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QTL mapping of partial resistance to basal stem rot in sunflower using recombinant inbred lines

ROBAB DAVAR[1], REZA DARVISHZADEH[2,3], AHMAD MAJD[1], YOUBERT GHOSTA[4] and AHMAD SARRAFI[5]

[1]Department of Biology, Faculty of Science, Tarbiat Moallem University, Tehran, Iran
[2]Institute of Biotechnology, Urmia University, Iran
[3]Department of Agronomy and Plant breeding, Faculty of Agriculture, Urmia University, Iran
[4]Department of Plant Protection, Urmia University, Urmia, Iran.
[5]INP-ENSAT, IFR 40, Laboratoire de Biotechnologie et Amélioration des Plantes (BAP), F–31326 Castanet Tolosan, France

Summary. Basal stem rot, caused by *Sclerotinia sclerotiorum* (Lib.) de Bary, is an important cause of yield loss in sunflower (*Helianthus annuus* L.). Quantitative trait loci (QTLs) implicated in partial resistance to basal stem rot disease were identified using 116 recombinant inbred lines (RILs) from the cross between the sunflower parental lines PAC2 and RHA266. The RILs and their parents were arranged in a completely randomized design with six replications and inoculated with a moderately aggressive isolate (SSU107) of *S. sclerotiorum* under controlled conditions. QTLs were mapped using a recently developed high-density simple sequence repeat/ amplified fragment length polymorphism (SSR/AFLP) sunflower linkage map. Analysis of variance showed highly significant differences among the sunflower genotypes for susceptibility to basal stem rot. The frequency distribution of genotypes for susceptibility to disease showed continuous patterns, suggesting that resistance is controlled by a polygenic system. Transgressive segregation for resistance occurred in this cross. Composite interval mapping analysis revealed 7 QTLs for percentage necrotic area, localized on 7 linkage groups. The effects of QTLs were small to moderate indicating a polygenic control of the studied character. However, like any other quantitative trait, it is necessary to confirm the position of the QTLs and to carry out fine-scale mapping before marker assisted selection (MAS) can be done. LG8 and LG16 are good candidates for further analysis to develop molecular markers for resistance to *Sclerotinia* disease.

Key words: *Helianthus annuus* L., partial resistance, QTL mapping, basal stem rot, *Sclerotinia sclerotiorum*.

Introduction

Sunflower, *Helianthus annuus* L. is a major oilseed crop widely cultivated throughout the globe. It is a diploid species with an estimated haploid genome size of about 3,000 Mb with 2n=2×=34 chromosomes (Arumuganathan and Earle, 1991). *Sclerotinia sclerotiorum* (Lib.) de Bary is a widespread pathogen infecting over 400 species of plants including many important crop species (Boland and Hall, 1994). White rot caused by *S. sclerotiorum* is a major yield-limiting factor of sunflower in the temperate regions of the world. The fungus attacks several plant parts and causes stalk rot/ wilt or head rot (Gulya *et al.*, 1997). Rapid drying of the leaves and development of lesions on the tap roots and basal portion of the stem cause plant death within a few days after the onset of wilting (Dorrell and Huang, 1978).When climatic conditions are favorable for fungus growth, yield losses can reach 100% (Sackston, 1992). In most cases, the fungus penetrates directly through the cuticle and not through the stomata (Boyle, 1921). Enzymatic digestion of the cuticle plays a role in the penetration process (Tariq and Jeffries, 1986). Infection of healthy tissue with the myceliogenic infection method depends on the formation of an

Corresponding author: R. Darvishzadeh
Fax: + 98 441 2779558
E-mail: r.darvishzadeh@mail.urmia.ac.ir

appressorium (Tariq and Jeffries, 1984). The Soil and climatic conditions of sunflower growing areas influence which sunflower part is most attacked (Tourvieille de Labrouhe *et al.,* 1992). In Iran, infections of the sunflower basal stem are considered a potential threat to the entire crop. The available chemical controls are either ineffective or difficult to apply (Hahn, 2002). Genetic control is therefore of considerable importance, the aim being to select cultivars with high resistance to all forms of *S. sclerotiorum* found in the regions in which they are cultivated. To date, sunflower genotypes with different levels of resistance to stem rot have been identified, but no fully resistant genotypes are available (Hahn, 2002), so that breeding resistant varieties is an important objective.

