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P.-F. Bert · I. Jouan · D. Tourvieille de Labrouhe
F. Serre · P. Nicolas · F. Vear

Comparative genetic analysis of quantitative traits in sunflower (*Helianthus annuus* L.) 1. QTL involved in resistance to *Sclerotinia sclerotiorum* and *Diaporthe helianthi*

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Abstract *Sclerotinia sclerotiorum* and *Diaporthe helianthi* are important pathogens of sunflower (*Helianthus annuus* L.). Two hundred and twenty F2–F3 families were developed from an intraspecific cross between two inbred sunflower lines XRQ and PSC8. Using this segregating population a genetic map of 19 linkage groups with 290 molecular markers covering 2,318 cM was constructed. Disease resistances were measured in field experiments during 3 years (1998, 1999 and 2000) for phomopsis and 2 years for *S. sclerotiorum* (1997 and 1999). QTL were detected using the interval mapping method at a LOD threshold of 3. A total of 15 QTL for each pathogen resistance were detected across several linkage groups, confirming the polygenic nature of the resistances. These QTL explained from 7 to 41% of the phenotypic variability. The QTL for phomopsis resistance, in the 3 years of tests, mapped in the same region, and this was also true for some forms of *S. sclerotiorum* resistance in the 2 years of tests. On linkage group 8, QTL affecting resistance to both *S. sclerotiorum* and *D. helianthi* mycelium extension on leaves colocalised, suggesting a common component in the mechanism of resistance for these two pathogens. The colocalisation of QTL and breeding for resistance to *S. sclerotiorum* and to *D. helianthi* by pyramiding QTL in sunflower are discussed.

Keywords Sunflower · Genetic map · QTL · Disease resistance · *Sclerotinia sclerotiorum* · *Diaporthe helianthi*

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P.-F. Bert · I. Jouan · P. Nicolas
UMR 1095 INRA –
Université Blaise Pascal "Amélioration et Santé des Plantes",
24, avenue des Landais, 63177 Aubière Cedex, France

D. Tourvieille de Labrouhe · F. Serre · F. Vear (✉)
UMR 1095 INRA –
Université Blaise Pascal "Amélioration et Santé des Plantes",
234, avenue du Brézet, 63039 Clermont-Ferrand Cedex 2, France
e-mail: vear@clermont.inra.fr

Introduction

Sclerotinia sclerotiorum white rot and phomopsis stem canker are two important diseases of cultivated sunflower (*Helianthus annuus* L.) causing severe and worldwide crop losses. *S. sclerotiorum* (Lib.) de Bary is to-day considered as one of the most damaging pathogens of sunflower. It is widespread, it persists for many years in the soil and has a very wide host range (Masirevic and Gulya 1992). Although the fungus can infect different plant parts, roots, stem bases, leaves, terminal buds and capitula, the infections on capitula probably produce the greatest loss because they occur at the end of the season when no compensation by surrounding plants is possible. Resistance to *S. sclerotiorum* is under polygenic control (Castaño et al. 1993) but to-date, no complete resistance to *S. sclerotiorum* is available in cultivated sunflower, even if differences in susceptibility exist (Tourvieille et al. 1996).

Phomopsis (*Diaporthe helianthi* Munt-Cvet et al.) was first mentioned and identified in Yugoslavia in 1980 (Muntanola-Cvetkovic et al. 1981). It spread quickly and was found rapidly in the neighbouring countries (Vranceanu et al. 1983) and in France (Regnault 1985). It was also reported in the USA (Yang et al. 1984). Since this pathogen cannot always be efficiently controlled by chemicals, breeding for resistance has to be employed to offset the disease. Like the resistance to *S. sclerotiorum*, phomopsis resistance shows continuous variation (Vear et al. 1997). Also, different genotypes appear to possess resistances to different parts of the disease cycle (Viguié et al. 2000).

The development of molecular markers makes it possible to investigate the inheritance of complex traits and to locate genetic factors underlying these quantitative traits. During the past two decades, DNA molecular markers have been successfully used to map loci controlling quantitative trait variations. Depending on the host-parasite interaction considered, from two (Landry et al. 1992) to seven (Schön et al. 1993) QTL were identified, contributing to quantitative variations in disease resis-

tance. Analyses of QTL associated with resistance to extension of the *S. sclerotiorum* mycelium on sunflower capitula and leaves, using molecular markers, have already been made by Mestries et al. (1998). They used 73 RFLP probes to construct a genetic map in order to identify loci involved in resistance to *S. sclerotiorum*. Individual QTL explained between 9% and 48% of the phenotypic variability, confirming the polygenic basis of the trait. One of these regions appeared to be involved in resistance in both plant parts to *S. sclerotiorum* attack, while the others appeared specific for resistance to one part of the plant. An important step in QTL analysis is the determination of the relationships between putative candidate genes and loci underlying quantitative traits. Gentzbittel et al. (1998) presented a candidate-gene approach to analyse resistance to pathogenic fungi in sunflower in three crosses. They showed that a Protein-Kinase-like gene was a marker co-segregating with a locus which explained up to 50% of the phenotypic variation and thus was a possible candidate to control some types of resistance to *S. sclerotiorum* in sunflower.

To confirm the first studies on *S. sclerotiorum* resistance and to determine their transportability and possibly to find new QTL, it appeared of interest to identify QTL for the same characters in other crosses. In addition, determination of QTL for resistance to the two pathogens in the same genotypes is important to improve the efficiency of resistance breeding and should provide a better understanding of the genetic organisation of the quantitative resistance in plants.

