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ORIGINAL PAPER

B. Pérez-Vich · B. Akhtouch · S. J. Knapp · A. J. Leon ·
L. Velasco · J. M. Fernández-Martínez · S. T. Berry

Quantitative trait loci for broomrape (*Orobanche cumana* Wallr.) resistance in sunflower

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Abstract Broomrape (*Orobanche cumana* Wallr.) is a root parasite of sunflower that is regarded as one of the most important constraints of sunflower production in the Mediterranean region. Breeding for resistance is the most effective method of control. P-96 is a sunflower line which shows dominant resistance to broomrape race E and recessive resistance to the very new race F. The objective of this study was to map and characterize quantitative trait loci (QTL) for resistance to race E and to race F of broomrape in P-96. A population from a cross between P-96 and the susceptible line P-21 was phenotyped for broomrape resistance in four experiments, two for race E and two for race F, by measuring different resistance parameters (resistance or susceptibility, number of broomrape per plant, and proportion of resistant plants per F_3 family). This population was also genotyped with microsatellite and RFLP markers. A linkage map comprising 103 marker loci distributed on 17 linkage groups was developed, and composite interval mapping analyses were performed. In total, five QTL (*or1.1*, *or3.1*, *or7.1* *or13.1* and *or13.2*) for resistance to race E and six QTL (*or1.1*, *or4.1*, *or5.1*, *or13.1*, *or13.2* and *or16.1*) for resistance to race F of broomrape were detected on 7 of the 17 linkage groups. Phenotypic variance for race E resistance was mainly explained by the major QTL *or3.1* associated to the resistance or susceptibility character (R^2=59%), while race F resistance was explained by QTL with a small to moderate effect (R^2 from 15.0% to 38.7%), mainly associated with the number of broomrape per plant. *Or3.1* was race E-specific, while *or1.1*, *or13.1* and *or13.2* of were non-race specific. *Or13.1*, and *or13.2* were stable across the four experiments. *Or3.1*, and *or7.1* were stable over the two race E experiments and *or1.1* and *or5.1* over the two race F experiments. The results from this study suggest that resistance to broomrape in sunflower is controlled by a combination of qualitative, race-specific resistance affecting the presence or absence of broomrape and a quantitative non-race specific resistance affecting their number.

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B. Pérez-Vich (✉) · B. Akhtouch · L. Velasco ·
J. M. Fernández-Martínez
Instituto de Agricultura Sostenible (CSIC),
Apartado 4084, 14080 Córdoba, Spain
e-mail: bperez@cica.es
Tel.: +34-957-499210
Fax: +34-957-499252

S. J. Knapp
Department of Crop and Soil Science,
Oregon State University,
Corvallis, OR 97331, USA

A. J. Leon
Advanta Semillas,
Ruta 226, Km 60, 7620 Balcarce, Buenos Aires, Argentina

S. T. Berry
Advanta Biotechnology Laboratory, SES-Europe,
3300 Tienen, Belgium

Present address:
S. T. Berry, Advanta Seeds UK Ltd.,
Station Road, Docking, King's Lynn, Norfolk, PE31 8LS, UK

Introduction

Sunflower broomrape (*Orobanche cumana* Wallr.) is an obligate, holoparasitic angiosperm that lives attached to the roots of sunflower (*Helianthus annuus* L.), depleting the plant of nutrients and water. Sunflower broomrape is nowadays regarded as one of the most important constraints of sunflower production in countries surrounding the Black Sea, as well as in central Europe, Spain and Israel (Bülbül et al. 1991; Shindrova 1994; Alonso et al. 1996; Domínguez et al. 1996). Attacks are frequently severe and yield losses can reach 50% (Domínguez 1996a). Control of this parasite remains extremely difficult because the thousands of tiny seeds produced by a single broomrape plant can be easily dispersed by wind, water, animals, humans, machinery or soil attached to agricultural products. The seeds may remain viable for 15–20 years and will germinate in the presence of the host plant (Skoric 1988). Several control methods have been

investigated including the use of herbicides (García-Torres et al. 1994, 1995), soil solarization (Sauerborn et al. 1989), crop rotation with pepper as a catch crop (Hershenhorn et al. 1996), modifying sunflower planting times (Aydin and Mutlu 1996) and the use of biological agents (Thomas et al. 1999; Klein and Kroschel 2002; Shabana et al. 2003).

Breeding for resistance is considered the most effective and feasible method of controlling sunflower broomrape. Genetic resistance to broomrape was introduced into sunflower in the early breeding programs in the former USSR (Pustovoit 1966). However, the widespread use of resistant cultivars has led to the appearance of new races of the parasite, which overcome existing resistance genes (Skoric 1988), thus there is a continuous need for new resistance sources. Races A to E of broomrape have been described (reviewed in Alonso 1998), and these can be identified using a set of sunflower differentials, each carrying a single dominant gene (*Or1* through *Or5*, respectively) (Vrânceanu et al. 1980). Although a monogenic and dominant inheritance of resistance to races A to E was found in most genetic studies (Pogorletsky and Geshele 1976; Vrânceanu et al. 1980; Ish-Shalom-Gordon et al. 1993; Sukno et al. 1999), some reports pointed to a more complex inheritance of the trait, including two dominant genes (Domínguez 1996b), one recessive gene (Ramaiah 1987), double recessive epistasis (Kirichenco et al. 1987) or even quantitative inheritance (Pustovoit 1966).