Two general types of disease resistance have long been recognized in plants (van der Plank, 1968): (i) complete resistance, conditioned by major genes and (ii) incomplete resistance, conditioned by multiple genes each having a partial effect. A variety of terms has been used to refer to this perceived dichotomy, including vertical versus horizontal, complete versus incomplete, major-gene versus minor-gene and narrow-spectrum versus broad-spectrum. One limitation of the major gene in resistance breeding is its lack of durability; resistance can break down because of a change in the population of the pathogen, overcoming the specific plant resistance gene (Talukder *et al.,* 2004). In this type of interaction, there is either a compatible interaction between the host and the pathogen, allowing the pathogen to infect, grow and reproduce in the host, or an incompatible interaction, in which a hypersensitive reaction causes the death of the invading pathogen. It should be possible to extend the durability of major genes by pyramiding multiple resistance genes (Hittalmani *et al.,* 2000), as some varieties have durable resistance (they maintain resistance for many years in the field under conditions of high disease pressure). Durable resistance is considered to be a combination of race-specific major genes and non race-specific minor (partial resistance) genes (Wang *et al.,* 1994). Partial (also known as horizontal) resistance reduces pathogen growth and reproduction without the hypersensitive reaction that is indicative of the incompatibility conferred by the major resistance genes. Partial resistance involves many genes, each of which singly makes a relatively small contribution to overall resistance. Since resistance can be measured quantitatively, this genes are known as quantitative resistance characters, and the genetic loci associated with them are called quantitative resistance loci and in general, quantitative trait loci (QTLs) (Young, 1996). Partial resistance to Phoma black stem disease has been demonstrated to be present in a limited number of sunflower genotypes (Darvishzadeh *et al.,* 2007a).

As mentioned above, quantitative inheritance often results from the segregation of multiple genetic factors, and such polygenic characters are strongly influenced by environmental conditions (Kearsey and Pooni, 1996). Earlier studies suggested that inheritance of resistance to *S. sclerotiorum* is polygenic (Bert *et al.,* 2004). Mechanical infection under controlled conditions is essential for evaluating inbred lines and hybrids in order to select individual plants and families in segregating generations, because natural epidemics of white rot are strongly affected by environmental factors (Tourvieille de Labrouhe *et al.,* 1978). Field tests with natural and/or artificial infections are extensively practiced but they are laborious, time consuming and not always reliable indicators of disease development because of variations in inoculum pressure and in environmental conditions (Hahn, 2000).

The recent development of molecular markers has been a major factor in establishing saturated molecular maps. Species with a large genome, such as sunflower (2n=34), need techniques with a great number of markers. Amplified fragment length polymorphism (AFLP) is an efficient marker technology on account of its high multiple ratios (Pejic *et al.,* 1998). AFLP is a powerful technique for the genetic fingerprinting of sunflower (Hongtrakul *et al.,* 1997). Simple sequence repeats (SSRs) are used as another molecular marker and their polymorphisms have been very effective in genetic mapping, evolutionary studies and fingerprinting, as well as in pedigree analysis (Paniego *et al.*, 2002; Tang *et al.,* 2003).

With a dense molecular linkage map, polygenic quantitative traits can be resolved into discrete Mendelian factors. With QTL mapping, individual resistance loci can be identified and located on the chromosomes. This is a highly effective tool for studying genetically complex disease resistance such as partial resistance (Young, 1996).

Many studies have been conducted on sunflower to determine the QTLs for important agronomic and developmental traits such as 'days to flowering' and photoperiod response (Leon *et al.,* 2001) and somatic embryogenesis (Flores Berrios *et al.,* 2000) as well as for resistance to *Diaporthe helianthi* (Bert *et al.,* 2002), *S. sclerotiorum* (Mestries, 1998; Bert *et al.,* 2002; Bert *et al.,* 2004; Micic *et al.,* 2005a,b), phoma black stem (*Phoma macdonaldii*) (Rachid Al-Chaarani *et al*., 2002; Bert *et al.,* 2004; Darvishzadeh *et al.,* 2007b), and downy mildew (*Plasmopara halstedii*) (Rachid Al-Chaarani *et al*., 2002).

In the present work, we studied a recombinant inbred population (116 RILs) derived from a cross of "PAC2 × RHA266" and mapped the QTLs for partial resistance to basal stem rot in the sunflower genome using a high-density SSR/AFLP map.

Materials and methods

Fungal isolates and pathogenicity tests

Basal stem sections were collected from field grown sunflower plants affected with basal stem rot in different regions of northwestern Iran, and were used to obtain *S. sclerotiorum* isolates. Sclerotia were obtained from infected plants and were then immersed in a mixture of 4% sodium hypochlorite and 70% ethanol for 2 min, rinsed in sterile distilled water, blotted dry on sterile filter paper, plated onto potato dextrose agar (PDA 39 g L^{-1}, pH 6) medium and grown in the dark at room temperature (25±1°C). Pure cultures were obtained by the hyphal tip method and stored at 4°C until used.

