J. Agr. Sci. Tech. (2015) Vol. 17: 213-226

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

M. Amoozadeh[1], R. Darvishzadeh[1, 2*], R. Davar[2], B. Abdollahi Mandoulakani[1, 2], P. Haddadi[3], and A. Basirnia[1]

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_9 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 gene-derived 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. Co-localized QTLs on LG 1 were linked to the glutathione S-transferase gene (GST). The co-localized 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

[1] Department of Plant Breeding and Biotechnology, Urmia University, P. O. Box 165, km 12 Nazlou Road, Urmia, Islamic Republic of Iran.
[2] Institute of Biotechnology, Urmia University, Shahid Beheshti St., Urmia, Islamic Republic of Iran.
[3] INRA, Institute Jean-Pierre Bourgin (IJPB), 78026, Versailles Cedex, France.
*Corresponding author; e-mail: r.darvishzadeh@ urmia.ac.ir

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_9 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 separate experiments at Institute of Biotechnology, Urmia, Iran in 2010-2011. The experiments were conducted 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 $\mu Em^{-2}\ s^{-1}$, and 65% relative humidity). In the first 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^{-1}, 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™ 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 µL water) and 2 µL of diluted PCR products mixed with 7.94 µL Formamide HiDi™ and 0.06 µL GS™ 500 LIZ™ 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 Biosystems). Chi-square tests were 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 bioinformatics portal 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

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.[a]

Target gene	Accession		Sequence of primer (5'-3')	
	AGI-*Arabidobsis*	Homologue with Heliagene cluster	5' M13F-Fwd 3'	5' M13R-Rev 3'
Tocopherol pathway-related genes				
VTE4	AT1G64970	HuCL02246C001	TGAATCTGACGGTTTAGAAC ATCCGTATGATTGAACAAGC	AAACTCCGTTCAGAAAGCG ATGTGCTCTCCACTCTCCATTG
VTE2	AT2G18950	HuCL02840C003	TGCCACAAGAGCAAATCGCTTC	TTTGGGCACTCTTCATAAG
HMBPP	AT5G60600	HuCL00358C002	TGTGCTTGGTATGCCATTC	CCCTTTGGGAATGTTATGTGG
Enzymatic antioxidant 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	ATGATAACCGTGTGGATAGC	ATGCTAAACTGGAGGAAAGC
SFH3	AT2G21540	HuCL00667C001	CAAGGAAGGATTTCACCGTG	AAGGCGGTTGATGCTTTACG
Phytosterol pathway-related genes				
SMT2	AT1G20330	HuCL02933C001	CCTTCTACAACCTCGTAACCG	ATCCTTCTCTTTCACCACCTC

[a] Forward primers were tailed at 5' with M13-Fwd tail (5'-CACGACGTTGTAAAACGAC-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 and deletions) and SNP-based CAPS (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 (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 ($\overline{X}$ RILs) and the disease severity mean of their parents ($\overline{X}_P$) 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 ($\overline{X}_{RIL}$) for SSU107 with that for SSKH41 was significant (Table 3). $\overline{X}_{RILs}$ 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 sliced by isolate for genotype		
SSKH41	89	377.01*
SSU107	89	1256.75**
CV		19.98

[a] Degrees of freedom, [b] Mean of Squares.
* and **: significant at 0.05 and 0.01 probability level, respectively.

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

Items	Isolates		t-test
	SSKH41	SSU107	
PAC2 (P1)	64.99	65.33	[a]0.02 [ns]
RHA266 (P2)	89.16	76.00	1.51 [ns]
P1-P2	-24.19	-10.67	
$\bar{X}_P = (P_1+P_2)/2$	77.07	70.67	
$\bar{X}_{RILs}$ [a]	78.78	88.04	7.77**
$\bar{X}_{RILs} - \bar{X}_P$ [b]	1.38[ns]	18.20 [ns]	
BRIL[c] $- \bar{X}_P$	-32.07*	-6.53[ns]	
$LSD_{0.05}$ [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[ns]
C39	98.54	63.83	3.01*

[a] Mean of all recombinant inbred lines; [b] Mean of the parents; [c] The best RIL, [d] Least Significant Difference calculated using $t_{0.05}$ and the error mean square of each experiment.
* and **: Significant at 0·05 and 0·01 probability level, respectively, ns: Non-significant,
[a]$t = \frac{\overline{X}_{PAC2 \rightarrow SSKH41} - \overline{X}_{PAC2 \rightarrow SSU107}}{S_{\overline{X}_{PAC2 \rightarrow SSKH41} - \overline{X}_{PAC2 \rightarrow SSU107}}}$.

