QTL for capitulum resistance to *Sclerotinia sclerotiorum* in sunflower

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ABSTRACT

Quantitative trait loci (QTL) for sunflower capitulum resistance to head rot (*Sclerotinia sclerotiorum*) were studied on a population of recombinant inbred lines (RIL), by infection at flowering with suspensions of ascospores. In addition, hybrids made between the RIL and 2 tester lines were observed under natural *Sclerotinia* attack in yield trials and under re-enforced *Sclerotinia* attack in trials made specially to observe disease reaction**.** The inbred lines were genotyped with RFLP and SSR markers which were mapped to give correspondence between different widely published maps and so permit comparison of QTL results in different sunflower populations. Two or three QTL were shown for each character, generally each representing only 8-15% of phenotypic variance. Mostly the same QTL were found for percent infection and latency index as in results for F3 families of the same population or other populations of F3 progenies or RIL. They were also observed in the hybrids, but the latter also showed significant QTL on other linkage groups.

Key words: ascospores – branching– hybrids – natural attack – recombinant inbred lines– tester

INTRODUCTION

Improved knowledge of the inheritance of quantitative characters is most often obtained by studies of QTL, number, proportion of phenotypic variance explained and whether they are the same or different in different genotypes and for characters observed in different environments.

From 1995 to 2000, a recombinant inbred line (RIL) population was developed from 2 inbred sunflower lines, XRQ and PSC8, which not only showed considerable polymorphism for resistance to diseases including *Sclerotinia*, *sclerotiorum*, Phomopsis (*Diaporthe helianthi)* and downy mildew (*Plasmopara halstedii*) and to drought but also gave some of the best yields of varieties registered in France in that period. An AFLP map, anchored to the Cartisol map (Gentzbittel et al, 1995) with RFLP was developed for the F2 generation and permitted mapping of the downy mildew resistance gene *Pl5* (Bert *et al*, 2001) and of QTL for a wide range of agronomic and disease resistance characters of the F3 families (Bert et al, 2002 a and b). To enable more detailed studies, in particular of yield characteristics of hybrids, RIL were obtained, and to develop a map of this cross comparable with other sunflower genetic maps, these lines were genotyped with SSR, but also with RFLP to enable comparison with, and continued use, of QTL and genes mapped previously. This paper reports QTL for resistance to *Sclerotinia* head rot, not only concerning reactions of inbred lines to a resistance test but also for hybrids under natural attack, for which little data has so far been published.

MATERIALS AND METHODS

Sunflower recombinant inbred lines: 279 RIL were obtained by single seed descent from a cross of INRA lines XRQ (bred from a cross of USDA line HA89 and the Russian open pollinated variety Progress) and PSC8 (bred from a populations under recurrent selection for *Sclerotinia* resistance). These RIL were studied *per se* and were crossed with 2 tester lines representing maintainer (F) and restorer (R) sunflower populations, according to their restorer (*Rf1*) and recessive branching (*b1*) gene status, in order to obtain male fertile unbranched hybrids.

Tester F- CMS line PGF650 (Soltis): crossed with branched and unbranched RIL carrying*Rf1*:181 hybrids
Tester R- Branched genic male sterile line 83HR4gms(INRA): crossed with unbranched RIL: 130 hybrids

Measurements of *Sclerotinia* resistance: The RIL were subjected to *Sclerotinia* ascospore infections at flowering (2 replications of 25 plants) following the method described by Tourvieille and Vear (1984). Observations of first symptoms were made twice a week to obtain the mean percentage attack for each

line and a latency index, which is the mean delay from infection to symptom appearance compared with check lines infected at the same time. QTL were calculated for the 2 characters. The hybrids were observed in 2001 and 2002 in trials (2 replications of 50 plants) in a fields devoted to *Sclerotinia* observations, with natural attack re-enforced by provision of sclerotia in the soil and irrigation at flowering to maintain liquid water on the florets for at least 48h for all plants, whatever their flowering date (Vear and Tourvieille, 1982). Observations were made at maturity of percentage attack, compared with check hybrids (trials 01ROPGF, 01RO83HR4, 02ROPGF and 02RO83HR4). In addition, in 2001, observations were made of a significant natural attack on yield trials (2 replications of 100 plants) in Eure et Loir department, near Paris (trials 01RNPGF and 01RN83HR4). The 3 series of data were analysed for QTL, but the mean was studied only for hybrids with tester 83HR4 since not all the hybrids with PGF650 were present in the re-enforced *Sclerotinia* field.