The aggressiveness of 108 isolates was determined on the sunflower hybrid cv. Iroflor (kindly provided by The Seed and Plant Improvement Institute (SPII), Karaj, Iran). This hybrid comes from a single cross and was registered in 1988 in France. It is one of the most widely planted sunflower hybrids in northwestern Iran. Seeds were sown in 10×12 cm pots filled with sterilized soil collected from the research farm of Urmia University, Iran. The soil was a silty clay with a pH of 7.6 and an EC of 0.6 dSm^{-1}. Table 1 summarizes the properties of the soil used in the experiments. Plants were grown in a controlled environment at temperatures of 24±1°C, 65% relative humidity, with a 12 h day having a light intensity of 200 $mEm^{-2}s^{-1}$, for 4 weeks, until growth stage V6–V8 (sunflower plants with at least six to eight leaves) (Schneiter and Miller, 1981). At this growth stage, mycelial plugs of each isolate (3 mm diameter) were cut from the growing edge of the colony (2 days old on PDA) and were placed against the basal stem of the sunflower plants. The stem and mycelial plug were wrapped with Parafilm for 48 h to preserve humidity, following the method of Price and Colhoun (1975). The percentage of necrotic area on 1 cm of the stem base and all around it was measured visually 3 days after inoculation. The experiment was arranged in a completely randomized design with three replications per isolate.

Sunflower genotypes and disease assessment

A population of RILs was developed through single-seed descent from a cross between the sunflower parental lines PAC2 and RHA266. RHA266 was obtained from a cross between *Helianthus annuus* and *Peredovik* by USDA, and PAC2 is an inbred line from a cross between *H. petiolaris* and 'HA61' developed by INRA-France (Gentzbittel *et al.,* 1995). This public RILs population has been

Table 1. Some physical and chemical properties of the soil used in this study (average values).

Soil parameters														
pH	EC×10^3 (dS m^{-1})[a]	P (mg kg^{-1})	K (mg kg^{-1})	N (Tot.) (%)	Mg (meqL^{-1})	Ca (meqL^{-1})	Cl (meqL^{-1})	OC[b] (%)	SP (%)	$CaCO_3$ (%)	HCO_3 (%)	Sand (%)	Silt (%)	Clay (%)
7.60	0.60	39.3	565	0.11	1.87	1.89	0.80	0.73	49.9	11.84	3.57	16	44	40

[a] EC×10^3, electrical conductivity.
[b] OC, organic carbon.
[c] SP, saturation percentage.

widely used for genetic analysis of complex traits in sunflower (Rachid Al-Chaarani *et al.,* 2004; 2005; Abou Al Fadil *et al.,* 2007; Darvishzadeh *et al.,* 2007b; Poormohammad Kiani *et al.,* 2007a, b; 2008; 2009). Seeds of RILs and their two parents kindly provided by INRA (France) were planted in pots filled with sterilized soil (Table 1). Plants were grown in a growth chamber with the same controlled conditions as for the pathogenicity test with the selected isolate (SSU107). The RILs and their parental lines were evaluated in a completely randomized design with six replications. This fungal isolate was moderately aggressive so that partial resistance could not be concealed by a rapid development of severe symptoms. For artificial inoculation, the same procedure was used as for the pathogenicity test.

Statistical analysis

The normality of the disease severity data was assessed according to the Shapiro and Wilk (1965) test in SAS (PROC UNIVARIATE; SAS Institute Inc., Cary, NC, USA). Analysis of variance (ANOVA) of the disease severity data was performed using the general linear model (GLM) procedure in the SAS software (SAS Institute Inc.). The function "FREQ" of SPSS software (SPSS/PC-17, SPSS Inc., Chicago, IL, USA; http://www.spss.com) was used to analyze the frequency distribution of RILs and their parents for partial resistance to *S. sclerotiorum*, scored 3 days after basal stem rot inoculation. Compared to SAS, the SPSS graphs had a high quality. The mean of the RILs and that of the parents were compared. Genetic gain was determined by subtracting the mean of the parents from the mean of the 10% most resistant RILs. Genotypic and environmental variances as well as board-sense heritability were calculated according to Kearsey and Pooni (1996) using least-square estimates of genetic parameters as follows:

$$V_g = \frac{MSG - MSE}{r} \qquad V_e = MSE$$

$$V_{ph} = V_g + V_e \qquad h_b^2 = \frac{V_g}{V_{ph}}$$

where V_g, V_e, and V_{ph} are the genotypic, environmental and phenotypic variance respectively, *MSG* and *MSE* the mean square for genotype and error respectively, and r the number of replication.