In Spain, broomrape has been traditionally restricted to limited areas cropped with confectionary sunflower. From the early 1970s onward, the parasite quickly spread to central and southern Spain, causing serious infections in oilseed cultivars. The first racial studies in this country identified races overcoming *Or1*, *Or3* and *Or4*, but not *Or2* or *Or5* (reviewed in Melero-Vara et al. 2000). More recent studies have shown the presence of a new race, named race F, which overcomes all the known resistance genes, including *Or2* and *Or5* (Alonso et al. 1996; Domínguez 1999). Resistance to this new race has been found in both cultivated and wild sunflower (Domínguez 1999; Sukno et al. 1999; Fernández-Martínez et al. 2000; Jan et al. 2002). Resistance to race F of broomrape in germplasm derived from cultivated sunflower has been reported to be recessive and controlled by alleles at two loci (Rodríguez-Ojeda et al. 2001; Akhtouch et al. 2002).

The development of broomrape-resistant inbred lines is not an easy task, mainly due to the difficulties in assessing resistance under experimental conditions, where the presence of escapes, genetic background effects, and genotype×environment interactions lead to a sometimes inefficient selection. Therefore, *Orobanche* resistance genes are outstanding targets for molecular breeding (Tang et al. 2003). DNA marker studies for broomrape resistance in sunflower have been focused on the identification of molecular markers linked to the *Or5* gene, which confers resistance to race E of broomrape. Lu et al. (2000), using bulked segregant analysis (BSA), developed a linkage map containing *Or5* flanked 22.5 cM by a distal random amplified polymorphic DNA (RAPD) marker, and from 5.6 cM to 39.4 cM by five proximal DNA sequence characterized amplified regions (SCAR) markers. This linkage group (LG) was integrated with the LG17 of the GIE Cartisol restriction fragment length polymorphism (RFLP) map (Lu et al. 1999). Recently, Tang et al. (2003), also using BSA, placed the *Or5* gene in a telomeric region of LG3 of the public simple sequence repeat (SSR) map of sunflower (Tang et al. 2002), with the closest SSR marker mapping 6.2 cM proximal to the *Or5* locus. To date, no molecular markers linked to genes conferring resistance to the most recent broomrape race F have been identified.

The objective of the present research was to identify and characterize quantitative trait loci (QTL) linked to genes for resistance to races E and F of sunflower broomrape.

Materials and methods

Plant materials and segregating populations

The sunflower lines used to generate the F_2 mapping population were P-96, an inbred line resistant to races E and F of broomrape developed from cultivated sunflower of Yugoslavian origin (Akhtouch et al. 2002) and P-21, a genetic male sterile (*GMS*) line of sunflower, which is highly susceptible to broomrape. P-21 was used as female for crosses with P-96. The F_1 together with both parents were planted in the glasshouse during the spring of 1999. F_2 seeds were produced by self-pollinating the F_1 plants. In the spring of 2000, 113 F_2 plants were sown in the field. About 25% of the F_2 plants were sterile due to the segregation of the GMS gene from P-21. Fertile F_2 plants were self-pollinated to produce F_3 seeds.

Broomrape populations

Two different race E broomrape populations were used in this study: SE-194, collected in southern Spain in 1994 (Sukno et al. 1999), and CU-796, collected in central Spain in 1996 (J.M. Melero-Vara, personal communication). Both SE-194 and CU-796 were classified as race E by artificial inoculation on sunflower differentials carrying the resistance genes *Or1*, *Or4* and *Or5*. The race F broomrape population used in this study was SE-296, collected in southern Spain in 1999 from broomrape plants attacking cultivars which incorporated genes of resistance to race E. SE-296 was confirmed as race F by artificial inoculation on sunflower differentials carrying the resistance genes *Or1* and *Or5* (Akhtouch et al. 2002).

Phenotypic evaluation

Four different experiments were conducted to test the reaction of populations derived from the cross P-21×P-96 to broomrape races E and F:

Experiment 1. Evaluation of 80 F_3 families artificially inoculated with broomrape population SE-194 (race E), conducted in pots in the glasshouse in the winter of 2000/2001.

Experiment 2. Evaluation of 60 F_3 families artificially inoculated with broomrape population CU-796 (race E), conducted in pots in a mesh cage in the spring of 2001.

Experiment 3. Evaluation of 113 F_2 plants artificially inoculated with broomrape population SE-296 (race F), conducted in the field in the spring of 2000.

Experiment 4. Evaluation of 52 F_3 families artificially inoculated with broomrape population SE-296 (race F), conducted in the field in the spring of 2001.

F_3 families consisted of 15 to 30 plants. Plants of the parents as well as F_1 plants from the P-21×P-96 cross were also tested. Additionally, plants of the R-5 line, which carries the resistance gene *Or5* (Sukno et al. 1999), were used as check.