In the present paper we report the identification, localisation and genetic effects of QTL involved in resistance to *S. sclerotiorum* and *D. helianthi* using several resistance tests on a set of F3 families derived from a cross between inbred sunflower lines.

Materials and methods

Sunflower genotypes

The two parental inbred lines of the cross were bred by INRA (Institut National de la Recherche Agronomique, France). XRQ was a selection for downy mildew resistance from a cross between HA89 and the Russian population 'Progress' (Vear et al. 1998). PSC8 was selected from a population made from a wide range of sunflower lines and subjected to recurrent selection for capitulum resistance to *S. sclerotiorum* (Vear et al. 1992). XRQ, an unbranched PET-1 CMS maintainer, shows a medium level of resistance to *S. sclerotiorum*, but a high level of resistance to phomopsis. PSC8, a male-fertility restorer line, exhibits the apical branching gene (*b1*) phenotype, a high level for *S. sclerotiorum* resistance and a medium level for phomopsis resistance. The male-fertile forms of the two lines were crossed in both directions. The F1 plants were selfed by covering the capitula with grease-proof paper bags a few days before flowering to obtain the 220 F2 generation, which was in turn selfed to obtain the 220 F3 families.

Experimental plan and resistance tests

Field trials were conducted in randomised block designs with two replicates. The *S. sclerotiorum* ascospore test was that described by Tourvieille and Vear (1984). Capitula were infected at the beginning of flowering with an ascospore suspension. The test measured both a percentage attack (on two replications of 25 plants) and a latency index, which was the number of days between infection and symptom appearance compared with the mean delay for two control inbreds infected on the same day. *S. sclerotiorum* ascospore tests were made in 1997 on all the 220 F3 families, but, to reduce the amount and cost of field work in 1999, only 180 F3 families were tested. Because previous results (Mestries et al. 1998) had shown that many characters, including *S.sclerotiorum* resistance, were linked to the recessive branching gene *b1*, to facilitate demonstration of QTL on other linkage groups, it was decided to eliminate 20 homozygous branched and 20 heterozygous families for which phomopsis resistance studies had not been started. These families were chosen according to the geographical position in the field of the F2 plants in 1996. The 180 families retained were 51 homozygous unbranched, 108 segregating and 21 homozygous branched.

S. sclerotiorum mycelium tests on leaves and capitula were described by Castaño et al. (1993). The leaf test gave a lesion length from the mycelium explant infection on the leaf tip along the main vein. Two replications of five plants were infected at 1-week intervals. The test using the mycelium on the capitulum measured the lesion area 3 days after infection of the dorsal surface of capitula with mycelium explants. The test gave an index which was the lesion area divided by the mean lesion area of a control variety infected at the same time. Three explants were used to infect each of five capitula with two replications for each F3 family. The mycelium tests on leaves and capitula were made in 1997 on all 220 families.

Developed by Bertrand and Tourvieille de Labrouhe (1987), the test for resistance to *D. helianthi* mycelium extension on the leaves measures the rate of lesion development along the main vein from a mycelial explant placed on the upper side of the leaf tip. Two leaves per plant were infected. Since all the families could not be infected on the same day, results are given as an index which was the lesion length of the plants tested divided by the mean lesion length of the control genotypes infected on the same day. This test was made twice for each of the 180 F3 families used for the *S. sclerotiorum* ascospore tests in 1999, spread over 1998, 1999 and 2000. Ninety eight families were tested in 1998, 155 in 1999 and 104 in 2000. Seventy three were common to 1998 and 1999, 79 to 1999 and 2000, and 26 to 1998 and 2000. Analyses were made separately for each year, but to be able to treat all the families simultaneously, an overall index was obtained by multiplying the 1998 and 2000 values by the ratios of the means of these years with the mean of 1999.

The *D. helianthi* semi-natural attack methodology described by Tourvieille (1989) consists in placing infected sunflower stems regularly throughout the trial at the starbud stage. The observations of reaction to semi-natural phomopsis attack were started in 1997 in trials of two replications of 50 plants in several locations in south-west France. They continued each year until 2000, but only those showing sufficient levels of attack and a significant *F* genotype were retained. For each F3 family, one to four valid trial data were obtained. As for the mycelium tests, families were grouped in trials according to F2 plant positions and a general index was calculated as the means of the indices for each trial.

Molecular analysis and map construction

RFLP probes (available upon request) and candidate genes, protein kinase-like (PK-like), NBS-LRR type Resistance Gene Analogues (NBS-Like), phenylalanine ammonia lyase (PAL), anther-specific gene (SF3), heat shock protein (HSP70) and ubiquitin, were chosen among those used for the sunflower CARTISOL linkage map (Gentzbittel et al. 1995, 1998, 1999), both for their polymorphism between XRQ and PSC8 and their regular repartition over all linkage groups. DNA extraction, digestion by restriction enzymes (*Eco*RI and *Hin*dIII) and Southern hybridisation were carried out as described previously (Gentzbittel et al. 1995). The

Pl5 locus for resistance to downy mildew, a fertility restorer locus *Rf1*, and the apical branching gene *b1* were added to marker data.

The AFLP protocol developed by Vos et al. (1995) was followed with minor modifications as described in Bert et al. (1999). PCR products were visualised using the silver-nitrate staining method as described by Tixier et al. (1997). AFLP markers were named according to their primer-pair combination and their estimated molecular size. RFLP markers were dominantly (59%) and codominantly (41%) scored and AFLP markers were dominantly scored.

