18950	HuCL02840C003	TGCCACAAGAGCAAATCGCTTC	TTTGGGCACTCTTCATAAG
HMBPP	AT5G60600	HuCL00358C002	TGTGCTTGGTATGCCATTC	CCCTTTGGGGAATGTTATGTGG
Enzymatic a	ntioxidant related gene			
POD	AT1G14540	HuCL03143C001	TCGTCGGGATAGTCTTTAC	CGATAGGTAGAGGACTGTTG
CAT	AT1G20620	HuCL00001C054	AAACTACCCTGAGTGGAAG	AATGAATCGTTCTTGCCTG
GST	AT1G02930	HuCL00790C003	AAAGAGCACAAGAGTCCTG	ACTTATTTGAGTGGGCAAC
PAT2	AT1G22530	HuCL00156C004	CTTGGAACAACTGAAGAGC	TGAGTTTACTGCTGTTCCG
SEC14-1	AT1G75170	HuCL10527C001	TATGTCCATCTTTCGGCGTC	ATGGTGTCTTTAGCGGTTC
SEC14-2	AT3G24840	HuCL09897C001	ATGATAACCGTGTGGGATAGC	ATGCTAAACTGGAGGAAAGC
SFH3	AT2G21540	HuCL00667C001	CAAGGAAGGATTTCACCGTG	AAGGCGGTTGATGCTTTACG
Phytosterol 1	pathway-related genes			
SMT2	AT1G20330	HuCL02933C001	CCTTCTACAACCTCGTAACCG	ATCCTTCTTTCACCACCTC
<sup>a</sup> Forward p	vrimers were tailed at 5'	with M13-Fwd tail (5'-CACGACGT	TGTAAAACGAC-3') and reverse primers	were tailed at 5' with M13-Rev tail (5'-

ACAGGAAACAGCTATGAC-3'). These tails were used for sequencing. The candidate genes are: tocopherol methyl-transferase (VTE4), homogenitisate phytyltransferase (VTE2), 4-hydroxy-3-methylbut-2-enyl diphosphate (HMBPP), peroxidase (POD), catalase (CAT), glutation s-transferase (GST), pattelin 2 (PAT2), phosphogelyceride transfer (SEC14-1, SEC14-2), phosphatidylinositol transporter (SFH3), sterol methyltransferase II (SMT2). (5'CACGACGTTGTAAAACGAC3') and reverse primers were tailed at 5' with M13-Rev tail

(5'ACAGGAAACAGCTATGAC3').

Between 2 and 4 different primer combinations per each candidate gene were tested on agarose gel. The PCR program was: 4 minutes at 94°C followed by 35 cycles; 30 seconds at 94°C, 30 seconds at 55 or 58°C, 1 minute at 72°C and final extension of 5 minutes at 72°C. One PCR fragment per gene was sequenced using M13-Fwd and M13-Rev primers (Table 1). After sequencing; SNP-PHAGE (SNP discovery Pipeline with additional features for identification of common haplotypes within a sequence tagged site (Haplotype Analysis) and GenBank (-dbSNP) submissions), through the website at http://www.heliagene.org/, was applied for analyzing sequence traces from both parents to identify SNPs. Several types of markers such as dominant, co-dominant, HRM (high resolution melting), InDel (short insertions deletions) and SNP-based CAPS and (cleaved amplified polymorphic sequences) markers are developed for genotyping of the studied candidate genes.

# QTL Mapping

QTL mapping for partial resistance to isolates was performed by composite interval mapping (CIM) in Windows QTL Cartographer Version 2.5 (Wang et al., 2011) with LOD ( $Log_{10}$  likelihood ratio: Likelihood that the effect occurs by linkage/likelihood that the effect occurs by chance) score of 2.5 determined by permutation testing (n= 1,000 permutations) (Churchill and Doerge, 1994). The genome was scanned at 2 cM intervals with a window size of 15 cM and up to 15 background markers were used as cofactors in the CIM analysis. The percentage of phenotypic variance  $(\mathbf{R}^2)$  explained by each QTL was estimated at the peak of the LOD curve by Windows QTL Cartographer. Map Chart (Voorrips, 2002) was used to draw a graphical presentation of linkage groups and map position of QTLs.