In all the experiments, artificial inoculation was carried out by planting 2-day-old sunflower seedlings in small pots (7×7×8 cm) containing a mixture of sand and peat (1:1, v/v). Each pot (approximately 180 g of the mixture) was carefully mixed with 50 mg of broomrape seeds to obtain a homogeneously infested substrate. The plants were kept in a growth chamber for 15–20 days for incubation at 25°C/18°C (day/night) using a 14-h photoperiod. For experiments in the glasshouse and mesh cage, the plants were then transplanted to larger pots containing 3 l of fertilized and uninfected sand/silt/peat (2:1:1, v/v/v) soil mixture. For the field experiments, the plants were transplanted into a field plot artificially infected with race F of broomrape.

Disease reactions were assessed at physiological maturity by counting the number of emerged broomrape shoots around each sunflower plant. In order to minimize escapes, resistant plants were carefully uprooted to observe any non-emerged broomrape and nodules or stalks. Phenotypic characterization of the F_2 plants was made by classifying them as susceptible (S), when plants showed emerged or underground broomrape, or resistant (R), when they showed no infection (RS trait) and by considering the total number of broomrape per F_2 plant (NBr trait). Phenotypic characterization of F_3 families was made by considering both the average number of broomrape per F_3 family (NBr trait), as well as the proportion of resistant sunflower plants within each F_3 family (PR trait). F_3 families were also classified as segregating (i.e. those with both resistant and susceptible plants, scored as 'H'), susceptible (all plants susceptible, scored as 'A'), or resistant (all plants resistant, scored as 'B').

Molecular data collection

For the molecular markers analyses, three fully expanded leaves were cut from each of the 113 F_2 plants grown in the spring of 2000 and frozen at −70°C. The leaf tissue was then lyophilised and ground to a fine powder in a laboratory mill. RFLP marker analysis was carried out as described by Berry et al. (1995), using the RFLP probes developed and mapped by Berry et al. (1994, 1995, 1996). Microsatellite SSR-marker data collection was performed using primers from various sources including those described by Paniego et al. (2002), Tang et al. (2002) and Yu et al. (2003). PCRs were performed by using 30 μl of reaction mixture containing 1× PCR buffer, 1.5 mM $MgCl_2$, 0.2 mM each of dNTPs, 0.3 μM each of 3′- and 5′-end primers, 0.7 U of Platinum *Taq* DNA polymerase (Invitrogen, Carlsbad, Calif., USA), and 50 ng of genomic DNA. To reduce non-specific amplification, touchdown PCR was used with an initial denaturation at 94°C for 2 min, followed by one cycle at 94°C for 30 s, final annealing temperature (T_A)+10° for 30 s and 72°C for 30 s. The annealing temperature was decreased 1°C per cycle during each of the nine following cycles, at which time the products were amplified for 32 cycles at 94°C for 30 s, T_A for 30 s and 72°C for 30 s with a final extension of 20 min at 72°C. Final annealing temperatures varied from 52°C to 60°C. After the PCR reaction, the amplification products were resolved by electrophoresis on denaturing polyacrylamide gels (4% acrylamide/bisacrylamide, 19:1, 7 M urea in TBE). Bands were visualised using a silver staining kit (Promega, Madison, Wis., USA).

Linkage map construction

Chi-square analyses were carried out on each locus to detect deviations from the expected Mendelian ratios for codominant (1:2:1) or dominant (3:1) markers. RFLP-SSR linkage maps were constructed using the software MAPMAKER/EXP version 3.0b (Whitehead Institute, Cambridge, Mass., USA) (Lander et al. 1987). Two-point analysis was used to identify LGs at an LOD score of 3 and a maximum recombination frequency of 0.40. Three-point and multi-point analyses were used to determine the order and interval distances between the markers in each LG. The Haldane mapping function was used to compute the map distances in centiMorgans from the recombination fractions. The LG nomenclature follows Berry et al. (1997) and Tang et al. (2002). Multiple loci detected by a single probe were coded with the probe name plus the suffix 'A', 'B', or 'C'. Based on clearly visible differences in signal intensity on Southern blots, A was the suffix given to the primary locus (strong hybridisation signal), and B and C to the secondary loci (fainter hybridisation signal). LG maps were drawn using the MapChart software (Voorrips 2002).