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).

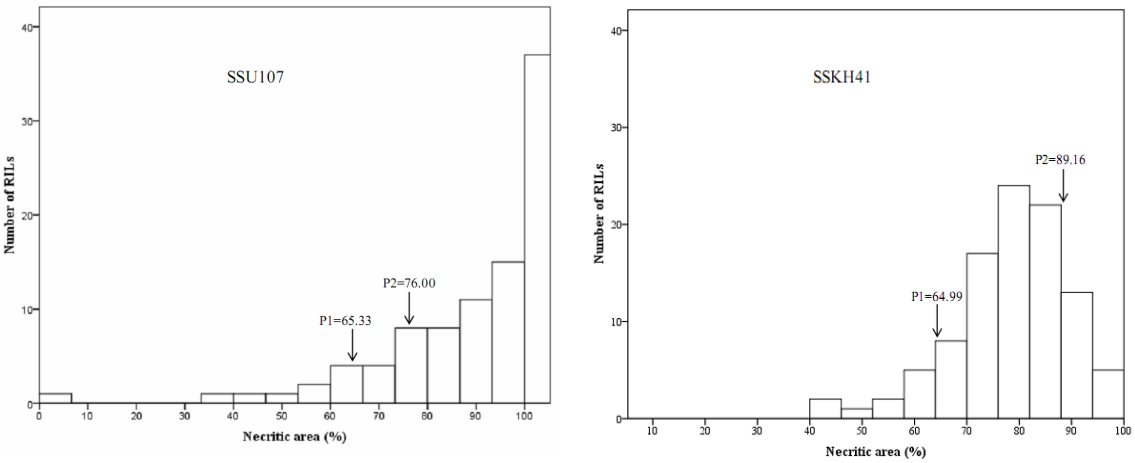


Figure 1. Frequency distribution of sunflower recombinant inbred lines (RILs) and their parents for partial resistance to *Sclerotinia sclerotiorum* isolates (SSKH41 and SSU107), scored 3 days after basal stem inoculation and based on the percentage of basal stem area exhibiting necrosis symptoms. Arrows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

Table 4. Updated RILs genetic linkage map of PAC2×RHA266 cross in sunflower by SSR (simple sequence repeat) and SNP (single nucleotide polymorphism) markers.

LG[a]	No. SSRs	No. genes	Total markers	Length (cM)	Mean density	No. New SSR	New SSR name					
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						