Marker analysis: DNA extraction was performed from young leaves of greenhouse-grown plants using a CTAB method (Rogers and Bendich, 1985). Digestion by restriction enzyme (*Eco*R1 and *Hin*d3) and Southern hybridization were carried out as described by Gentzbittel *et al.* (1999). RFLP probes and candidates genes (anther specific gene SF3, heat shock protein HSP70, Ubiquitin, Calmodulin, protein-kinase-like PK, NBS-LRR type Resistance Gene analogues L3) were chosen from the F2 XRQ × PSC8 linkage map (Bert *et al*., 2002) for their location in order to assign chromosomes according to the Cartisol consensus map (Gentzbittel *et al.*, 1999). Therefore, only 48 RILs were genotyped with these markers. Morphological markers *Pl2* and *Pl5* loci (downy mildew resistance) *Rf1* (male fertility restoration) and *b1* (apical branching) were also added to marker data. A set of 212 SSR markers named 'ORS', 'SSL' and 'SSU', provided by GIE Cartisol (public and available upon request) was genotyped on the RIL population. Microsatellite amplifications and detections were obtained either by standard PCR method or by an M13 tailing scheme. In the first procedure, amplification reactions were performed using a Perkin Elmer 9600 or Biorad thermocycler, in a final volume of 20µl containing 50ng of template DNA, 1x PCR reaction buffer, 1.5mM MgCl2, 0.2mM dNTPs mix, 250nM of each primer, and 0.5 units of *Taq*-DNA polymerase (Qiagen). Amplification conditions consisted of 95°C for 2min, 37 cycles of denaturing at 94°C for 30s, annealing at 54°C for 30s, and elongation 25s at 72°C. Microsatellite pattern were then visualised on polyacrylamide gel by silver-nitrate staining method (Tixier *et al*., 1997). In the second procedure, M13 tailing required adding the M13 forward consensus sequence to the 5' end of each forward primer (Boutin-Ganache *et al*., 2001). Then, the M13-forward primers were used in combination with a 10-fold excess of a fluorescently labelled M13 forward primers. The PCR conditions were in 13µl mixes containing 50ng of genomic DNA, 1x PCR reaction buffer, 1.5mM MgCl2, 0.2mM dNTPs mix, 500nM of fluorescently labelled M13 forward primer, 50nM of M13-tailed forward primer and 500nM of reverse primer and 0.2 units of *Taq*-DNA polymerase (Qiagen). Thermal conditions included 5 min denaturation at 95°C, 30 cycles of 30s at 95°C, 30s at Tm°C and 30s at 72°C, followed by 8 cycles 30s at 95°C, 30s at Tm-4°C and 30s at 72°C, and a final extension of 5min at 72°C. Amplified fragment sizes polymorphisms were detected using fluorescent capillary electrophoresis on an ABI PRISM 3100 Genetic Analyzer. The use of three different dyes (Pham, Hex and Ned) allowed for the pool-plexing of samples during separation and allele sizing.

QTL analysis: The map built for QTL detection included 39 RFLP, 162 SSR, and 4 Mendelian traits (*Pl2, Pl5, Rf1, b1*). It was developed with the CARTHAGENE software (de Givry *et al*.,2005) with the commands [group 0.4 4], then [buildfw 3 3 {} 0] to build a framework for each of the groups identified, and finally [build] to add the remnant markers. It spans over 1666cM, with an average of 12.2 markers per linkage group. QTL detection was performed with the software MCQTL ® (Jourjon et al., 2005) under the "forward" algorithm and with the "iQTL" option (Charcosset *et al*, 2001). The level of significance was determined through 3000 permutations for each trait. As several traits related with resistance to *S. sclerotiorum* were recorded, we used the software BIOMERCATOR ® (Arcade *et al*, 2004) to map the different QTL and to check the hypothesis of a unique QTL associated with different related traits, recorded either on RIL's ("per se" value) or on testcrosses.

RESULTS

Figure 1 presents distributions for the *Sclerotinia* resistance observations. For the RIL, the mean percentage of plants showing *Sclerotinia* symptoms after ascospore infections was 70.8% and the latency index, calculated thus on a mean of 18 plants per plot, was 1.09. Distribution of RIL percent attack was not normal, but since no transformation rendered the data normal, QTL analyses were carried out on the