The software package JOINMAP 2.0 (Stam 1993) was used to estimate segregation distortion and determine linkage groups, and the software MAPMAKER 3.0b (Lander et al. 1987) was used to order loci and construct the map. Analyses were performed with a LOD score threshold of 4.0 and a maximum of recombination value $\theta = 0.40$ for grouping and ordering markers. Haldane's mapping function was applied for map-distance calculation.

Statistical analyses and QTL detection

Before analysis, normality of the different traits was assessed according to the Shapiro and Wilk test (PROC UNIVARIATE of SAS; SAS Institute Inc.). When necessary, data were converted using the arc-sin-square root (for percentage attack), Log (for mycelium index) and square root (for latency index) to fit a normal distribution.

Phenotypic correlation analyses among traits were also performed using Statgraphics/Plus to detect associations between resistance tests for the two pathogens.

Data sets were analysed by generalised linear modelling (GLM) using the SAS program and Statgraphics/Plus. Normality of the residuals and homogeneity of variances using Bartlett's test were also checked. Using the framework map orders, QTL were detected using the method of interval mapping of MAPMAKER/QTL (Lincoln et al. 1992) software with a LOD score threshold of 3. This value was chosen from the theoretical consideration of Lander and Botstein (1989).

Results

Frequency distributions of phenotypes

The frequency distributions of phenotypes of the F3 families for each resistance test are shown in Figs. 1 and 2. All traits showed approximately normal distributions. When parental values were measured, transgressive segregants were observed for all traits studied. For example, in *S. sclerotiorum* tests 9.1% of F3 families were more susceptible than the susceptible parent XRQ for mycelium extension on leaves, and 57.5% of the F3 families were more susceptible than PSC8, the parent more susceptible to mycelium extension on the capitula.

Phenotypic variation and correlations between traits

Results from statistical analyses (mean phenotypic values, *F*-values and broad-sense heritabilities) are presented in Table 1. For the *S. sclerotiorum* ascospore test, the mean percentage attack was greater (65%) in 1997 than in 1999 (41%). Since, in each family, more plants showed disease symptoms, this led to a more precise estimate of the latency index in 1997 than in 1999, where the *F* genotype was at the limit of significance. Nevertheless, for the 2 years, there were significant correlations between results for both characters. For the *S. sclerotiorum* mycelium tests, made only in 1997, those on leaves were more precise than those on capitula, as seen by the coefficients of variations (CV). Heritabilities were moderate, comprised between 0.30 and 0.62, except for the latency index for 1999 ($h^2 = 0.05$).

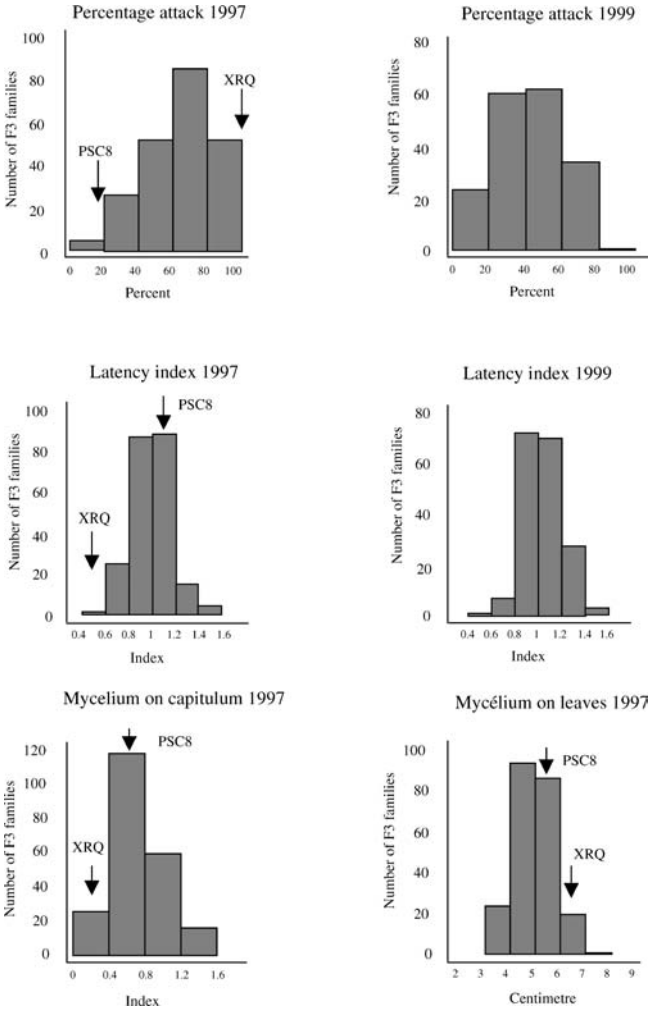


Fig. 1 Frequency distributions of phenotypes for *S. sclerotiorum* resistance in the XRQ × PSC8 F3 families. Phenotypes of parental lines (when available) are shown by *arrows*

Correlation coefficients between years for the tests carried out more than once, in addition to correlations between the different characters observed, were calculated by phenotypic regression. The significant correlation coefficients are presented in Table 2. *S. sclerotiorum* latency indices and percentage attacks were negatively correlated, as might be expected for two inversely related traits. Field trial data from the two seasons 1997 and 1999 were significantly correlated. For phomopsis mycelium tests on leaves, close correlations were observed between the 3 years of tests, especially 1998–1999 and 1999–2000, and also with the results of semi-natural attack.