[a] 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

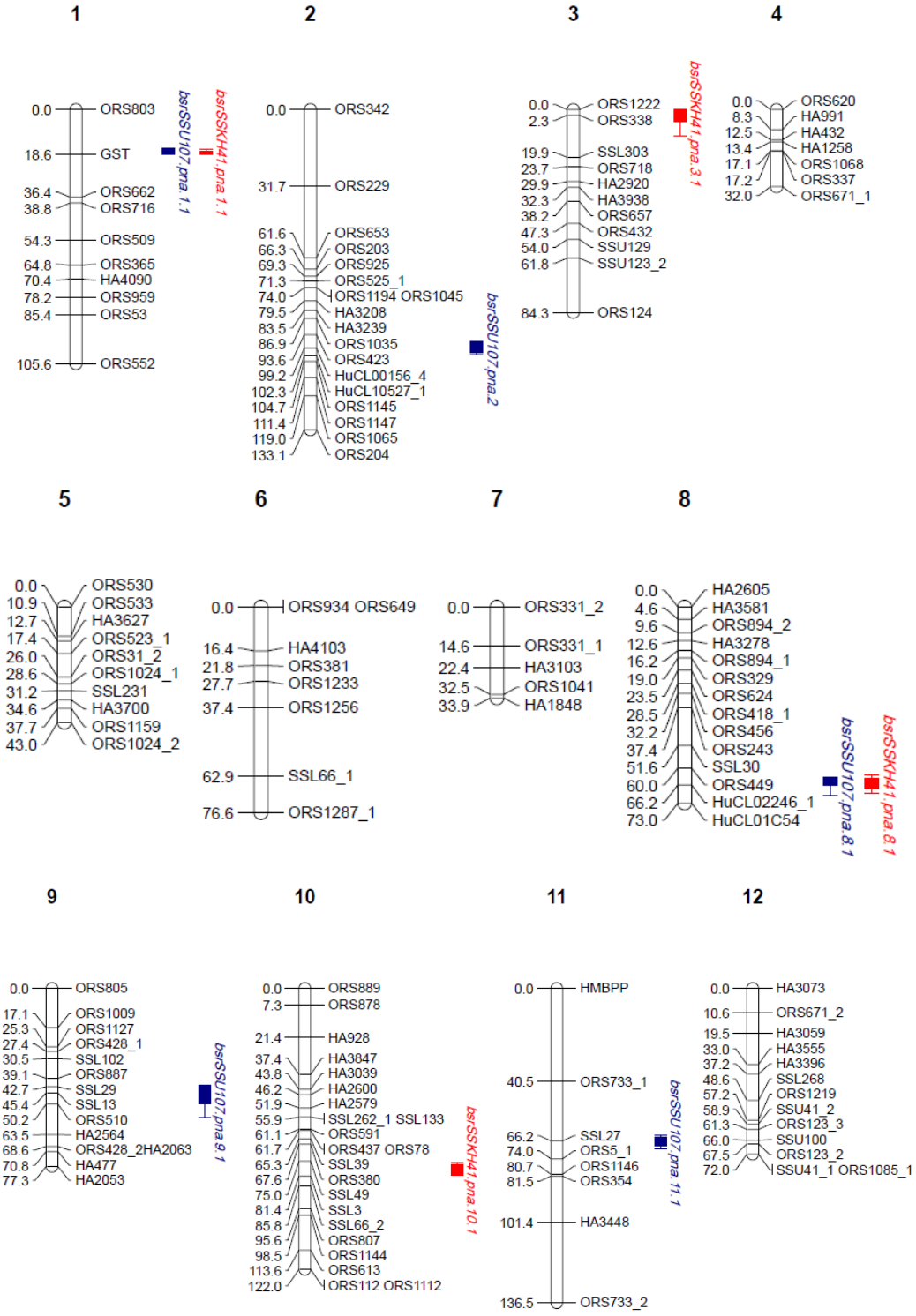
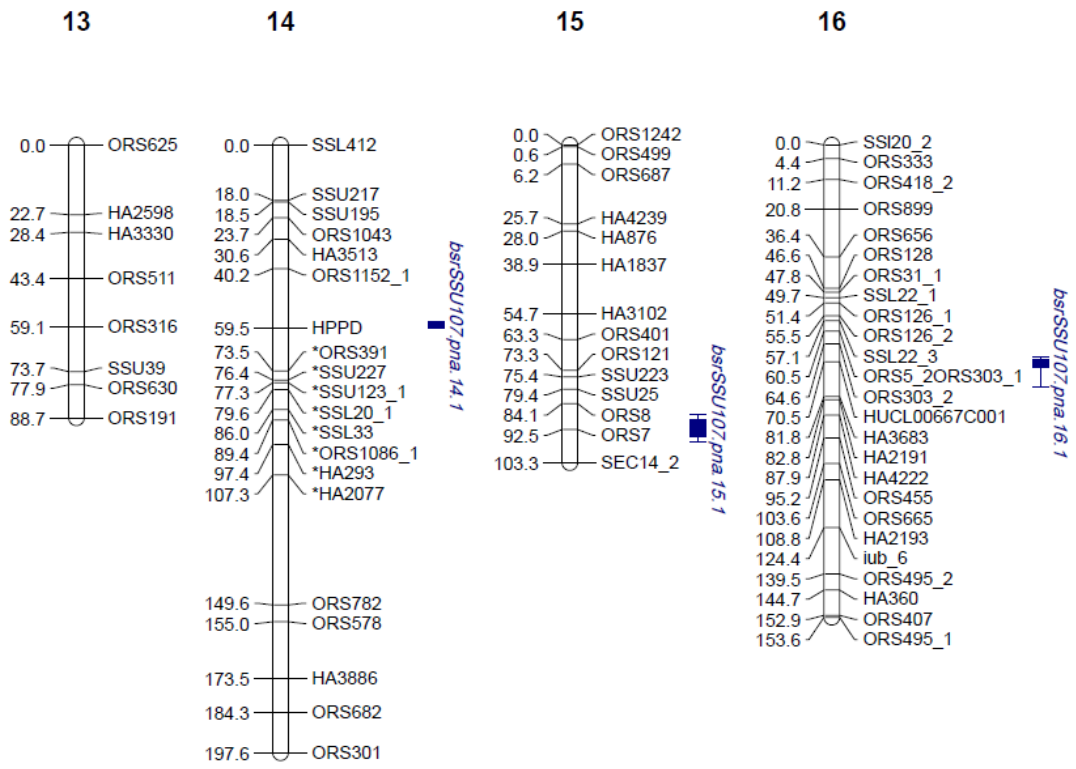
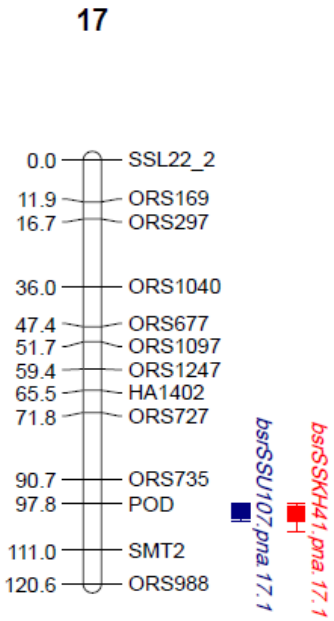