QTL analyses

For mapping of QTL and estimation of their effects, the method of composite interval mapping (CIM) (Jansen and Stam 1994; Zeng 1994) was used. Computations were carried out using the software PLABQTL version 1.1 (Utz and Melchinger 1996), which combines interval mapping by the regression approach (Haley and Knott 1992) with the use of selected markers as cofactors. The phenotypic data consisted on trait values for each F_2 plant (tested for race F, broomrape population SE-296) and for each F_3 family (tested for race E, broomrape populations SE-194 and CU-796 and race F, broomrape population SE-296). Trait values for the QTL analysis were: (1) NBr: total number of broomrape per F_2 plant or F_3 family averaged (2) RS: F_2 plants scored as resistant (scale 0 = no broomrape per plant) or susceptible (scale 1 = one or more broomrape per plant), and F_3 families scored as resistant (scale 0 = family with all plants resistant), heterozygous (scale 1 = family with both resistant and susceptible plants) or susceptible (scale 2 = family with all plants susceptible) and (3) PR: proportion of resistant plants for each F_3 family (= number of resistant plants per F_3 family/total number of plants evaluated per F_3 family). Analyses were made initially with the first statement to check the database for errors and outliers. Next, CIM analysis was done with cofactors chosen for each trait by a stepwise regression procedure (*F* to enter: 3.5, *F* to drop: 3.5) with the procedure 'cov select'. Genome-wide threshold values (α=0.05) for declaring the presence of QTL were estimated from 1,000 permutations of each phenotypic trait (Churchill and Doerge 1994; Doerge and Churchill 1996). The threshold of the LOD score was 2.6. Estimates of QTL positions were obtained at the point where the LOD score reaches its maximum in the region under consideration. One-LOD support limits for the position of each QTL were also calculated (Bohn et al. 1996).

The proportion of phenotypic variance explained by each individual QTL was calculated as the square of the partial correlation coefficient (R^2). Estimates of the additive (a_i) and dominance (d_i) effects, as defined by Falconer (1989), for the *i*th putative QTL, the total LOD score, as well as the total proportion of the phenotypic variance explained by all QTL, were obtained by fitting a multiple regression model including all putative QTL for the respective trait simultaneously (Bohn et al. 1996). Following Bohn et al. (1996), the ratio $DR = (|d_i|/|a_i|)$ was used to describe the type of gene action at each QTL: additive for DR<0.2, partial dominance for 0.2≤DR<0.8, dominance for 0.8≤DR<1.2, and overdominance for DR≥1.2. The occurrence of QTL×QTL interactions was tested by adding digenic epistatic effects to the model (add×add, add×dom, dom×add and dom×dom).

Results

Phenotypic segregations

All the plants of the resistant parent P-96 were resistant to all broomrape populations. Similarly, all the plants of the susceptible parent P-21 were totally susceptible to the three broomrape populations (Table 1). Additionally, the differential line R-5 was resistant to the race E broomrape populations SE-194 and CU-796, and susceptible to the race F population SE-296 (Table 1). F_1 plants were resistant to race E populations and susceptible to the race F population (Table 1).

Resistance to race E of broomrape followed a 1:2:1 (R:H:S) ratio for broomrape population CU-726 (18R:32H:10S, χ^2=2.4, P=0.30). The observed segregation ratio for the race E SE-194 population (40R:32H:8S) was significantly different from the expected segregation ratio 1:2:1 (20R:40H:20S). There was a deficiency in the number of both susceptible (−12) and segregating (−8) F_3 families and a corresponding excess of resistant (+20) F_3 families. Resistance to race F fitted a 1:15 (R:S) ratio for the F_2 generation (10R:103S, χ^2=1.30, P=0.25) and a 1:8:7 ratio for the F_3 generation (4R:27H:21S, χ^2=0.35, P=0.84).

The number of broomrape per plant showed continuous distributions for both race E and F populations. For race E, the average number of broomrape per plant was similar with the two broomrape populations used, 0.7 for SE-194 and 0.8 for CU-796 (Table 2). For race F, the average number of broomrape per plant was 13.2 in the F_2 and 8.6 in the F_3 evaluation (Table 2).

The genetic map

The parents of the mapping population were screened for RFLPs using 772 probe-enzyme combinations (193 RFLP probes×*Eco*RI, *Eco*RV, *Hin*dIII and *Dra*I restriction enzyme digests) and for SSRs polymorphisms using 82 primer pairs. Sixty codominant and 19 dominant RFLP, and 35 codominant and 4 dominant SSR-marker loci were polymorphic between P-21 and P-96. SCAR markers and SSR markers closest to the *Or5* gene conferring resistance to race E developed by Lu et al. (2000) and Tang et al. (2003), respectively, were not polymorphic in the P-21×P-96 population.

After the removal of closely linked marker loci (<1 cM), the RFLP-SSR linkage map used for QTL mapping comprised 103 marker loci (66 codominant RFLP + 11 dominant RFLP + 24 codominant SSR + 2 dominant SSR-marker loci). The linkage map spanned a distance of 1,144.4 cM with an average marker interval of 13.3 cM. There were 17 LGs, corresponding to the haploid chromosome number in sunflower. Marker coverage by LG is shown in Table 3. There were a number of regions exceeding 20 cM that were devoid of markers; however, 97.4% of the mapped genome was within 20 cM to the nearest marker. None of the RFLP or SSR-marker loci deviated significantly from the expected segregation ratios (P<0.001).