Map construction and QTL analysis

The linkage map used in this study is the improved map recently described by Poormohammad Kiani *et al*. (2007b). Briefly, the parental lines (PAC2 and RHA266) were screened for polymorphisms with 190 'SSL' and 'SSU' SSR markers (GIE CARTISOL, Paris, France), 507 'ORS' SSR markers from the SSR database (Tang *et al.,* 2002, Tang *et al.,* 2003) and 463 'HA' SSR markers developed by INTA (Paniego *et al.,* 2002). The genomic DNA of PAC2, RHA266 and 123 F9 was isolated according to the method of extraction and purification described by Fulton *et al*. (1995). Electrophoresis was performed using denaturing polyacrylamide gels and silver-staining and/or SYBR gold detection protocols (Molecular Probes, Eugene, OR, USA) (Paniego *et al.,* 2002) or by multiplex PCR assays using modified forward primers by adding fluorophores (6FAM, HEX and NED) according to the method described by Tang *et al*. (2003). PCR fragments were resolved using electrophoresis through an ABI Prism 377 DNA analyzer (Applied Biosystems, Foster City, CA, USA). Fragment sizing was done using the GeneScan Filter. Set D, the ROX 500 internal-lane standard (Applied Biosystems; ROX, 6-carboxy-X-rhodamine) and the GeneScan 3.5 programme (Applied Biosystems). Genotyper 3.6 (Applied Biosystems) was used to score SSR alleles. The map was constructed using Mapmaker 3.0 (Lander *et al.,* 1987) and CarthaGene 0.999 (Schiex and Gaspin, 1997). Chi-square-tests were performed to determine the segregation distortion of each locus. Loci were assembled into groups using likelihood odds (LOD) ratios with a LOD threshold of 4.0 (Gentzbittel *et al.,* 1995; Bert *et al.,* 2003; Bert *et al.,* 2004; Rachid Al-Chaarani *et al.,* 2004) and a maximum recombination frequency threshold of 0.35. Multiple locus orders were estimated for each linkage group using CarthaGene 0.999. The likelihood of different locus orders was compared and the locus-order with the greatest likelihood for each linkage group was selected. The Kosambi mapping function (Kosambi, 1944) was used to calculate map distances (cM) from recombination frequencies, where a map function is a mathematical relationship between recombination probabilities and map distances measured in centi Morgans. Mapchart 2.1 was used for the graphical presentation of linkage groups and the map position of the SSR and AFLP markers. The improved map incorporated 157 more

microsatellite markers than the old version (Rachid Al-Chaarani *et al*., 2004). Each linkage group was numbered according to the sunflower reference map (Tang *et al*., 2002) and was presumed to correspond to one of the 17 chromosomes in the haploid sunflower genome (x=17). The total map length was 1824.6 cM with a mean density of 3.7 cM per locus.

QTL mapping of the studied traits was performed by composite interval mapping (CIM), conducted with the QTL Cartographer programme, version 1.16 (Basten *et al.,* 2002) using mean values of six replications for each RIL. The genome was scanned at 2-cM intervals with a window size of 15 cM. Up to 15 background markers were used as cofactors in the CIM analysis identified with the programme module Srmapqtl (model 6; Basten *et al.,* 2002). A QTL was considered significant if the LOD threshold exceeded 3.0 (Bert *et al.,* 2003; Bert *et al.,* 2004; Rachid Al-Chaarani *et al*., 2004). A decrease in the LOD score of 1.0 represented the end point of the support interval for each QTL (Lander and Botestein, 1989). Additive effects of the QTLs detected were estimated with the Zmapqtl programme (Basten *et al.,* 2002). The percentage of phenotypic variance (R^2) explained by the QTLs was estimated at the peak QTL position by QTL Cartographer (Basten *et al.,* 2002).

Results

Aggressivity test

The 108 isolates differed significantly in their capacity to cause disease on the basal stems of the sunflower hybrid cv. Iroflor. The percentage of lesions ranged from 8 to 100% depending on the iso-

Table 2. Isolate mean lesion percentage on sunflower cv. Iroflor measured 3 days post basal stem inoculation under controlled conditions.