Figure 2. Linkage groups of the sunflower genome presenting QTLs for partial resistance to *Sclerotinia sclerotiorum* isolates (SSKH41 and SSU107). Marker names are listed in the right side of each linkage group. Position of markers in centi Morgans (cM) are listed in the left side of linkage group. 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**….





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

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.

Isolate	QTL[a]	Nearest marker	Linkage group	Position of QTL[b]	Position of nearest markers	LOD score	Additive effect	R^2 (%)[c]
SSKH41	BsrSSKH41.1.1	**GST**	1	18.0	18.6	3.03	0.0023	1.30
	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

[a] 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); [b] Expressed in Kosambi cM, from the north of linkage group (LG), [c] 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, 15, and 16 (BsrSSU107.2.1, BsrSSU107.9.1, BsrSSU107.11.1, BsrSSU107.14.1, BsrSSU107.15.1 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). Co-localized 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

against ozone damages, heavy metals and xenobiotics (Mohsenzadeh *et al.,* 2011). Co-localized 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, cross-linking 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 QTLs 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 cross CM625 (susceptible)×TUB-5-3234 (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 QTLs for the mycelium extension *S. 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 isolate-specific 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).

ACKNOWLEDGEMENTS

The authors thank Dr Y. Ghosta (Department of Plant Pathology, Urmia University, Iran) who helped to gather the collection of *Sclerotinia sclerotiorum* isolates and the Institute of Biotechnology, Urmia University, Iran, for financial support.