The codominant phenotypic score of race E for both the SE-194 and the CU-796 broomrape populations mapped on top of LG3. The map distances in centiMorgans are shown in Fig. 1. Both scores mapped distal to the markers of this LG. The closest marker (*ZVG406*) was 4.2 cM downstream from the score of the CU-796 population and 22.8 cM downstream from the score of the SE-194 population (Fig. 1). The map distance in the SE-

Table 1 Segregation of resistance and susceptibility in the parents, F_1, F_2 and F_3 generations of the cross P-21×P-96. The R-5 line, which carries the resistance gene *Or5*, is also included as a check

Generation	Number of F_2 plants or F_3 families[a]											
	Race E						Race F					
	SE-194			CU-796			SE-296					
	Winter-00/01			Spring-01			Spring-00			Spring-01		
	Res	Seg	Sus	Res	Seg	Sus	Res	Seg	Sus	Res	Seg	Sus
P-21	0	-	15	0	-	11	0	-	10	0	-	15
P-96	10	-	0	15	-	0	10	-	0	15	-	0
F_1 (P-21×P-96)	10	-	0	-	-	-	0	-	10	-	-	-
F_2 (P-21×P-96)	-	-	-	-	-	-	10	-	103	-	-	-
F_3 (P-21×P-96)	40	32	8	18	32	10	-	-	-	4	27	21
R-5	10	-	0	7	-	0	0	-	10	0	-	15

[a] *Res* Resistant, *Seg* segregating, *Sus* susceptible

Table 2 Mean±standard deviation (SD) and range for the total number of broomrape per F_2 plant or F_3 family from the cross between P-21 and P-96

Generation	Broomrape race and population	No. of F_2 plants or F_3 families	No. of broomrape	
			Mean±SD	Range
F_2 (P-21×P-96)	Race F (SE-296)	113	13.22±11.93	0.0–47.0
F_3 (P-21×P-96)	Race F (SE-296)	52	8.57±7.72	0.0–28.8
F_3 (P-21×P-96)	Race E (SE-194)	80	0.70±1.31	0.0–5.7
F_3 (P-21×P-96)	Race E (CU-796)	60	0.80±1.14	0.0–4.4

Table 3 Genome coverage offered by the marker set used for quantitative trait loci (QTL) analysis in the P-21×P-96 F_2 population

LG[a]	Number of marker loci[b]		LG coverage (cM)		
	RFLP	SSR	Mean	Largest interval	Total
1	3	2	16.1	29.7	64.5
2	7	0	10.2	19.2	61.1
3	6	0	22.6	38.2	112.9
4	3	2	18.0	34.9	72.0
5	4	0	13.8	25.6	41.4
6	4	0	17.8	48.3[c]	53.3
7	4	3	12.4	41.8	74.2
8	3	4	7.9	24.2	47.5
9	5	1	16.7	60.1[c]	83.3
10	6	3	7.3	15.5	58.4
11	4	0	25.8	39.9	77.4
12	4	1	14.3	33.8	57.3
13	8	3	11.0	30.2	110.2
14	1	5	7.7	19.5	38.7
15	3	0	6.2	9.5	12.3
16	5	2	14.3	36.2	85.8
17	7	0	15.7	37.2	94.0
Total	77	26	14.0	60.1	1144.3

[a] *LG* Linkage group. LG nomenclature is according to Berry et al. (1997)
[b] *RFLP* Restriction fragment length polymorphism, *SSR* simple-sequence repeat
[c] One marker on top of LG6 and one marker on top of LG9 were initially unlinked and they were mapped using the 'near' command of MAPMAKER/EXP by increasing the recombination default

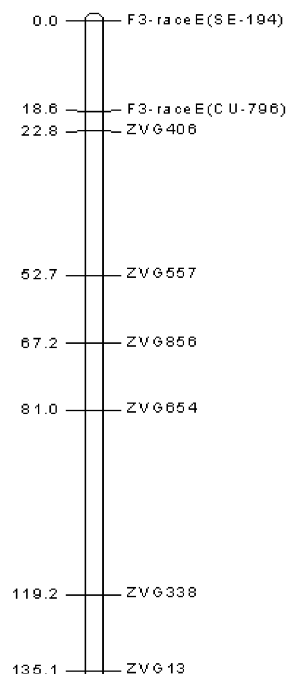

Fig. 1 Molecular map of linkage group 3 (*LG03*) of sunflower containing the codominant phenotypic score for race E of broomrape (populations SE-194 and CU-796) in F_3 families from the cross P-21×P-96. The scores for race E [*F_3-raceE (SE-194)* and *F_3-raceE (CU-796)*] and marker names are listed to the *right* of the map. The cumulative map distances in centiMorgans are shown at the *left* of the map

194 experiment could be upwardly biased by phenotyping errors that inflate map distances (Tang et al. 2003). The phenotypic scores for race F (dominant from the F_2 data and codominant from the F_3 data) were not linked to any of the 17 LGs of the P-21×P-96 map.

QTL analysis

Several QTL associated with resistance to race E and/or race F were identified. The number, magnitude of effect and direction of effect of QTL identified are summarised in Table 4 and Fig. 2. Altogether, five QTL for the three parameters evaluated for resistance to race E of broomrape (NBr, RS and PR) and six QTL for the same three parameters evaluated for resistance to race F of broomrape were detected on 7 of the 17 LGs. Three of these QTL were associated with resistance to both race E and race F.