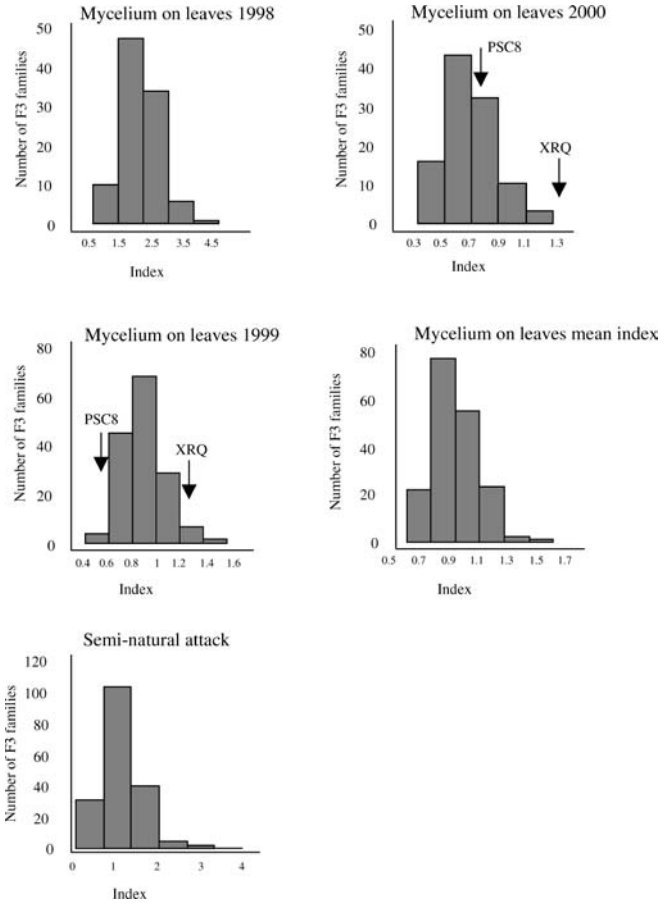


Fig. 2 Frequency distributions of phenotypes for *D. helianthi* resistance in the XRQ × PSC8 F3 families. Phenotypes of parental lines (when available) are shown by *arrows*

Molecular analysis and map construction

Eighty two *Eco*RI and 110 *Hin*dIII probes were chosen among those used for the CARTISOL sunflower linkage map, and checked for their polymorphism between the parental lines XRQ and PSC8. Thirty one of the *Eco*RI and 45 of the *Hin*dIII probes were polymorphic, including PK-like, NBS-Like, HSP70, SF3 and ubiquitin. Segregation analysis was performed on 220 F2 plants. Sixty four AFLP primer combinations were tested in order to compare fingerprint patterns and determine those which produced clearly detectable bands, and revealed high levels of polymorphism. Of the 64 primer pairs tested, 11 were selected (E32/M48, E32/M49, E32/M50, E32/M59, E32/M61, E32/M62, E33/M48, E33/M49; E33/M59, E33/M61 and E35/M48) for mapping. For data scoring, segregation distortion was checked and highly significantly ($P < 0.001$) skewed markers were removed from further analysis in order to avoid false linkages in map construction. Overall, a total of 290 markers were mapped on 19 linkage groups which were identified using concordance of RFLP loci on the CARTISOL map (Fig. 3). The total map length covered by all the 290 markers was 2,318 cM. The average density of markers was approximately one per 8.0 cM. Linkage group 9 with 25 markers was the longest (210 cM) while linkage group 15 was the smallest with only 19 cM and four markers.

Quantitative Trait Loci detection for resistance to *S. sclerotiorum*

Table 3 presents QTL that affected resistance of sunflower to *S. sclerotiorum* and *D. helianthi*. A total of 30 QTL were identified in this study, ranging from 1 to 5 per trait (Fig. 3 and Table 3). Altogether, 15 significant QTL for the four parameters for resistance to *S. sclerotiorum* and 15 QTL for the two parameters for resistance to *D. helianthi* were detected, on 11 of the 19 linkage groups.

Percentage attack

Two QTL were identified on the basis of data from both 1997 and 1999, with the same chromosomal location (group 6) and relative magnitude effect (R^2 = 12%). They were mapped in the 20-cM E32M59-240 *Pl5* interval. Four additional QTL were identified on linkage groups 5, 7, 8 and 13 in 1997, and two others on linkage

Table 1 Summary of the statistical results of disease resistance tests for the XRQ × PSC8 F3 families

Character		Number of F3 families	Mean	CV (%)	*F* genotype	Heritabilities
Sclerotinia	Mycelium on leaves 1997	220	5.36 (cm)	14.62	1.87***	0.30
	Mycelium on capitulum 1997	220	0.54 (index)	42.52	2.49***	0.43
	Percentage attack 1997	220	64.73 (%)	31.39	4.31***	0.62
	Percentage attack 1999	180	41.78 (%)	42.25	3.05***	0.51
	Latency index 1997	220	1.04 (index)	10.96	2.52***	0.43
	Latency index 1999	180	0.91 (index)	10.93	1.11 ns	0.05
Phomopsis	Mycelium on leaves 1998	98	2.04 (index)	21.39	1.69**	0.26
	Mycelium on leaves 1999	155	0.88 (index)	16.93	2.58***	0.44
	Mycelium on leaves 2000	104	0.72 (index)	20.66	1.82**	0.29
	Mycelium on leaves mean	179	0.88 (index)	17.04	–	–
	Semi-natural attack	178	1.55 (index)	42.72	–	–