Isolate	Mean lesion percentage	Isolate	Mean lesion percentage	Isolate	Mean lesion percentage	Isolate[a]	Mean lesion percentage
SSU82	66	SSS55	41	SSU28	45	SSU1	61
SSU83	52	SSKH56	47	SSKH29	98	SSKH2	58
SSKH84	68	SSU57	77	SSU30	85	SSKH3	25
SSKH85	42	SSU58	40	SSU31	63	SSU4	20
SSU86	71	SSU59	75	SSU32	70	SSU5	33
SSU87	94	SSKH60	44	SSU33	31	SSS6	75
SSKH88	47	SSKH61	53	SSU34	33	SSKH7	32
SSU89	60	SSU62	20	SSU35	86	SSKH8	32
SSU90	47	SSU63	63	SSU36	50	SSKH9	53
SSKH91	92	SSU64	17	SSKH37	41	SSKH10	47
SSU92	76	SSU65	67	SSKH38	8	SSKH11	40
SSU93	42	SSKH66	15	SSU39	57	SSKH12	20
SSU94	62	SSKH67	77	SSU40	53	SSKH13	21
SSU95	64	SSU78	56	SSKH41	71	SSU14	22
SSU96	32	SSKH69	17	SSU42	76	SSKH15	51
SSU97	71	SSU70	35	SSU43	20	SSU16	48
SSU98	54	SSU71	38	SSU44	75	SSU17	79
SSU99	54	SSKH72	48	SSS45	61	SSU18	25
SSU100	60	SSU73	100	SSKH46	16	SSKH19	40
SSU101	60	SSKH74	79	SSS47	27	SSKH20	25
SSKH102	12	SSU75	42	SSU48	57	SSS21	33
SSKH103	61	SSKH76	26	SSU49	58	SSS22	33
SSU104	73	SSKH77	51	SSKH50	27	SSKH23	24
SSKH105	65	SSKH78	56	SSS51	66	SSU24	45
SSKH106	45	SSU79	63	SSU52	38	SSU25	47
SSU107	86	SSU80	64	SSU53	77	SSKH26	85
SSKH108	69	SSU81	10	SSU54	32	SSU27	63

[a] For each isolate the first two letters refer to *Sclerotinia sclerotiorum* Lib. de Bary. The third and fourth letters show the abbreviated name of the locations where the isolates were collected. S, Salmas; KH, Khoy; U, Urmia. The locations were ~200 km apart.

Table 3. ANOVA and genetic parameters for partial resistance to *Sclerotinia sclerotiorum* in sunflower recombinant inbred lines (RILs) under controlled conditions.

S.O.V	df	MS_{PNA}	Item	PNA[a]	Item[b]	PNA[a]
Genotype	114	1299.78**	PAC2 (P_1)	65.33	RILs -P	18.20 ns
			RHA266 (P_2)	76.00	10%SRILs	50.75
Residual	529	279.79	P_1-P_2	–10.67	GG10%=10%SRIL-P	–19.92*
			P= (P_1+P_2)/2	70.67	LSD 0.05	18.80
Total	643		RILs	88.87	h^2	37.80

[a]PNA, percentage of necrotic area.
[b]RILs, mean of all recombinant inbred lines (RILs); P, mean of parents; 10%SRILs, mean of the 10% Selected RILs; GG10%, genetic gain when the mean of 10% selected RILs is compared with the mean of the parents. $LSD_{0.05}$, least significant differences calculated using $t_{0.05}$ and the error mean square of each experiment; h^2, Board-sense heritability.
**, *, Significant at 0.01 and 0.05 probability level; ns: non significant.

late (Table 2). In view of these findings, one moderately aggressive isolate (SSU107) was selected for the QTL mapping program. This isolate came from a Sclerotinia-infected sunflower sample collected from the Urmia region, where the present study was carried out.

Disease assessment

Three days after mechanical inoculation, the majority of inoculated plants showed *S. sclerotiorum* symptoms on the basal stem.

As reports on resistance to basal stem rot in sunflower so far do not indicate full resistance in any sunflower genotypes, all plantlets with no visible lesions on the basal stem were taken to be not infected, and were excluded from the experiment. Since normalizing data through transformation may misrepresent differences among individuals by pulling skewed tails toward the centre of the distribution (Doerge and Churchill, 1994, Mutschler *et al.,* 1996; Poormohammad Kiani *et al.,* 2009), the phenotypic data analysis was performed on the untransformed data. ANOVA detected highly significant difference among the sunflower geno-

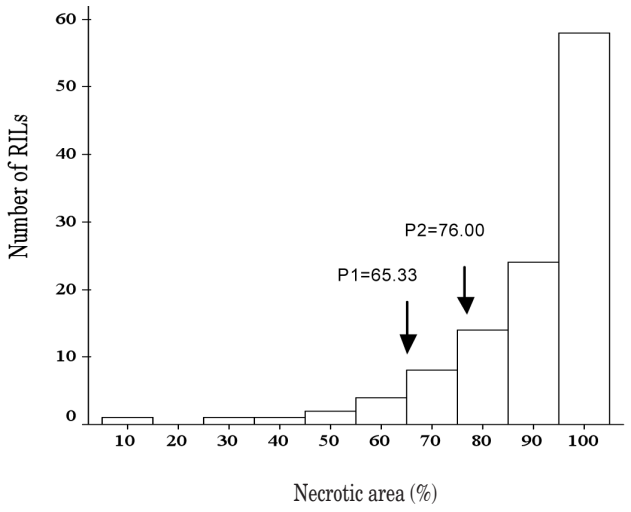

Figure 1. **Frequency distribution of sunflower recombinant inbred lines (RILs) and their parents for partial resistance** to *Sclerotinia sclerotiorum*, scored 3 days after basal stem inoculation and based on the percentage of the area exhibiting necrosis symptoms. Arrows show the phenotypic values of the parental lines (P_1=PAC2 and P_2= HA266).