Race E resistance

One QTL associated with resistance to race E of broomrape (RS trait) was detected on top of LG3 (*or3.1*). This QTL was consistently detected with both race E populations SE-194 and CU-796, in which it explained a phenotypic variance of 37.0% for SE-194 and 59.4% for CU-796.

When the number of broomrapes per plant were considered (NBr trait), two QTL were detected for each of the race E populations (Fig. 2 and Table 4), explaining 15.0% of the total phenotypic variation for SE-194 and 47.5% for CU-796. In both cases, the most significant QTL was *or3.1*, which showed R^2 values of 18.8% in the SE-194 test and 24.5% in the CU-796 test. These R^2 values of *or3.1* were significantly lower than those observed for the RS trait (Table 4). The second QTL detected was located on LG7 (*or7.1*) for SE-194 and on LG13 (*or13.2*) for CU-796. All the resistance-enhancing alleles at these QTL originated from the resistant parent P-96 (Table 4). *Or3.1* exhibited a partially dominant effect for SE-194 and a dominant effect for CU-796 (Table 4). The degree of dominance was 1.47 for *or7.1* (overdominant) and 0.49 for *or13.2* (partially dominant). The test for digenic epistatic interactions between *or3.1* and *or13.2* was significant (P<0.01, additive × additive and dominant × additive) in the CU-796 test. The inclusion of these epistatic effects into the model increased the R^2 to 65.2%, compared to 47.5% for the model without epistasis (Table 4).

Or3.1, as well as *or7.1* and *or13.2*, were also associated with the proportion of resistant plants (PR) in both the SE-194 and CU-796 race E tests (Table 4). Two

Table 4 QTL affecting broomrape race E (SE-194 and CU-796 broomrape populations) and race F (SE-296 broomrape population) resistance in the P-21×P-96 cross

Generation and broomrape race	Trait[a]	QTL	LG	Position (cM)[b]	Support interval (cM)[c]	Left locus[d]	LOD	R^2 (%)	Gene effects[e]		
									a	*d*	|*d*|/|*a*|
F_3-E (SE-194)	RS	*or3.1*	3	0	0–11	*ZVG406*	7.71	37.0	−0.72**	−0.17	0.24
	NBr	*or3.1*	3	0	0–16	*ZVG406*	3.47	18.8	−0.97*	−0.47	0.48
		or7.1	7	54	50–55	*ZVG398*	3.09	16.9	−0.17	0.25	1.47
			Total				2.72	15.0			
	PR	*or3.1*	3	0	0–19	*ZVG406*	2.76	15.2	0.15**	0.04	0.27
		or7.1	7	53	47–55	*ZVG398*	3.47	18.8	0.04	−0.03	0.75
		or13.1	13	15	6–17	*ZVG59*	2.72	15.0	0.07*	0.09*	1.28
		or13.2	13	81	74–101	*ZVG547*	2.91	18.1	0.09**	−0.02	0.22
			Total				7.32	35.5			
F_3-E (CU-796)	RS	*or3.1*	3	0	0–10	*ZVG406*	11.1	59.4	−0.88**	0.03	0.03
	NBr	*or3.1*	3	1	0–21	*ZVG406*	3.54	24.5	−1.37**	−1.47**	1.07
		or13.2	13	81	80–92	*ZVG547*	3.09	25.2	−0.49**	−0.24	0.49
			Total				8.12	47.5			
			Total epistasis				13.3	65.2			
	PR	*or.1.1*	1	39	29–54	*SAD17*	3.32	23.5	0.07	0.12	1.71
		or3.1	3	0	0–12	*ZVG406*	3.01	21.6	0.28**	0.17	0.61
		or7.1	7	55	54–63	*ZVG586*	3.74	26.5	0.12*	−0.07	0.58
		or13.1	13	54	49–63	*ZVG472*	3.44	24.3	0.01	0.09	9.00
		or13.2	13	87	71–96	*ZVG547*	4.31	33.3	0.16**	0.002	0.01
			Total				8.64	59.0			
			Total epistasis				17.2	75.2			
F_2-F (SE-296)	RS	*or1.1*	1	63	46–64	*ZVG1*	2.63	11.3	−0.11*	0.21**	1.91
	NBr	*or1.1*	1	54	39–60	*SAD17*	3.04	11.8	−4.00**	3.00	0.75
		or5.1	5	0	0–4	*ZVG418*	4.62	17.4	4.78**	−1.51	0.32
		or13.1	13	37	34–42	*ZVG524*	4.38	16.6	−5.22**	−2.17	0.42
			Total				7.89	27.9			
			Total epistasis				10.3	34.8			
F_3-F (SE-296)	RS	*or5.1*	5	7	6–26	*ZVG20*	3.00	24.6	0.36**	0.21	0.58
		or16.1	16	54	43–68	*MS771*	3.24	26.3	−0.33**	0.09	0.27
			Total				5.55	40.6			
	NBr	*or1.1*	1	30	27–55	*SAD17*	2.80	23.4	−1.91	−2.47	1.29
		or4.1	4	19	18–31	*MS534*	3.74	29.6	−3.82**	−1.48	0.39
		or13.2	13	84	71–95	*ZVG547*	2.63	25.6	−6.12**	−2.91	0.53
		or16.1	16	47	41–68	*MS711*	3.31	26.7	−5.49**	0.33	0.06
			Total				9.83	60.3			
		or13.1	13[f]	42	25–51	*ZVG524*	2.07	17.7	−1.75	−3.55	2.03
	PR	*or4.1*	4	23	18–38	*MS534*	4.82	36.4	0.18**	−0.01	0.05
		or13.2	13	83	80–93	*ZVG547*	4.46	38.7	0.17**	0.04	0.24
		or16.1	16	57	54–68	*ZVG1023*	4.45	34.2	0.19**	−0.08	0.42
			Total				10.5	62.8			