#### RESULTS

Analysis of variance showed significant interactions between sunflower genotypes and S. sclerotiorum isolates (Table 2) suggesting that the response to disease agent of a given genotype relative to other genotypes varies between isolates (Slicing: Table 2 and t-test: Table 3) and partial resistance to S. sclerotiorum could be isolate-specific in sunflower. Differences between the disease severity mean of RILs (X RILs) and the disease severity mean of their parents  $(X_{P})$  were not significant for both isolates (Table 3). Difference between the disease severity mean of best RIL (BRIL) and the disease severity mean of the parental lines was significant for SSKH41 isolate (Table 3). Difference between disease severity mean of RILs (  $X_{\rm RIL}$ ) for SSU107 with that for SSKH41 was significant (Table 3).  $X_{\text{RILs}}$  for isolate SSU107 (88.04) was more than that of isolate SSKH41 (78.78). The frequency

**Table 2.** Analysis of variance for disease severity data in sunflower recombinant inbred lines (RILs) and their two parents infected by *Sclerotinia sclerotiorum* isolates (SSU107 and SSKH41) in controlled conditions.

Source of variation	$df^a$	$MS^b$
Isolate	1	18320.95**
Genotype	89	$773.82^{**}$
Genotype×Isolate	89	$664.06^{**}$
Residual	604	289.59
Total	783	
Genotype×Isolate effect genotype	sliced	by isolate for
SSKH41	89	$377.01^{*}$
SSU107	89	1256.75**
CV		19.98
<sup>a</sup> Desman of free dams <sup>b</sup> N	<b>A</b>	

<sup>*a*</sup> Degrees of freedom, <sup>*b*</sup> Mean of Squares. <sup>\*</sup> and <sup>\*\*</sup>: significant at 0.05 and 0.01 probability level, respectively.



Items	Isolates		- t tost
-	SSKH41	SSU107	- t-test
PAC2 (P1)	64.99	65.33	<sup>a</sup> 0.02 <sup>ns</sup>
RHA266 (P2)	89.16	76.00	1.51 <sup>ns</sup>
P1-P2	-24.19	-10.67	
$\overline{X}_{\rm P} = ({\rm P}_1 + {\rm P}_2)/2$	77.07	70.67	
$\overline{\mathbf{X}}_{\mathrm{RILs}}{}^{a}$	78.78	88.04	7.77**
$\overline{\mathbf{X}}_{\mathrm{RILs}}$ - $\overline{\mathbf{X}}_{\mathrm{P}}{}^{b}$	1.38 <sup>ns</sup>	18.20 <sup>ns</sup>	
$BRIL^{c} - \overline{X}_{P}$	-32.07*	-6.53 <sup>ns</sup>	
$\mathrm{LSD}_{0.05}{}^d$	27.49	18.80	
Example of RILs with different susceptibility to			
Sclerotinia sclerotiorum			
C71	55.1	6.54	19.16**
C100	45	79	$3.14^{*}$
C38	45.55	93.33	3.23 <sup>ns</sup>
C39	98.54	63.83	3.01*

**Table 3.** Genetic parameters for partial resistance to *Sclerotinia sclerotiorum* isolates (SSKH41 and SSU107) in sunflower recombinant inbred lines (RILs) under controlled conditions.

<sup>*a*</sup> Mean of all recombinant inbred lines; <sup>*b*</sup> Mean of the parents; <sup>*c*</sup> The best RIL, <sup>*d*</sup> Least Significant Difference calculated using  $t_{0.05}$  and the error mean square of each experiment.

and <sup>\*\*</sup>: Significant at 0.05 and 0.01 probability level, respectively, ns: Non-significant,  $\overline{\mathbf{X}}_{PAC2} \rightarrow SSKU41 - \overline{\mathbf{X}}_{PAC2} \rightarrow SSKU47$ 

$$^{a}t = \frac{\Lambda PAC2 \rightarrow SSKH 41 - \Lambda PAC2 \rightarrow SSU 10}{S_{\overline{X} PAC2 \rightarrow SSKH 41 - \overline{X} PAC2 \rightarrow SSU 107}}$$

distributions of RILs and their parents for partial resistance to both isolates showed continuous patterns, suggesting that partial resistance was controlled by a polygenic system (Figure 1). For both isolates, especially for isolate SSU107, the frequency distribution of RILs and their parents for partial resistance were skewed toward susceptibility, indicating that SSU107 isolate was more aggressive than SSKH41 isolate on the studied genotypes.