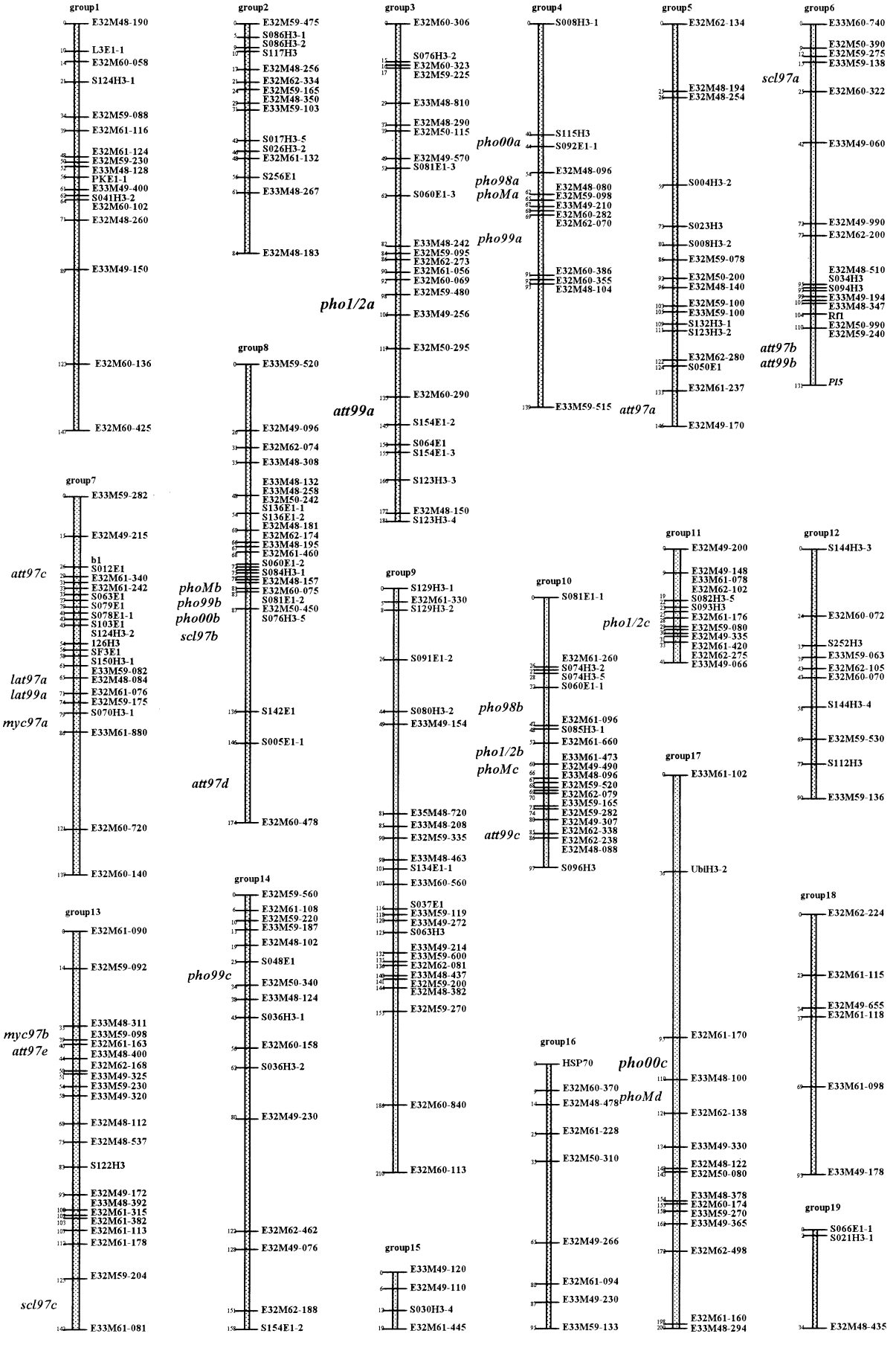
***$P > 0.0001$, **$P > 0.001$, ns = non significant

Table 2 Significant Pearson correlation coefficients among traits in XRQ × PSC8 F3 families. r = 0.132 at $P = 0.05$

Trait		Sclerotinia						Phomopsis			
		Myc/l	Myc/c	P.att97	P.att99	Lat97	Lat99	Myc98	Myc99	Myc00	MycM
Sclerotinia	Mycelium on leaves 1997										
	Mycelium on capitulum 1997	ns									
	Percentage attack 1997	–0.228	ns								
	Percentage attack 1999	–0.138	ns	0.310							
	Latency index 1997	ns	ns	–0.293	–0.477						
	Latency index 1999	ns	ns	ns	–0.401	0.320					
Phomopsis	Mycelium on leaves 1998	0.143	ns	ns	ns	ns	ns				
	Mycelium on leaves 1999	0.217	ns	ns	ns	ns	ns	0.615			
	Mycelium on leaves 2000	ns	ns	ns	ns	ns	ns	0.347	0.531		
	Mycelium on leaves mean	0.195	ns	ns	ns	ns	ns	0.906	0.865	0.875	
	Semi-natural attack	0.139	ns	–0.188	ns	ns	ns	ns	0.317	0.168	0.231

Table 3 Characteristics of QTL affecting *S. sclerotiorum* and *D. helianthi* resistance in the F3 population from the cross XRQ × PSC8. * = putative QTL with a 2 < LOD < 3. R^2 = phenotypic variation explained by each QTL for non-transformed data. Direction = the parent providing the best resistance