raw data. Latency index data were distributed normally, and significantly negatively correlated with percent attack (r= -0.674**), so that both characters were considered as representing resistance to *Sclerotinia* head-rot. In 2001, mean attacks in the re-enforced *Sclerotinia* trials were greater for hybrids with 83HR4gms (41%) than for those with PGF650 (25%) whereas in 2002 levels were very similar (83HR4: 52%, PGF650: 55%). In contrast, under 2001 natural attack, 83HR4 hybrids were less attacked (15%) than those made with PGF650 (37%). The data were normal for hybrids with the tester line PGF650, and close to normal for 83HR4gms. For each series of hybrids, the 3 observations were highly significantly correlated (PGF650: r= 0.375, 0.377, 0.378; 83HR4gms: r= 0.372, 0.397, 0.516). In contrast, the results for the 80 RIL crossed with the two tester lines in the yield trial with natural attack trial were not correlated, and in the re-enforced *Sclerotinia* trials, there were only 11 pairs of hybrids with the same RIL. In 2001, their reactions were not correlated, but were significantly in 2002 (r= 0.807**).

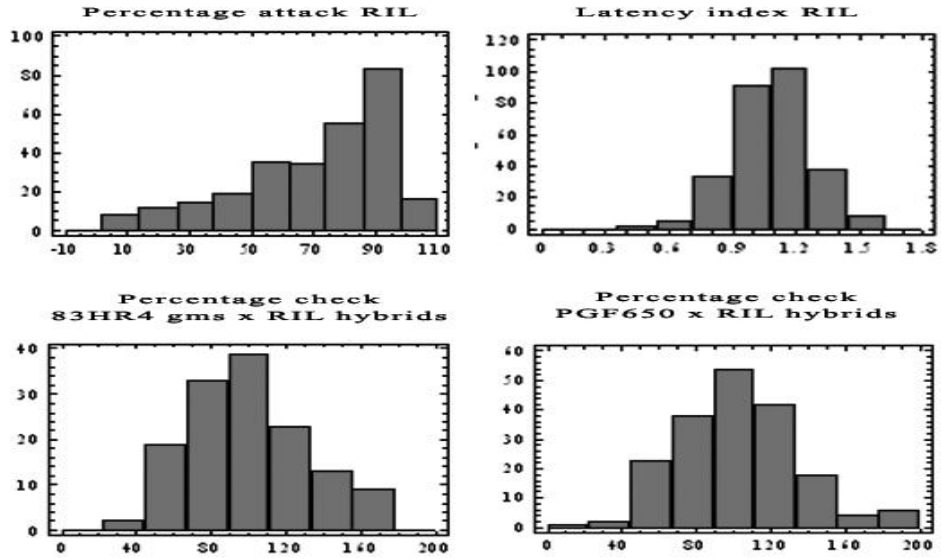

Fig. 1. Distributions of reactions of RIL in *Sclerotinia* ascospore tests and their hybrids under natural capitulum infection.

Table 1 presents correlations between mean data for the hybrids and either all the RIL or only the unbranched lines, for the hybrids made with the tester PGF650 and only for unbranched lines for the 83HR4gms hybrids, since only these were made. Coefficients were weak and similar for hybrids compared with unbranched RIL, but highly significant when both types of RIL are included. For the 3 locations taken separately, for the PGF650 hybrids, the reduced correlation for unbranched RIL was true for both locations in 2001 but not apparent for 2002.

Table 1. Correlations coefficients between results of *Sclerotinia* ascospore tests on RIL and means of re-enforced (2001, 2002) and natural (2001) *Sclerotinia* capitulum attack on hybrids between the RIL and with tester lines 83HR4 and PGF650.

Ascospore test	Percentage attack	Latency index
	Unbranched plants	unbranched plants
Mean percent check of 83HR4 hybrids	0.187*	- 0.177*
Mean percent check of PGF650 hybrids	*0.145ns*	- 0.172*
	all plants	all plants
Mean percent check of PGF650 hybrids	**0.251****	**- 0.309****

Table 2 presents the equivalent of Cartisol (1995 and 1998) and Tang *et al* (2002) linkage groups. Significant QTL for RIL and hybrid data are presented in Figure 2, and their details in Table 3. For each character, one to 3 QTL were identified, explaining from 9 to 29% of phenotypic variation. The parental line PSC8 provided generally the allele with better resistance (lower percent attack, longer latency period). For percentage attack and latency index, the same 2 QTL were shown, on LG 1 and 10 (Tang *et al*. 2002), but they explained only up to 30% of phenotypic variance. Checking with BIOMERCATOR a unique MetaQTL position for the QTL involved on LG10 for Latency Index and for Percent attack on

Table 2. Correspondence between Linkage Groups from Cartisol (Gentzbittel *et al*, 1995 and Mestries *et al*, 1998) and those of Tang *et al*, 2002)