#### Linkage Map and QTL Analysis

Of 114 SSR primer pairs tested, 32 SSR primers (28.07%) produced clear polymorphisms between the parental lines which segregated in a Mendelian manner (Table 4). Also, 19.5% of SSR markers detected two loci in sunflower genome (Figure 2). The SSR markers together with 11 candidate genes identified with the code

HuCL were assigned to the previously reported linkage map (Poormohammad Kiani et al., 2007). New SSR can be found in linkage groups 1, 2, 3, 5, 6, 8, 10, 11, 13, 14, 15, and 17 and candidate genes (HuCL) in linkage groups 1, 2, 8, 11, 14, 15, 16, and 17. The updated map consisted of 210 SSR loci and 11 gene-derived markers placed in 17 linkage groups. Linkage groups were named as 1 to 17 according to reference linkage map of sunflower (Tang et al., 2002). The total map length was 1,653.1 cM with a mean density of 1 marker per 7.44 cM compared to reference map with 1,368.3 cM long and a mean density of 3.1 cM per locus (Tang et al., 2002). Also, 3.62% of the markers were distorted. The distorted markers were clustered on linkage group 14 and denoted with an asterisk. The number of markers per linkage group ranged from 5 to 26. Linkage group 14 was the largest in terms of cM size (197.6 cM), while linkage group 4 (32 cM) was the smallest (Figure 2).



**Figure 1**. Frequency distribution of sunflower recombinant inbred lines (RILs) and their parents for partial resistance to *Sclerotinia sclerotiorum* isolates (SSKH41 and SSU107), scored 3 days after basal stem inoculation and based on the percentage of basal stem area exhibiting necrosis symptoms. Arrows show the mean values of the parental lines (P1= PAC2 and P2= RHA266).

Table 4.	Updated	RILs	genetic	linkage	map	of	PAC2×F	RHA266	cross	in	sunflower	by	SSR	(simple
sequence	repeat) ar	nd SNI	P (single	nucleot	ide po	olyn	norphisn	1) marke	rs.					

LG <sup>a</sup>	No.	No.	Total	Length	Mean	No.	New SSR na	me		
	SSRs	genes	markers	(cM)	density	New				
						SSR				
LG1	9	1	10	105.6	10.56	3	ORS662	ORS716	ORS552	
LG2	16	2	18	133.1	7.39444	4	ORS1145	ORS1147	ORS1065	ORS204
LG3	11		11	84.3	7.66364	3	ORS1222	ORS338	ORS124	
LG4	7		7	32	4.57143	0				
LG5	10		10	43	4.3	1	ORS530			
LG6	8		8	76.6	9.575	3	ORS934	ORS649	ORS1256	
LG7	5		5	33.9	6.78	0				
LG8	12	2	14	73	5.21429	2	ORS456	ORS449		
LG9	14		14	77.3	5.52143	0				
LG10	22		22	122	5.54545	3	ORS613	ORS112	ORS1112	
LG11	7	1	8	136.5	17.0625	2	ORS733_1	ORS733_2		
LG12	13		13	72	5.53846	0				
LG13	8		8	88.7	11.0875	2	ORS625	ORS191		
LG14	19	1	20	197.6	9.88	6	SSL412	ORS782	SSU217	SSU195 ORS1043 HA3513
LG15	13	1	14	103.3	7.37857	1	ORS7			
LG16	25	1	26	153.6	5.90769	0				
LG17	12	2	14	120.6	8.61429	2	ORS735	ORS988		
Total	210	11	222	1653.1	7.4464	32				

<sup>a</sup> Linkage Group.

A total of 14 QTLs were detected for partial resistance to two isolates. QTL involved in partial resistance to the SSKH41 isolate was located on LGs 1, 3, 8, 10, and 17; and those for partial resistance to SSU107 isolate were located on LGs 1, 2, 8, 9, 11, 14, 15, 16 and 17. The number of QTLs identified for partial resistance to SSU107 was more than SSKH41 isolate. The phenotypic variance explained by QTLs  $(R^2)$  ranged from 0.10 to 9.85. The sign of additive gene effects showed that favorable alleles for partial resistance to the isolates came from both parents. Six QTLs out of 14 QTLs, located two by two on LGs 1, 8 and 17 provided partial resistance to both



**Figure 2.** Linkage groups of the sunflower genome presenting QTLs for partial resistance to *Sclerotinia sclerotiorum* isolates (SSKH41 and SSU107). Marker names are listed in the right side of each linkage group. Position of markers in centi Morgans (cM) are listed in the left side of linkage group. Vertical bars represent the positions of QTLs with confidence interval and LOD peak values higher than 2.5 determined by 1,000-permutaion test. **Continued**....