Trait		QTL	Marker interval	Group	LOD	R^2 (%)	Direction
Sclerotinia	Mycelium on leaves 1997	*scl97a*	E33M59-138_E32M60-322	6	4.04	16.4	PSC8
		scl97b	S076H3-5_S142E1	8	3.63	17.4	XRQ
		scl97c	E32M59-204_E33M61-081	13	3.36	41.2	PSC8
				R^2 total (%)		56.1	
	Mycelium capitulum 1997	*Myc97a*	S070H3-1_E33M61-880	7	4.72	18.3	XRQ
		Myc97b	E33M48-311_E33M59-098	8	3.01	9.7	PSC8
				R^2 total (%)		25.6	
	Percentage attack 1997	*att97a*	E32M61-237_E32M49-170	5	3.29	19.9	PSC8
		att97b	E32M59-240_Pl5	6	3.64	12.7	PSC8
		att97c	b1_S012E1	7	7.28	14.8	PSC8
		att97d	S005E1-1_E32M60-478	8	3.10	9.0	PSC8
		att97e	E33M48-311_E33M59-098	13	4.24	11.2	XRQ
				R^2 total (%)		43.3	
	Percentage attack 1999	*att99a*	E32M60-290_S154E1-2	3	3.84	13.7	PSC8
		att99b	E32M59-240_Pl5	6	3.79	12.5	XRQ
		att99c	E32M62-338_E32M62-238	10	4.22	11.8	PSC8
				R^2 total (%)		31.8	
	Latency index 1997	*lat97-7*	E32M48-084_E32M61-076	7	3.67	9.9	PSC8
	Latency index 1999	*lat99-7*	E32M48-084_E32M61-076	7	3.57	10.4	PSC8
Phomopsis	Mycelium on leaves 1998	*pho98a**	E32M48-096_E32M48-080	4	2.15	22.9	XRQ
		pho98b	S060E1-1_E32M61-096	10	3.12	17.7	PSC8
				R^2 total (%)		27.7	
	Mycelium on leaves 1999	*pho99a*	E32M62-070_E32M60-386	4	5.28	34.7	XRQ
		*pho99b**	S081E1-2_E32M50-450	8	3.07	34.7	XRQ
		pho99c	S048E1_E32M50-340	14	3.39	12.0	PSC8
				R^2 total (%)		59.3	
	Mycelium on leaves 2000	*pho00a*	S115H3_S092E1-1	4	3.59	23.4	XRQ
		*pho00b**	S081E1-2_E32M50-450	8	2.42	12.1	XRQ
		*Pho00c**	E32M61-170_E33M48-100	17	2.42	29.0	PSC8
				R^2 total (%)		47.2	
	Mycelium on leaves mean	*phoMa*	E32M48-096_E32M48-080	4	6.02	30.5	XRQ
		phoMb	S081E1-2_E32M50-450	8	4.75	12.7	XRQ
		phoMc	E33M61-473_E32M49-490	10	3.09	9.9	PSC8
		phoMd	E33M48-100_E32M62-138	17	3.32	12.5	PSC8
				R^2 total (%)		49.2	
	Semi-natural attack	*pho1/2a*	E32M59-480_E33M49-256	3	3.09	15.7	XRQ
		*pho1/2b**	E32M61-660_E33M61-473	10	2.24	7.2	PSC8
		pho1/2c	E32M59-080_ E32M49-335	11	3.27	9.8	XRQ
				R^2 total (%)		21.3	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S124H3-1
E32M59-088
E32M61-116
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E32M59-230
E33M48-128
PKE1-1
E33M49-400
S041H3-2
E32M60-102
E32M48-260
E33M49-150
E32M60-136
E32M60-425
group2
E32M59-475
S086H3-1
S086H3-2
S117H3
E32M48-256
E32M62-334
E32M59-165
E32M48-350
E33M59-103
S017H3-5
S026H3-2
E32M61-132
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E32M49-570
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S064E1
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S123H3-3
E32M48-150
S123H3-4
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att99a
group4
S008H3-1
S115H3
S092E1-1
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E32M48-080
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E32M62-070
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E32M48-104
E33M59-515
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pho98a
phoMa
pho99a
group5
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scl97b
att97d
group9
S129H3-1
E32M61-330
S129H3-2
S091E1-2
S080H3-2
E33M49-154
E35M48-720
E33M48-208
E32M59-335
E33M48-463
S134E1-1
E33M60-560
S037E1
E33M59-119
E33M49-272
S063H3
E33M49-214
E33M59-600
E32M62-081
E33M48-437
E32M59-200
E32M48-382
E32M59-270
E32M60-840
E32M60-113
group10
S081E1-1
E32M61-260
S074H3-2
S074H3-5
S060E1-1
E32M61-096
S085H3-1
E32M61-660
E33M61-473
E32M49-490
E33M48-096
E32M59-520
E32M62-079
E33M59-165
E32M59-282
E32M49-307
E32M62-338
E32M62-238
E32M48-088
S096H3
pho1/2c
pho98b
pho1/2b
phoMc
att99c
group11
E32M49-200
E32M49-148
E33M61-078
E32M62-102
S082H3-5
S093H3
E32M61-176
E32M59-080
E32M49-335
E32M61-420
E32M62-275
E33M49-066
group12
S144H3-3
E32M60-072
S252H3
E33M59-063
E32M62-105
E32M60-070
S144H3-4
E32M59-530
S112H3
E33M59-136
group13
E32M61-090
E32M59-092
E33M48-311
E33M59-098
E32M61-163
E33M48-400
E32M62-168
E33M49-325
E33M59-230
E33M49-320
E32M48-112
E32M48-537
S122H3
E32M49-172
E33M48-392
E32M61-315
E32M61-382
E32M61-113
E32M61-178
E32M59-204
E33M61-081
myc97b
att97e
scl97c
group14
E32M59-560
E32M61-108
E32M59-220
E33M59-187
E32M48-102
S048E1
E32M50-340
E33M48-124
S036H3-1
E32M60-158
S036H3-2
E32M49-230
E32M62-462
E32M49-076
E32M62-188
S154E1-2
pho99c
group15
E33M49-120
E32M49-110
S030H3-4
E32M61-445
group16
HSP70
E32M60-370
E32M48-478
E32M61-228
E32M50-310
E32M49-266
E32M61-094
E33M49-230
E33M59-133
group17
E33M61-102
UbiH3-2
E32M61-170
E33M48-100
E32M62-138
E33M49-330
E32M48-122
E32M50-080
E33M48-378
E32M60-174
E33M59-270
E33M49-365
E32M62-498
E32M61-160
E33M48-294
pho00c
phoMd
group18
E32M62-224
E32M61-115
E32M49-655
E32M61-118
E33M61-098
E33M49-178
group19
S066E1-1
S021H3-1
E32M48-435