REFERENCES

1. Bert, P. F., Dechamp-Guillaume, G., Serre, F., Jouan, I., Tourvieille de Labrouhe, D., Nicolas, P. and Vear, F. 2004. Comparative Genetic Analysis of Quantitative Traits in Sunflower (*Helianthus annuus L.*) 3. Characterization of QTL involved in resistance to *Sclerotinia sclerotiorum* and *Phoma macdonaldii*. *Theor. Appl. Genet.,* **109:** 865–74.
2. Bert, P. F., Jouan, I., Tourvieille de Labrouhe, D., Serre, F., Nicolas, P. and Vear, F. 2002. Comparative Genetic Analysis of Quantitative Traits in Sunflower (*Helianthus annuus L.*). 1. QTL Involved in Resistance to *Sclerotinia sclerotiorum* and *Diaporthe helianthi*. *Theor. Appl. Genet.,* **105:** 985–993.
3. Boyle, C. 1921. Studies in the Physiology of Parasitism. VI. Infection by *Sclerotinia libertiana*. *Annals Bot.,* **35:** 337–347.
4. Chartrain, L., Brading, P. A., Makepeace, J. C. and Brown, J. K. M. 2004. Sources of Resistance to *septoria tritici blotch* and Implication for Wheat Breeding. *Plant Pathol.,* **53:** 454–60.
5. Churchill G. A. and Doerge, R.W. 1994. Empirical Threshold Values for Quantitative Trait Mapping. *Genetics,* **138:** 963–971
6. Darvishzadeh, R., Poormohammad Kiani, S., Dechamp-Guillaume, G., Gentzbittel, L. and Sarraf, A. 2007. Quantitative Trait Loci Associated with Isolate Specific and Isolate Nonspecific Partial Resistance to *Phoma macdonaldii* in Sunflower. *Plant Pathol.,* **56:** 855–861.
7. Davar, R., Darvishzadeh, R. and Majd, A. 2011. Genotype-isolate Interaction for Resistance to *Sclerotinia sclerotiorum* in Sunflower. *Phytopathol. mediterr.,* **50:** 442–449.
8. Davar, R., Darvishzadeh, R., Majd, A., Ghosta, Y. and Sarrafi, A. 2010. QTL Mapping of Partial Resistance to Basal Stem Rot in Sunflower Using Recombinant Inbred Lines. *Phytopathol. mediterr.,* **49:** 330–341.
9. Degener, J., Melchinger, A. E., Gumber, R. K. and Hahn, V. 1998. Breeding for *Sclerotinia* Resistance in Sunflower: A Modified Screening Test and Assessment of Genetic Variation in Current Germplasm. *Plant Breed.,* **117:** 367–372.
10. Degener, J., Melchinger, A. E. and Hahn, V. 1999. Optimal Allocation of Resources in Evaluating Current Sunflower Inbred Lines for Resistance to *Sclerotinia*. *Plant Breed.,* **118:** 157-160.
11. De Givry, S., Bouchez, M., Chabrier, P., Milan, D. and Schiex, T. 2005. Carthagene: Multi-population Integrated Genetic and Radiation Hybrid Mapping. *Bioinformatics,* **21:** 1703–1704.
12. Dorrell, D. G. and Huang H. C. 1978. Influence of *Sclerotinia* Wilt on Seed Yield and Quality of Sunflower Wilted at Different Stages of Development. *Crop Sci.,* **18:** 974–978.
13. Ekins, M. G., Aitken, E. A. B. and Goulter, K. C. 2007. Aggressiveness among Isolates of *Sclerotinia sclerotiorum* from Sunflower. *Australas. Plant Pathol.,* **36:** 580–586.
14. Gentzbittel, L., Vear, F., Zhang, Y.X., Berville, A. and Nicolas, P. 1995. Development of a Consensus Linkage RFLP Map of Cultivated Sunflower (*Helianthus annuus* L.). *Theor. Appl. Genet.,* **90:** 1079-1086.
15. Haddadi, P., Ebrahimi, A., Langlade, N. B., Yazdi-Samadi, B., Berger, M., Calmon, A., Naghavi, M. R., Vincourt, P. and Sarrafi A. 2012. Genetic Dissection of Tocopherol and Phytosterol in Recombinant Inbred Lines of Sunflower through QTL Analysis and the Candidate Gene Approach. *Mol. Breed.,* **29:** 717-729.
16. Hahn, V. 2002. Genetic Variation for Resistance to *Sclerotinia* Head Rot in Sunflower Inbred Lines. *Field Crop Res.,* **77:** 153–159.
17. Kawano, T. 2003. Roles of the Reactive Oxygen Species Generating Peroxidase Reactions in Plant Defense and Growth Induction. *Plant Cell Rep.,* **21:** 829-937.