17



**Continued of Figure 2** 

isolates tested (Figure 2). All QTLs identified for partial resistance to isolates had LOD score> 3.1, except for QTL BsrSSKH41.17.1 on linkage group 17 for partial resistance to SSKH41 isolate with LOD score= 2.72. Among the identified QTLs, the QTL on linkage group 11 for partial resistance to SSU107 isolate (BsrSSU107.11.1) had the highest LOD score as well as  $R^2$ = 9.85. Among the isolate-non-specific QTLs, the one on linkage group 8 (BsrSSKH41.8.1) had the highest LOD score and  $R^2$  (Table 5).

# DISCUSSION

The RILs exhibited significant variability for susceptibility to *S. sclerotiorum* isolates, indicating that partial resistance to basal stem rot isolates is genetically controlled. Genetic variability for partial resistance to *S. sclerotiorum* has been previously reported both

Isolate	$QTL^a$	Nearest marker	Linkage group	Position of QTL <sup>b</sup>	Position of nearest markers	LOD score	Additive effect	$R^2$ (%) <sup>c</sup>
SSKH41	BsrSSKH41.1.1	GST	1	18.0	18.6	3.03	0.0023	1.30
5511111	BsrSSKH41.3.1	ORS338	3	2.0	2.3	3.59	0.0213	2.97
	BsrSSKH41.8.1	HUCL02246_1	8	65.6	66.2	3.01	0.0092	0.50
	BsrSSKH41.10.1	SSL3	10	81.0	81.4	3.34	0.0084	0.83
	BsrSSKH41.17.1	POD	17	99.8	97.8	2.72	0.0079	3.16
SSU107	BsrSSU107.1.1	GST	1	18.0	18.6	6.25	2.0553	1.58
	BsrSSU107.2.1	HUCL00156_4	2	99.0	99.2	5.83	-2.7526	2.71
	BsrSSU107.8.1	HUCL02246_1	8	65.6	66.2	6.49	1.6074	6.39
	BsrSSU107.9.1	SSL13	9	44.0	45.4	4.87	-0.2382	1.01
	BsrSSU107.11.1	SSL27	11	66.2	66.2	7.03	2.8964	9.85
	BsrSSU107.14.1	HPPD	14	58.2	59.5	6.55	-3.1006	5.83
	BsrSSU107.15.1	ORS7	15	91.4	92.5	5.46	0.04	0.02
	BsrSSU107.16.1	HUCL00667C001	16	70.0	70.5	5.21	-2.502	0.90
	BsrSSU107.17.1	POD	17	99.8	97.8	5.48	9.009	0.10

**Table 5.** QTLs detected for partial resistance to *Sclerotinia sclerotiorum* isolates in sunflower recombinant inbred lines (RILs) using composite interval mapping (CIM). Markers linked to QTLs common for the two isolates are presented in bold.

<sup>*a*</sup> QTL were designated as Bsr corresponding to basal stem rot followed by the isolate name, linkage group and QTL number, respectively. A negative sign in additive effect indicates that the resistant allele is coming from the maternal line (PAC2) and a positive sign indicates that the resistant allele is from the paternal line (RHA266); <sup>*b*</sup> Expressed in Kosambi cM, from the north of linkage group (LG), <sup>*c*</sup> Percentage of individual phenotypic variance explained, value determined by Windows QTL Cartographer Version 2.5 (Wang *et al.*, 2011).

in field (Micic et al., 2005a, b) and controlled conditions (Davar et al., 2010, 2011). Combined analysis of experiments showed significant interactions between sunflower genotypes and basal stem rot isolates. In other host-pathogen systems such as sunflower-Phoma (Darvishzadeh et al., 2007), sunflower-Phomopsis (Viguie et al., 1999), rice-leaf blast (Roumen 1992; Zenbayashi et al., 2005) and wheat-Septoria tritici blotch (Chartrain et al., 2004) similar effects have been reported. The significant difference between the disease severity mean of the best RIL (BRIL) and the disease severity mean of parents is evidence for transgressive segregation for partial resistance to S. sclerotiorum isolates (Table 3). The positive and negative signs of additive effect at the different loci (Table 5) indicate the contribution of both parental lines to partial resistance and confirm the transgressive segregation observed at the phenotypic level. Transgressive segregation has previously been reported by Micic et al. (2004, 2005a, b), for partial resistance to S. sclerotiorum, as well as by Rachid Al-Chaarani et al. (2002), Bert et al.