groups 3 and 10 in 1999. Except for the QTL on groups 6 and 13, all alleles linked to resistance were from the resistant line PSC8.

Latency index

Only one QTL was identified for the latency index from each year with the same chromosomal location on linkage group 7 and the same relative importance (R^2 = 10%), and for both years resistance came from PSC8, so they may be considered to be the same QTL.

Mycelium on the capitulum

Two genetic regions were identified for resistance to the mycelium on the capitulum on linkage groups 7 and 13. These QTL explained from 18.3 and 9.7% of observed phenotypic variance and came from XRQ and PSC8 respectively. The QTL *myc97a* on linkage group 7 co-located with the QTL for latency index.

Mycelium on the leaves

Three QTL were significantly associated with resistance to *S. sclerotiorum* on the leaves. The genomic regions were identified on linkage groups 6, 8 and 13, accounting for 16.4 to 41.2% of the phenotypic variance.

Quantitative Trait Loci detection
for resistance to *D. helianthi*

Mycelium on the leaves

Two major QTL regions were identified for resistance to *D. helianthi* mycelium extension on the leaves, on linkage groups 4 (all years) and 8 (1999, 2000 and mean index). In both cases resistance came from XRQ. The QTL on linkage group 8 co-located with the QTL *scl97b* for resistance to *S. sclerotiorum* mycelium on the leaves, and also with resistance from XRQ. Additional year-specific phomopsis resistance QTL were identified on linkage groups 10, 14 and 17, in each case with resistance from PSC8.

Semi-natural attack

Two significant QTL were detected on linkage groups 3 and 11 with the resistant allele from XRQ. A putative QTL with a LOD below the significance threshold was also identified on linkage group 10 in the same interval which contained a QTL for resistance to *D. helianthi* on the leaves, with resistance from PSC8.

◀ **Fig. 3** Genetic linkage map of the sunflower genome based on an intraspecific F2 population of a cross between inbred lines XRQ and PSC8 at a LOD score of 4.0. Genetic distances are in Haldane cM. On the left of each linkage group, the QTL detected are indicated in *bold type*

Discussion

Breeding sunflower for resistance to *S. sclerotiorum* and *D. helianthi* is complicated by the fact the two pathogens can attack several parts of the plant and the level of resistance may be different for each plant part (Castano et al. 1993). In order to determine the underlying genetical mechanisms, several resistance parameters were measured by complementary tests: mycelium infection on leaves, ascospore and mycelium infection on capitula, and observations of semi-natural attacks on leaves and stems.

The length of the map was quite similar to that described recently by Flores Berrios et al. (2000) and significantly greater than those described by Berry et al. (1995), Gentzbittel et al. (1995, 1999) and the integrated RFLP-AFLP map described by Gedil et al. (2001). The map is 500-cM longer than the estimation of Gentzbittel et al. (1999). Genotyping errors may have partially inflated map distances (Lincoln and Lander 1992), since linkage groups 3, 8, and 17 which possess the same RFLP markers on their extremities as those on the corresponding CARTISOL groups, now appear longer. The use of a large proportion of dominant markers may also have affected local locus ordering and given some overestimation of distances between loci but a population of 220 genotypes should give small standard deviations for the estimation of the recombination frequencies (Lorieux and Gonzales de Leon 1993). Several regions were devoid of RFLP because they were not polymorphic and new areas were detected by AFLP markers locating beyond the RFLP of the CARTISOL map for linkage groups 1, 5, 6, 9 and 14. Thus, the true length of the sunflower genome remains to be confirmed.

The resistance tests used are well known and considered as the most-reliable available. The repeatability was satisfactory with significant correlations between the two replicates in the field (data not shown) and correlations between the years of experiments were also significant (Table 2). For phomopsis resistance, QTL analyses were made both on the data from single years and on an overall index for the 3 years. This made it possible to demonstrate general QTL and also to identify those involved in year-specific (environment linked) effects. Three major linkage groups seemed to be involved with common or close locations of QTL (Fig. 3). Linkage groups 4, 8 and 10 contained QTL for resistance to mycelium extension on leaves, detected for 1998, 1999 and 2000. On linkage group 10, a QTL for resistance to semi-natural attack was located close to one for resistance to mycelium on leaves. This suggests that there may be a common QTL for resistance to these two kinds of infection. The other QTL detected were specific to resistance to semi-natural attacks and may correspond to different factors of resistance, such

as those for ascospore infection, or for mycelium growth on stems, not measured by the mycelium test on leaves.