18. Kearsey, M. J. and Pooni, H. S. 1996. *The Genetical Analysis of Quantitative Traits*. Chapman and Hall, London, UK, 381 PP.
19. Lander, E. S., Green, P., Abrahamson, J., Barlow, A., Daly, M. J., Lincoln, S. E. and Newberg, L. A. 1987. Mapmaker: An Interactive Computer Package for Constructing Primary Genetic Linkage Maps of Experimental and Natural Populations. *Genomics,* **1:** 174–181
20. Mestries, E., Gentzbittel, L., Tourvieille de Labrouhe, D., Nicolas, P., and Vear, F. 1998. Analysis of Quantitative Trait Loci Associated with Resistance to *Sclerotinia sclerotiorum* in Sunflowers (*Helianthus annuus* L.) Using Molecular Markers. *Mol. Breed.,* **4:** 215–226.
21. Micic, Z., Hahn, V., Bauer, E., Schon, C. C., Knapp, S. J., Tang, S. and Melchinger, A. E. 2004. QTL Mapping of *Sclerotinia* Mid-stalk Rot Resistance in Sunflower. *Theor. Appl. Genet.,* **109:** 1474–1484.
22. Micic, Z., Hahn, V., Bauer, E., Schon, C. C. and Melchinger, A. E. 2005a. QTL Mapping of Resistance to *Sclerotinia* Mid-stalk Rot in RIL of Sunflower Population NDBLOSsel×CM625. *Theor. Appl. Genet.,* **110:** 1490–1498.
23. Micic, Z., Hahn, V., Bauer, E., Melchinger, A. E., Knapp, S. J., Tang, S. and Schön, C.C. 2005b. Identification and Validation of QTL for *Sclerotinia* Mid-stalk Rot Resistance in Sunflower by Selective Genotyping. *Theor. Appl. Genet.,* **111:** 233–242.
24. Mohsenzadeh, S., Esmaeili, M., Moosavi, F., Shahrtash, M., Saffari, A. and Mohabatkar, H. 2011. Plant Glutathione *S*-transferase Classification, Structure and Evolution. *Afr. J. Biotechnol.,* **10:** 8160-8165.
25. Paniego, N., Echaide, M., Munoz, M., Fernandez, L., Torales, S., Faccio, P., Fuxan, I., Crrera, M., Zandomeni, R., Syarez, E. Y. and Esteban Hopp, H. 2002. Microsatellite Isolation and Characterization in Sunflower (*Helianthus annuus* L.). *Genome,* **45:** 34-43.
26. Parts, E., Galindo, J.C., Bazzalo, M.E., Leon, A., Macias, F.A., Rubiales, D. and Jorrin, J. V. 2007. Antifungal Activity of a New Phenolic Compound from Capitulum of a Head Rot Resistant Sunflower Genotype. *J. Chem. Ecol.,* **33:** 2245–2253.
27. Poormohammad Kiani, S., Grieu, P., Maury, P., Hewezi, T., Gentzbittel, L. and Sarrafi, A. 2007b. Genetic Variability for Physiological Traits under Drought Conditions and Differential Expression of Water Stress-associated Genes in Sunflower (*Helianthus annuus* L.). *Theor. Appl. Genet.,* **114:** 193–207
28. Porebski, S., Bailey, L. and Baumm, B. 1997. Modification of a CTAB DNA Extraction Protocol for Plants Containing High Polysaccharide and Polyphenol Components. *Plant Mol. Biol. Rep.,* **15:** 8–15.
29. Rachid Al-Chaarani, G., Roustaee, A., Gentzbittel, L., Mokrani, L., Barrault, G., Dechamp-Guillaume, G. and Sarrafi, A. 2002. A QTL Analysis of Sunflower Partial Resistance to Downy Mildew (*Plasmopara halstedii*) and Black Stem (*Phoma macdonaldii*) by the Use of Recombinant Inbred Lines (RILs). *Theor. Appl. Genet.,* **104:** 490–6.
30. Robert, N., Vear, F., and Tourvieille de Labrouhe, D. 1987. The Inheritance of Resistance *Sclerotinia sclerotiorum (Lib.)* de Bary in Sunflower. I. Study of Reactions to Two Tests Mycelial. *Agronomie,* **7:** 423-429.
31. Roumen, E. C. 1992. Small Differential Interactions for Partial Resistance in Rice Cultivars to Virulent Isolates of the Blast Pathogen. *Euphytica,* **64:** 143-148.
32. Ronicke, S., Hahn, V., Horn, R., Grone, I., Brahm, L., Schnabel, H. and Friedt, W. 2004. Interspecifc Hybrids of Sunflower as a Source of *Sclerotinia* Resistance. *Plant Breed.,* **123:** 152-157.
33. Sackston, W. E. 1992. On a Treadmill: Breeding Sunflowers for Resistance to Disease. *Annu. Rev. Phytopathol.,* **30:** 529–551.
34. Schneiter, A. A. and Miller, J. F. 1981. Description of Sunflower Growth Stages. *Crop Sci.,* **21:** 901–903.
35. Schuelke M. 2000. An Economic Method for the Fluorescent Labeling of PCR Fragments. *Nat. Biotechnol.,* **18:** 233-234.
36. Tang, S., Yu, J. K., Slabaugh, M. B., Shintani, D. K. and Knapp, S. J. 2002. Simple Sequence Repeat Map of the Sunflower Genome. *Theor. Appl. Genet.,* **105:** 1124-1136.
37. Tariq, V. N. and Jeffries, P. 1986. Ultra Structure of Penetration of *Pharsalus* spp. by *Sclerotinia sclerotiorum*. *Can. J. Bot.,* **64:** 2909–2915.
38. Tourvieille de Labrouhe, D., Vear, F. and Achbani, E. H. 1992. Attack of Sunflower Terminal Buds by *Sclerotinia sclerotiorum* Symptoms and Resistance. *In Proceedings of 13th International Sunflower Conference*, 8–10 September 1992, Pisa, Italy, PP. 859–864.