	LG								
Gentzbittel et al (1995)	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
Tang *et al* (2002)	8	14	11	17	6	13	10	9	16
Mestries et al *(1998)*	*G*	*F*	*E*	*D*	*C*	*B*	*A*	*H*	*I*
Gentzbittel *et al* (1995)	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	
Tang *et al* (2002)	2	5	7	1	4	12	3	15	
Mestries et al *(1998)*	*J*	*K*	*L*	*M*	*N*	*O*	*Q*	*P*	

RIL, and for some of the susceptibility indices observed on hybrids, showed a lightly strongest likelihood for one QTL, with an Akaike criterion (AIC value) of 38.53, when compared with two different positions (AIC=38.68). For LG1 and LG2, the same approach produced rather two different positions for RIL and hybrids, but the AIC value bkaike criterion (AIC value) is not very different between the hypothesis "One QTL" versus "Two QTL". QTL on LG16, 5 and LG 11 appear for both two sets of test crosses although with LOD of 2.7 to 3.3.

Table 3. Significant QTL for capitulum resistance to *Sclerotinia* in XRQ x PSC8 RIL and hybrids.

	TRAIT	Linkage Group	LOD	R^2	Local max. position	INF position	SUP position	PSC8 "allele" value
RIL	Latency Index	10	13.90	21%	88	84	92	8.3
		1	6.10	10%	15	3	22	5.5
	Percent Attack	2	5.40	9%	54	23	54	-9.2
		10	7.20	12%	88	82	96	-8.2
		1	4.60	8%	15	0	66	-6.6
Hybrids: % attack compared with checks								
Trials	01RNPGF650	16	3.20	8%	92	78	105	-5.0
		10	2.90	8%	90	75	153	-4.7
	01ROPGF650	10	2.67	12%	71	5	88	-9.0
	02ROPGF650	5	2.70	12%	12	2	38	-5.3
		11	2.74	12%	69	10	86	-5.7
	01RN83HR4	16	3.60	13%	80	48	105	-8.7
		3	2.93	11%	35	9	83	-7.2
		1	2.96	11%	49	23	67	-7.2
	01RO83HR4	5	3.89	14%	19	11	31	-8.1
	02RO83HR4	2	3.04	11%	29	0	54	-5.2
		10	2.76	10%	50	19	86	6.5
	Mean83HR4	5	3.15	12%	19	4	32	-4.7
		2	5.06	29%	29	26	54	-5.7
		16	3.32	13%	69	47	104	-4.9
		11	2.83	11%	67	20	98	-4.5

DISCUSSION

With a mean of 70% attack, and only 2 replications available, the percentage attack from the ascospore tests was not very precise, whereas the latency index, calculated from a mean of 2 replications of 18 diseased plants gave a good idea of the resistance levels of the RIL. For the 2 series of hybrids, the 3 locations of trials were all correlated highly significantly, so it could be expected that they also gave a satisfactory representation of the *Sclerotinia* reaction.

Bert *et al* (2002a), on F3 of the same population demonstrated for ascospore tests, the same QTL on LG 10 and 1 for percent attack, but not that on LG 2 or for latency index on LG1. They reported significant QTL on LG 6, 9 and 13. The LG most frequently shown in this and other populations to carry significant QTL for capitulum resistance is that carrying the branching gene *b1* (Mestries *et al*, 1998, Bert *et al*, 2003, Ronicke *et al*, 2005) and it appeared possible that the morphology of branched inbred lines compared with unbranched lines directly influenced results of the resistance tests. However, the hybrids