In contrast, for *S. sclerotiorum*, due to considerable differences in the mean percentage attack between 1997 and 1999, which also led to differences in the precision of latency indices and the different numbers of progeny studied, the data from the 2 years were only analysed separately. QTL for percentage attack after the ascospore test were mapped on linkage groups 5, 6, 7, 8 and 13 for 1997 and linkage groups 3, 6 and 10 for 1999, the only common QTL to 1997 and 1999 being that on linkage group 6. It may be noted that Mestries (1996) observed QTL for percentage attack on linkage groups 7, 10, 15 and 17 from a cross between inbred lines GH and PAC2. The QTL *att97c* was detected in the same marker interval as that reported by Mestries (1996), close to the *b1* branching gene on linkage group 7. The lack of the apparent effect of this QTL in 1999 could be due to the absence of 20 branched F3 families. However, in contrast, the one QTL detected for the latency index was located in the same interval for the 2 years, also on chromosome 7, close to that reported by Mestries et al. (1998) in GH × PAC2. A separate study is in progress to determine the effect (linkage or pleiotropy) of the *b1* branching gene on *S. sclerotiorum* resistance. It is possible that other QTL for the latency index will be demonstrated by analyses of unbranched plants only. The same chromosome also carries a QTL for resistance to mycelium extension on capitula in our study, common with that detected by Mestries et al. (1998). No QTL for resistance to *S. sclerotiorum* progression on leaves co-located with any QTL for this character detected by Mestries et al. (1988). No relationships between candidate genes and QTL were detected. The PK-like locus on linkage group 1 found to be associated with the phenotypic variation for percentage attack, latency index and mycelium on leaves by Gentzbittel et al. (1998) using different crosses between sunflower inbred lines, did not reach the significance threshold in the cross presented here. This suggests that sunflower resistance to *S. sclerotiorum* is controlled by several resistance factors which differ according to the genotype tested.

Co-localisation of QTL often reflects the level of correlation between the various traits. Identification of such QTL in the same genome region can result from pleiotropy or linkage. However, the distinction between the two possibilities requires high-resolution maps. There was coincidence in the map position on chromosome 8 for a QTL affecting resistance to *S. sclerotiorum* mycelium on leaves, and another for a leaf QTL for resistance to *D. helianthi*. The correlation between them was significant (Table 2) over different years. A clear physiological relationship between traits such as resistance to the progression of *S. sclerotiorum* or *D. helianthi* on leaves suggests that this QTL could result from the same components in the mechanism of resistance to these two facultative parasites.

Another co-localisation was observed between the QTL for *S. sclerotiorum* ascospore attacks on the capitulum and the *Pl5* locus conferring resistance to *Plasmopara halstedii* on chromosome 6 (Bert et al. 2001). Genetic co-localisation of resistance genes to different pathogens in *Asteraceae* has also been reported in lettuce (Witsenboer et al. 1995). In tomato, a 20-cM area contains specific resistance genes to five different pathogens and a QTL for resistance to *Pseudomonas solanacearum* (Thoquet et al. 1996). Other examples in *Arabidopsis thaliana* (Kunkel et al. 1996) and maize (Mcmullen and Simcox 1995) showed that resistance loci are not randomly distributed throughout the genome but are grouped in particular regions.

The cross XRQ × PSC8 was chosen as polymorphic for resistance to *S. sclerotiorum* capitulum attack as PSC8 is particularly resistant whereas XRQ is quite average in its reaction. This was confirmed by the origin of the alleles linked to resistance for nearly all the QTL for percentage attack and latency index which came from PSC8. For *S. sclerotiorum* mycelium extension on leaves, the two parents were quite similar and each has alleles linked to resistance. For capitulum resistance to mycelium extension, XRQ is more resistant than PSC8 but, again, each provides a marker allele linked to resistance. For phomopsis, both parents are of interest, but with XRQ giving the best levels of resistance to semi-natural attack. However, it was unexpected that the allele linked to resistance for the stable QTL should come from this line since tests on the parental lines (Fig. 2) showed XRQ to be more susceptible than PSC8, and Viguié et al. (2000) found that hybrids made with XRQ did not show good resistance to mycelium extension on leaves, although they were highly resistant to semi-natural attack.

In conclusion, the main aim of this QTL determination was to provide markers that would help to improve the efficiency of resistance breeding programmes in sunflower. As considerable variation between crosses, and for some characters variation between years is observed, validation of the QTL reported here is necessary. This will be undertaken with recombinant inbred lines developed from the F3 reported in this paper. Nevertheless, it should be possible already to exploit the present data. Since, in the present cross, the main QTL for *S. sclerotiorum* resistance appears different from those reported by Mestries (1996) and Gentzbittel et al. (1998), it can be suggested that pyramiding QTL from the different crosses should be possible. For example, as a first step it would be useful to determine whether a combination of the resistances found from PAC2 (Mestries 1996) and PAC1 (Gentzbittel et al. 1998) on linkage group 1 with those from XRQ × PSC8 on linkage groups 5 and 6, would reduce the percentage *S. sclerotiorum* attack on sunflower capitula.

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