[a] *RS* Resistance or susceptibility, *NBr* number of broomrape per F_2 plant or F_3 family, *PR* proportion of resistant plants within each F_3 family
[b] Absolute position from the top of the LG
[c] Region flanking each QTL peak in which LOD scores decline by one
[d] Left locus flanking the likelihood peak for a putative QTL. The *MS* prefix denotes SSR marker loci and the *ZVG* prefix denotes RFLP marker loci
[e] *a* Additive effect. For the traits NBr and RS, (–) means a decrease of the trait value due to P-96 alleles. For the trait PR, (+) means an increase of the trait value due to P-96 alleles (an increase in the proportion of resistant plants). *d* Dominant effect. |*d*|/|*a*| = type of gene action at each QTL. *a* and *d* estimates, as well as total R^2 and LOD score values were obtained from a simultaneous fit of all putative QTL using multiple regression
[f] QTL detected below the LOD threshold
Significance levels: **0.01 probability level, *0.05 probability level

additional QTL for the PR trait were detected, the first one (*or13.1*) located on LG13 linked to *or13.2* in both the SE-194 and the CU-796 populations and the second one (*or1.1*) located on LG1, but only detected in the CU-794 test. *Or13.1* had an overdominant effect and explained 15.0% and 24.3% of the phenotypic variance for PR in the SE-194 and the CU-794 tests, respectively (Table 4). *Or1.1* also had an overdominant effect and explained 23.5% of the phenotypic variance for PR in the CU-794 test. Simultaneous fit of the detected QTL for PR explained 35.5% of the phenotypic variation for SE-194 and 59.0% for CU-794. The test for digenic epistatic interactions was also significant (P<0.01, additive × additive) for *or3.1* and *or13.2* in the CU-796 test. The inclusion of the epistatic effects increased the R^2 to 75.2% (Table 4).