(2004) and Darvishzadeh *et al.* (2007) for partial resistance to *Phoma* black stem disease in sunflower.

A total of 14 QTLs were detected for partial resistance to SSU107 and SSKH41 isolates (Table 5). Among the identified QTLs, two QTLs on LGs 3 and 10 (BsrSSKH41.3.1 and BsrSSKH41.10.1) were isolate-specific for isolate SSKH41 and six QTLs on LGs 2, 9, 11, 14, (BsrSSU107.2.1, 15, and 16 BsrSSU107.9.1, BsrSSU107.11.1, BsrSSU107.15.1 BsrSSU107.14.1, and BsrSSU107.16.1) were specific for isolate SSU107. Six QTLs (BsrSSKH41.1.1 with BsrSSU107.1.1, BsrSSKH41.8.1 with BsrSSU107.8.1 and BsrSSKH41.17.1 with BsrSSU107.17.1) were two by two co-localized on LGs 1, 8 and 17 (isolate-nonspecific). Colocalized QTLs on LG 1 were linked to the glutathione S-transferase gene (GST), at the interval of 0.6 cM. Glutathione S-transferases are multifunctional proteins involved in diverse intracellular events such as primary and secondary metabolisms, stress metabolism, herbicide detoxification and plant protection

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against ozone damages, heavy metals and xenobiotics (Mohsenzadeh *et al.*, 2011). Colocalized QTLs on LG 17 were linked to the peroxidase gene (POD), at the interval of 2 cM. Studies have suggested that POD plays an important role in lignification process, crosslinking of cell wall structural proteins, and defense against pathogens (Kawano, 2003).

Several studies have been undertaken for mapping QTLs associating with partial resistance to *S. sclerotiorum* (stem lesion, leaf lesion, head rot) in sunflower (Bert *et al.*, 2002; Mestries *et al.*, 1998; Micic *et al.*, 2004; Micic *et al.*, 2005a, b; Rönicke *et al.*, 2004; Yue *et al.*, 2008). However, it is difficult to compare QTLs detected for partial resistance in the present study with most of the previous results due to different molecular markers employed.

Mestries et al. (1998) by using F2, F3, and F4 generations from the cross GH×PAC2, identified six QTLs for partial resistance to mycelial extension of S. sclerotiorum on leaves and capitula. The detected QTLs were located on different linkage groups according to generation: on the linkage group A in F2, on the groups G and P in F3, and on the group I in F4. However, the QTL located on linkage group G from the F3 data was also detected in the F4 by interval mapping. The favorable parent was PAC2 (partial resistant parent), for the F2 and F4 QTLs; however, GH (the susceptible parent) provided favorable alleles for the F3 QTLs. Rönicke et al. (2004) detected five OTLs for partial resistance to head rot. However, the lack of SSR markers and the common linkage group nomenclatures in their map made it difficult to compare the location of the QTLs detected in the present study with those identified by Mestries et al. (1998) and Rönicke et al. (2004).

Micic et al. (2004), by studying an F3 population from the cross between a resistant inbred line (NDBLOS) and a susceptible one (CM625) under filed conditions, identified seven QTLs for partial resistance to stem lesions on LGs 2, 3, 4, 6, 8, 15, and 16. Their QTLs on LGs 2, 3, 8, 15, and 16 were linked with ORS836 (position: 2 cM from the top of linkage group), ORS390 (58cM), ORS145 (20cM), ORS1040b (40cM) and ORS455 (4cM) markers, respectively. This research team (Micic et al. 2005a), by using the RIL population of the same cross

(NDBLOS×CM625), identified two QTLs for stem lesions on LGs 8 (ORS623, 16cM) and 16 (ORS31, 4cM). In another study, Micic et al. (2005b), by studying an F3 population from the (susceptible)×TUB-5-3234 cross CM625 (resistant) under artificial infection in field experiments across two environments, identified six QTLs for partial resistance to stem lesions on LGs 4, 10 (ORS1129, 38cM) and 17 (ORS588, 56cM). Two QTLs for stem lesion corroborated their earlier findings from the cross **NDBLOS** (resistant)×CM625 (susceptible). Bert et al. (2002) identified three mycelium extension S. OTLs for the sclerotiorum on leaves on LGs 1, 9, 13.