Table 4. QTLs conferring partial resistant to *Sclerotinia sclerotiorum* in sunflower recombinant inbred lines (RILs) and detected using composite interval mapping (CIM).

QTL	LG	Marker	Position [a]	LOD [b]	Additive effect	R^2 [c]
bsr.P.N.A.1.1	1	ORS803	4.01	4.46	4.37	0.06
bsr.P.N.A.2.1	2	E38M60-10	7.41	4.50	-3.91	0.05
bsr.P.N.A.4.1	4	E33M60-8	13.31	6.46	4.91	0.08
bsr.P.N.A.6.1	6	ORS1287-1	62.31	5.07	-4.45	0.07
bsr.P.N.A.8.1	8	ORS418-1	75.66	4.06	3.92	0.05
bsr.P.N.A.14.1	14	E37M61-3	118.41	5.30	4.01	0.05
bsr.P.N.A.17.1	17	E35M62-5	42.61	4.12	-4.45	0.07

[a]Expressed in Kosambi cM, from the top of linkage group (LG).
[b]LOD, likelihood that the effect occurs by linkage/ likelihood that the effect occurs by chance. A negative sign in additive effect indicates that the resistant allele comes from the maternal line (PAC2); a positive sign indicates that the resistant allele is from the paternal line (RHA266).
[c] R^2 is the percentage of individual phenotypic variance explained by each QTL.

types for susceptibility to basal stem rot (Table 3). The disease severity of the RILs caused by SSU107 ranged from 7 to 100% (Figure 1). A RIL such as C71 showed high partial resistance, while C146 was susceptible.

Differences between the mean disease severity of the RILs (RILs) and the mean of their parents (P) were not significant (Table 3). The difference between the mean of the 10% most resistant lines (10%SRILs = 50.75) and the mean of the parental lines (P=70.67), considered as genetic gain, was significant (GG10% = -19.92, P<0.05) (Table 3). Several studies used the LSD as a critical value to compare the mean of selected lines and the mean of their parents (Rachid Al-Chaarani *et al.,* 2004, 2005; Abou Al Fadil *et al.,* 2007; Poormohammad Kiani *et al.,* 2007a, 2007b, 2008, 2009). The frequency distribution of the RILs and their parents for partial resistance to basal stem rot showed a continuous pattern (Figure 1). Some RILs showed a lower disease severity than their parents, but most RILs had a higher disease severity.

Linkage map and QTL analysis

Composite interval mapping analysis revealed 7 putative QTLs, localized on seven linkage groups, and involved in basal stem rot resistance (Table 4, Figure 2).

The QTLs involved in partial resistance to basal stem rot were located in linkage groups 1, 2, 4, 6, 8, 14 and 17. All the 7 detected QTLs were located in different linkage groups (Figure 2). The phenotypic variance explained by each QTL (R^2) ranged from 5% to 8% (Table 4). The sign of additive gene effects showed that the additive gene for partial resistance to basal stem rot came from both parents.

Discussion

The RILs studied had a large genetic variation for partial resistance to *S. sclerotiorum* on the basal stem, indicating that there is a potential for enhancing resistance to mycelial extension. These findings are consistent with previous reports on *Sclerotinia* basal stem rot resistance (Micic *et al.,* 2005a, b). The frequency distribution of RILs and their parents for susceptibility to basal stem rot showed a continuous pattern, suggesting that resistance is controlled by a polygenic system. Their distributions were slightly skewed towards the higher values (susceptibility). Some RILs produced a lower disease severity than their parents, but others produced higher disease severity than the parents, suggesting that transgressive segregation for resistance occurred in this cross. This finding was supported by QTL mapping, since the sign of the gene effects showed that both parental lines contributed to positive alleles for resistance to basal stem rot. Trangressive segregation in sunflower has previously been reported by Micic *et al*. (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*. (2007b) for partial resistance to Phoma black stem. Mestries *et al*. (1998) analyzed QTLs associated with resistance to *S. sclerotiorum* in sunflower and found transgressive segregation for the capitulum's index. This suggests that each parent possesses resistance and susceptibility alleles for the loci controlling this character. The resistant parent probably possesses susceptibility alleles for loci involved in this trait, since transgressions occurred towards a greater susceptibility.