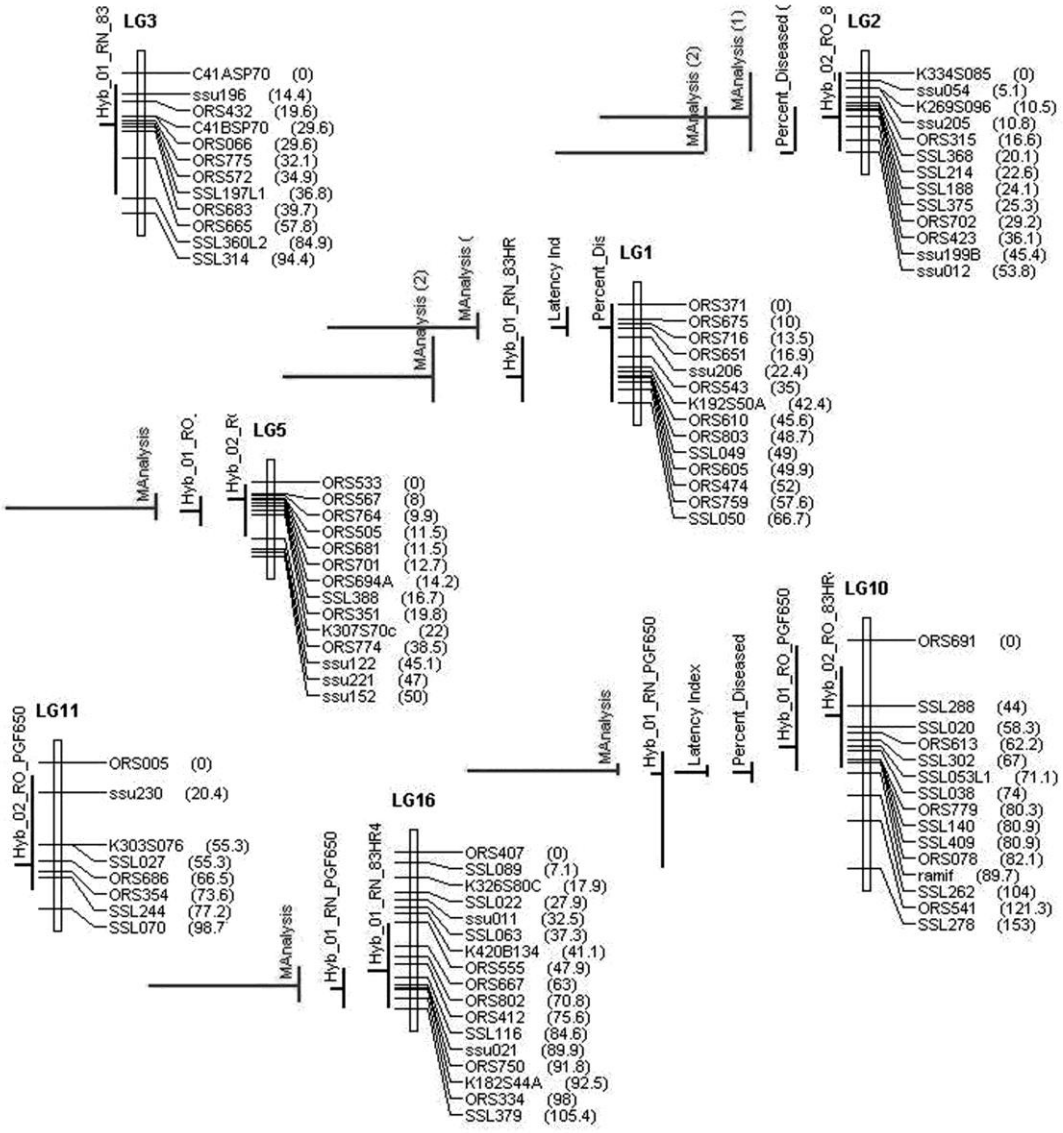

Fig. 2: Linkage groups with QTL and MetaQTL analysis

made between the tester line PGF650 and both branched and unbranched RIL showed closer correlations with results of the ascospore test than the hybrids made only with unbranched RIL and either of the tester lines. The QTL on LG10 carrying the recessive branching gene thus appears more likely to be a genetic linkage than a pleiotropic effect. Jouan *et al* (2000) suggested the same conclusion from tests of *Sclerotinia* mycelium extension on capitula of F3 families of the same population, since in this case PSC8, provided a susceptible allele linked to *b1*.

Many of the QTL were only significant at $p<0.05$ or $p<0.1$ and only a relatively small part of phenotypic variance was explained, as has generally been the case in the past (Bert *et al*, 2002a, Ronicke *et al*, 2005), except for the strong QTL shown by Gentzbittel *et al* (1998), but which appears to be specific to the line PAC1. The XRQ x PSC8 RIL segregated for the PK locus linked with this strong QTL, but showed no linkage with *Sclerotinia* reaction. Overall, the large number of LG which have been shown to carry QTL for *Sclerotinia* resistance are evidence that capitulum resistance to *Sclerotinia* is truly "polygenic", and it may be that, with a large number of small QTL, it is difficult to show significant linkages with genetic markers.

Since the ascospore test represents only part of "natural attack", it could be expected that there would be more QTL for hybrids, although with smaller effects since the tester lines provide half of the genotype. In the present study, QTL on LG 5, 11 and 16 were observed on both series of hybrids but not on the RIL

and it will be interesting to make further studies on them, first to check whether these effects are linked to characters such as height, maturity date or capitulum size or whether they concern some part of host-parasite relations which are not measured by artificial ascospore infections on inbred lines.

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