39. Viguie, A., Vear, F. and Tourvieille de Labrouhe, D. 1999. Interactions between French Isolate of *Phomopsis*/*Diaporthe helianthi* Munt.-Cvet *et al*. and Sunflower (*Helianthus annuus* L.) Genotypes. *Eur. J. Plant Pathol.,* **105:** 693–702.
40. Voorrips, R. 2002. Map Chart: Software for the Graphical Presentation of Linkage Maps and QTLs. *J. Hered*., **93:** 77–78.
41. Wang, S., Basten, C. J. and Zeng, Z. 2011. *Windows QTL Cartographer Version 2.5*. Department of Statistics, North Carolina State University, Raleigh, NC.
42. Yue, B., Radi, S. A., Vick, B. A., Cai, X., Tang, S., Knapp, S. J., Gulya, T. J., Miller J. F. and Hu, J. 2008. Identifying Quantitative Trait Loci Resistance to *Sclerotinia* Head Rot in Two USDA Sunflower Germplasms. *Phytopathol.,* **98:** 926-931
43. Zenbayashi-Sawata, K., Ashizawa, T. and Koizumi, S. 2005. Pi34-AVRPi34: A New Gene-for-gene Interaction for Partial Resistance in Rice to Blast Caused by *Magnaporthe grisea*. *J. Gen. Plant Pathol.,* **71:** 395–401.

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

م. عموزاده، ر. درویش زاده، ر. داور، ب. عبدالهی مندولکانی، پ. حدادی، ا. بصیرنیا

چکیده

پوسیدگی اسکلروتینیایی طوقه یکی از مهم‌ترین بیماری‌های آفتابگردان در جهان است که عامل آن قارچ *Sclerotinia sclerotiorum* (Lib.) de Bary می‌باشد. مکان های ژنی دخیل در مقاومت جزئی آفتابگردان به دو جدایه قارچ اسکلروتینیا با استفاده از جمعیت لاین های اینبرد نوترکیب (RILs) حاصل از تلاقی بین دو ژنوتیپ 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 اندازه‌گیری درصد ناحیه نکروز شده یقه ساقه سه روز پس از آلودگی ارزیابی شد. مکان‌یابی QTL ها با استفاده از یک نقشه پیوستگی نشانگرهای SSR و SNPصورت گرفت. نقشه پیوستگی 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 ژنوتیپ × جدایه قارچ معنی دار است که نشان می دهد مقاومت جزئی به بیماری پوسیدگی اسکلروتینیایی طوقه آفتابگردان می تواند اختصاصی–جدایه باشد. با استفاده از روش نقشه‌یابی فاصله‌ایی مرکب در مجموع ۱۴ مکان‌ژنی برای مقاومت جزئی به ۲ ایزوله شناسایی شد. واریانس فنوتیپی توجیه شده(R^2) توسط این مکان‌های ژنی از ۰.۱۰ تا ۹.۸۵ درصد متغییر بود. علامت اثرات افزایشی برای مکان‌های ژنی شناسایی شده مثبت و منفی بود که نشان می دهد آلل‌های مقاومت جزئی به بیماری هم از والد پدری (RH266) و هم از والد مادری (PAC2) می‌آیند. شش QTL از مجموع ۱۴ QTL شناسایی شده که در روی گروه‌های لینکاژی ۱، ۸، ۱۷ قرار داشتند دخیل در مقاومت جزئی به هر دو جدایه قارچ اسکلروتینیا بودند. QTL های هم مکان در روی گروه لینکاژی ۱ با ژن گلوتاتیون اس–ترانسفراز پیوستگی داشتند. QTL های هم مکان کاندیدای خوبی برای توسعه گزینش به کمک نشانگر در برنامه های اصلاحی مقاومت به بیماری پوسیدگی اسکلروتینیایی طوقه آفتابگردان هستند.