Broad-sense heritability for partial resistance to basal stem rot was 0.37, indicating that selecting for resistance to basal stem rot by segregating populations through conventional phenotypic selection would most likely be a complex undertaking and that marker-based selection (MAS) should therefore be an effective tool in breeding programs. In this study, seven genomic regions containing putative QTLs associated with partial resistance to basal stem rot were identified on linkage groups (LGs) 1, 2, 4, 6, 8, 14 and 17, but the estimated effect of each of these QTLs was small. These low values support the hypothesis that genetic mechanisms controlling resistance to basal stem rot in sunflower are complex. Since we identified genetic

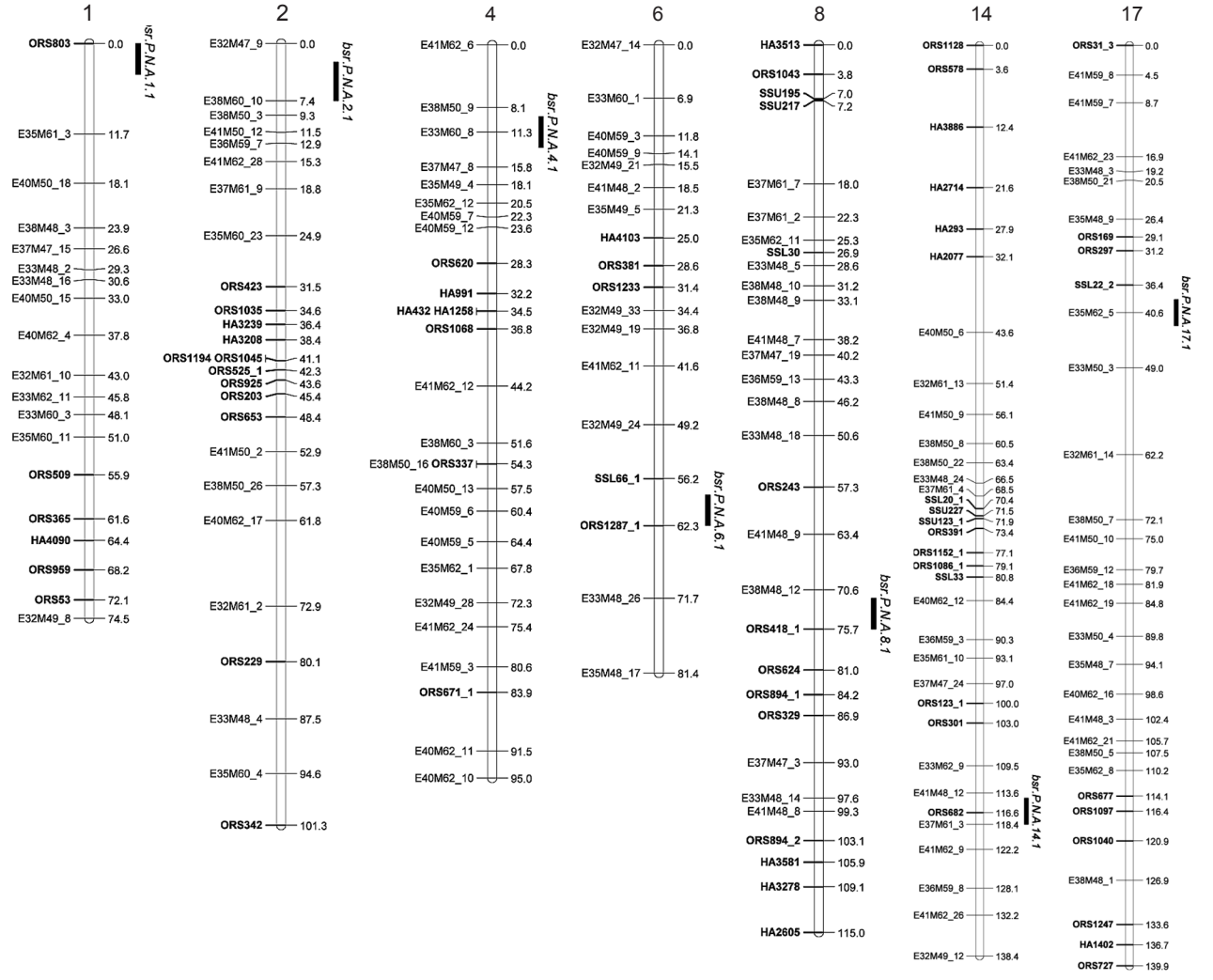

Figure 2. Linkage groups of the sunflower genome presenting QTLs for partial resistance to *Sclerotinia sclerotiorum*. The positions of the QTLs are shown on the right side of the linkage groups. Vertical bars represent intervals associated with the QTLs. Each QTL decrease in the LOD score of 1.0 determined the end point of the support interval.