The limited congruency of QTLs for partial resistance to basal stem rot is an evidence of the complex inheritance of resistance to disease, as well as indicating that resistance is controlled by a polygenic system. In addition, this could be a result of the different environmental conditions under which the experiments were conducted. Different resistance mechanisms can be activated in different environments (Micic et al., (2005a). Comparing the chromosomal positions of the QTLs detected for partial resistance to S. sclerotiorum in the present study with those of previous studies show that the linkage group 8 are good candidates for further analyses to develop molecular markers for Sclerotinia reaction, as QTLs for resistance are identified in different independent studies on this linkage group. QTLs for partial resistance to Sclerotinia disease on LG 8 were detected by Micic et al. (2004), Micic et al. (2005a), Davar et al. (2010), Mestries et al. (1998), and Yue et al. (2008). These reports could be a result of high power of LG8 for partial resistance to S. sclerotiorum.

In conclusion, regarding isolate-nonspecific and isolate-specific QTLs detected for partial resistance to two *S. sclerotiorum* isolates, it is suggested that both specific and nonspecific genes and genomic regions associate with partial resistance to *S. sclerotiorum* isolates. The results of this study confirm the need to consider different isolates in the basal stem rot resistance breeding programs. Pyramiding isolate-nonspecific together with isolatespecific QTLs could increase the level of resistance to a wide range of isolates. The markers such as GST, HUCL02246 1 and POD linked to QTLs for partial resistance to basal stem rot isolates could be good candidates for marker assisted selection (MAS).

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شناسایی مکانهای ژنی کنترل کننده مقاومت جزئی اختصاصی و غیر اختصاصی جدایه برای پوسیدگی اسکلروتینیایی طوقه در آفتابگردان (.*Helianthus annus* L)

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# چکیدہ

یوسیدگی اسکلرو تینیابی طوقه بکی از مهمترین بیماری های آفتانگردان در جهان است که عامل آن قارچ Sclerotinia sclerotiorum (Lib.) de Bary می باشد. مکان های ژنی دخیل در مقاومت جزئی آفتابگردان به دو جدایه قارج اسکلروتینیا با استفاده از جمعیت لاین های اینبرد نوترکیب (RILs) حاصل از تلاقی بین دو ژنوتیب PAC2 و RHA266 شناسایی شدند. آزمایشات در قالب طرح کاملا تصادفی با ۳ و ۶ تکرار در شرایط کنترل شده انجام گرفت. واکنش به بیماری با اندازه گیری درصد ناحبه نکروز شده یقه ساقه سه روز پس از آلودگی ارزیابی شد. مکان بایی QTL ها با استفاده از یک نقشه ییوستگی نشانگرهای SSR و SNPصورت گرفت. نقشه ییوستگی شامل ۲۱۰ نشانگر SSR و ۱۱ SNP در ۱۷ گروه لینکاژی بود. طول نقشه ۱۶۵۳.۱ سانتی مورگان و متوسط فاصله نشانگر ها از هم ۷.۴۴ سانتی مورگان بود. تجزیه واریانس مرکب آزمایش نشان داد که اثر متقابل ژنو تیپ × جدایه قارچ معنی دار است که نشان می دهد مقاومت جزئي به بيماري يوسيدگي اسكلروتينيايي طوقه آفتابگردان مي تواند اختصاصي–جدايه باشد. با استفاده از روش نقشهيابي فاصلهایی مرکب در مجموع ۱۴ مکانژنی برای مقاومت جزئی به ۲ ایزوله شناسایی شد. واریانس فنوتییی توجیه شده(R<sup>2</sup>) توسط این مکان های ژنی از ۱۰.۰ تا ۹۸۵ درصد متغییر بود. علامت اثرات افزایشی برای مکان های ژنی شناسایی شده مثبت و منفي بود که نشان مي دهد آلل هاي مقاومت جزئي به بيماري هم از والد پدري (RH266) و هم از والد مادري (PAC2) می آیند. شش QTL از مجموع QTL ۱۴ شناسایی شده که در روی گروههای لینکاژی ۱، ۸ ۷۷ قرار داشتند دخیل در مقاومت جزئي به هر دو جدايه قارچ اسكلروتينيا بودند. QTL هاي هم مكان در روى گروه لينكاژي ۱ با ژن گلوتاتيون اس-ترانسفراز پیوستگی داشتند. QTL های هم مکان کاندیدای خوبی برای توسعه گزینش به کمک نشانگر در برنامه های اصلاحي مقاومت به بيماري يوسيدگي اسكلرو تينيايي طوقه آفتابگر دان هستند.