markers that are linked to partial resistance factors, indirect selection can be directed to detect markers of interest in breeding lines. However, the QTLs and related markers need to be validated in other genetic backgrounds prior to their application in MAS. Some successful MAS has already been reported in rice breeding programmes. For example, Cho *et al*. (1994) used molecular markers to select for the semi-dwarf characteristic in rice. Wang *et al*. (2005) introgressed three QTLs into near isogenic lines using MAS, with large effects on spikelet fertility.

So far, several studies have been undertaken to map QTLs controlling partial resistance to *Sclerotinia* disease in sunflower (Mestries *et al*., 1998; Bert *et al*., 2002; Micic *et al*., 2004; Micic *et al*., 2005a, b; Rönicke *et al*., 2005). Bert *et al*. (2002) found three QTLs, explaining about 56% of the phenotypic variance for the mycelium trait on the leaves on LGs 6, 8, and 13, which coincided with LGs 13, 9, and 1 of the sunflower reference map (Tang *et al*., 2002). One of the seven QTLs identified as conferring partial resistance to basal stem rot in the present study, and one QTL reported by Bert *et al*. (2002) as having the the mycelium trait on the leaves, were both located on linkage group 1. Micic *et al*. (2004) using an SSR map of F2/F3 families developed from a cross between a resistant inbred line from the germplasm pool NDBLOS and the susceptible line CM625, identified seven QTLs as conferring partial resistance to stem lesions with phenotypic variance (R^2) ranging from 3.3 to 36.7% on the linkage groups 2, 3, 4, 6, 8, 15, and 16 of the sunflower reference map (Tang *et al*., 2002). In another study, QTLs identified as conferring partial resistance to stem lesion on linkage groups 8 and 16 were reconfirmed by Micic *et al*. (2005a). In our study, four of the seven QTLs here conferring partial resistance to basal stem rot disease, and 4 QTLs reported by Micic *et al*. (2004) as conferring partial resistance stem lesions, were located on the same linkage groups. Micic *et al*. (2005b), using selective genotyping of F2/F3 families developed from a cross between CM625 (susceptible) × TUB-5-3234 (resistant) by SSR markers, identified 3 QTLs as conferring partial resistance to stem lesions with phenotypic variance (R^2), ranging from 16.1 to 24.0%, on LGs 4, 10 and 17 of the sunflower reference map (Tang *et al*., 2002). In the present study it was found that 2 of the 7 QTLs identified as conferring partial resistance to basal stem rot, and 2 QTLs reported by Micic *et al*. (2005b) as conferring partial resistance to stem lesions, were located on the same linkage groups.

Mestries *et al*. (1998) identified 3 QTLs as conferring resistance to head rot, each explaining 12.3 to 17.5% of phenotypic variance. Rönicke *et al*. (2005) detected 5 QTLs governing resistance to head rot, each explaining 10.6 to 17.1% of total phenotypic variance. However, the lack of SSR markers and common linkage group nomenclature in their map made it difficult to compare the location of the QTLs detected in the present study and those detected by Mestries *et al*. (1998) and by Rönicke *et al*. (2005).

We also compared the chromosomal positions of the QTLs detected in this study with those of previous studies on the partial resistance to *P. macdonaldii* in sunflower (Darvishzadeh *et al*., 2007b). One of the QTLs conferring partial resistance to *S. sclerotiorum* in that study (LG2, E38M60_10 marker) was co-localized with a QTL conferring partial resistance to the *P. macdonaldii* in sunflower (LG2, E38M60_10 marker). Because of this co-localization, the same or similar resistance genes may be involved. These QTLs may be important in general defense mechanisms.

Marker assisted selection (MAS) for resistance to *S. sclerotiorum* (Lib.) de Bary, is not yet possible because of a lack of effective molecular markers. Further studies are necessary to develop such closely linked markers. By using larger population sizes and a greater number of markers, it will be possible to identify more tightly-linked markers; this process is termed "high-resolution mapping" (also "fine mapping"). Therefore, high-resolution mapping of the QTLs may be used to develop reliable markers for MAS (at least <5 cM but ideally <1 cM away from the gene) and also to discriminate between a single gene or several linked genes (Mohan *et al*., 1997). LG8 and LG16 are good candidates for further analyses to develop molecular markers for resistance to *Sclerotinia* disease, since QTLs conferring resistance to *S. sclerotiorum* have already been identified on these two linkage groups in various independent studies.

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