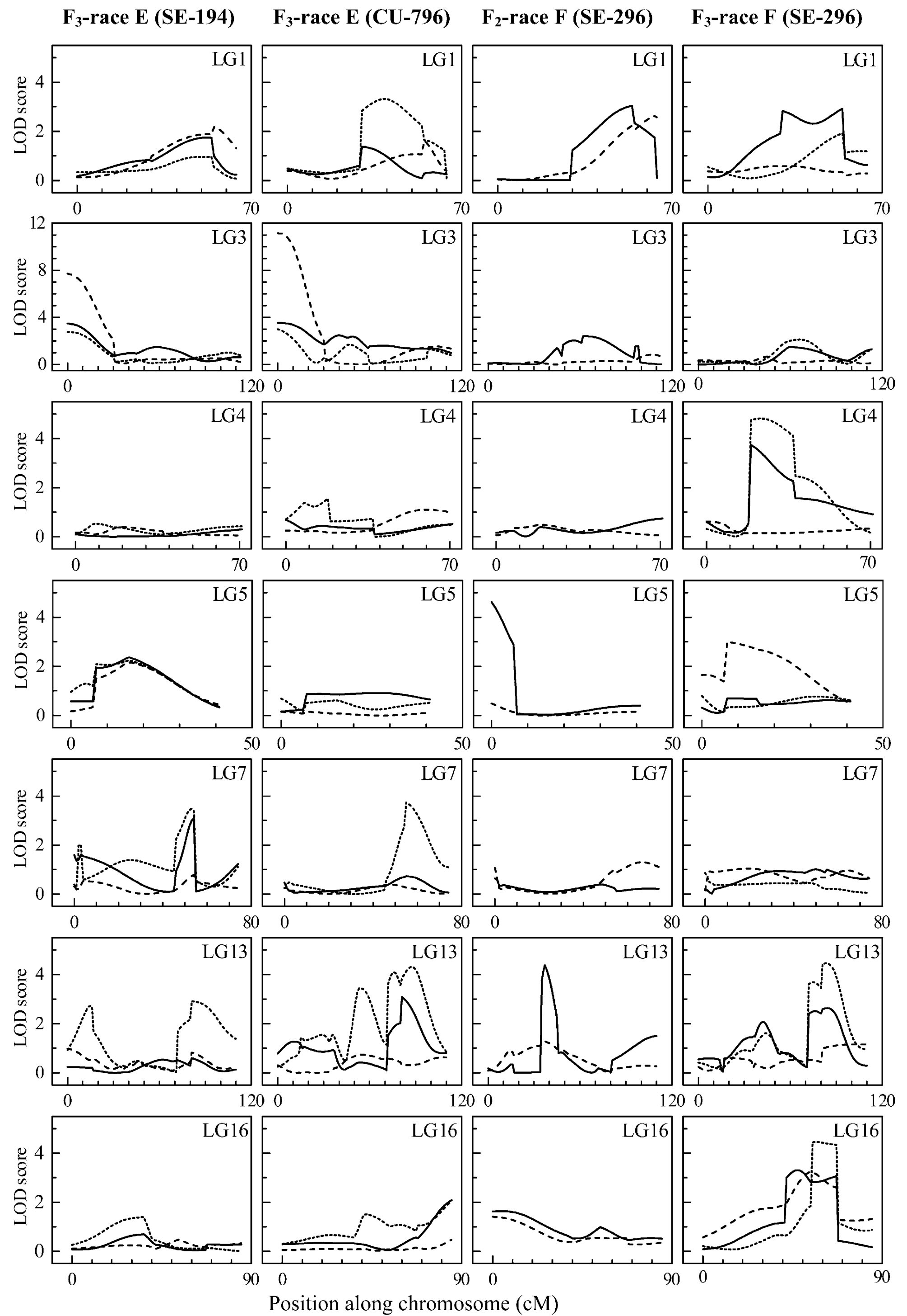

Fig. 2 Quantitative trait loci (QTL) map for broomrape resistance in the P-21×P-96 population for the traits (1) total number of broomrape, NBr (*continuous lines*), (2) resistance or susceptibility, RS (*dashed lines*), and (3) proportion of resistant plants, PR (*dotted lines*). The QTL map generated by CIM shows the likelihood of odds (*LOD*) score on the *y*-axis along each chromosome on the *x*-axis. The axes are independently scaled to accommodate chromosome length and maximum LOD scores. The columns represent results of the different evaluations as follows: *first column* F_3 race E evaluation with broomrape population SE-194, *second column* F_3 race E evaluation with broomrape population CU-796, *third column* F_2 race F evaluation with broomrape population SE-296, and *fourth column* F_3 race F evaluation with broomrape population SE-296

Race F resistance

Three QTL associated with NBr were identified on LG1 (*or1.1*), LG5 (*or5.1*) and LG13 (*or13.1*) in the F_2 evaluation for race F. The most significant QTL were on LG5 (R^2=17.4%) and LG13 (R^2=16.6%). The other QTL showed an R^2 value of 11.8% (Table 4). The three QTL explained 27.9% of the phenotypic variation for NBr. QTL on LG1 and LG13 were attributable to the resistant parent (P-96) alleles, which served to decrease the total number of broomrape (Table 4), whereas those on LG5 was attributable to the susceptible parent (P-21). The degree of dominance ranged from 0.75 to 0.32, indicating that the three QTL were partially dominant. The LOD support limit of 1.0 ranged from 21 cM for *or1.1* to 4 cM for *or5.1* (Table 4). The test for digenic

epistatic interactions was significant ($P<0.05$, additive × dominant) for *or5.1* and *or1.1* (Table 4). Additionally, another significant QTL on LG 13 was detected when the variation associated with *or13.1* was removed in the F_2 test (data not shown). According to its position and effect, this QTL might correspond to *or13.2*.

Four QTL affecting NBr were identified on LG1 (*or1.1*), LG4 (*or4.1*), LG13 (*or13.2*) and LG16 (*or16.1*) in the F_3 evaluation for race F. The four QTL jointly explained 60.3% of the phenotypic variation. Individual R^2 estimates for the four QTL were similar, ranging from 29.6% to 23.4% (Table 4). All the resistant-enhancing alleles originated from the resistant parent P-96. A gene action value of 1.29 was calculated for *or1.1*, which indicated that this QTL might be overdominant in effect. Partially dominant gene action was identified for *or4.1* and *or13.2*, while *or16.1* was additive in effect. The *or13.1* QTL was detected in the F_3 evaluation with an LOD score below the threshold (Table 4).

Only *or1.1* (R^2=11.3%) was detected for the RS trait in the F_2 evaluation for race F resistance (Table 4). In the F_3 evaluation, *or5.1* (R^2=24.6%) and *or16.1* (R^2=26.3%) were associated with the same trait (Table 4). *Or1.1* and *or16.1* were attributable to the resistant parent (P-96) alleles, whereas *or5.1* was attributable to the susceptible parent (P-21).

Three QTL for PR in the F_3 evaluation for race F resistance were located on LG4 (*or4.1*, R^2=36.4%), LG13 (*or13.2*, R^2=38.7%) and LG16 (*or16.1*, R^2=34.2%). The positions of these QTL were similar to those of QTL detected for the total number of broomrape. Increases in the proportion of resistant plants were all due to P-96 alleles (Table 4). The effect of *or4.1* was additive ($|d|/|a| = 0.05$), while a partially dominant gene action was identified for *or13.2* ($|d|/|a| = 0.24$) and *or16.1* ($|d|/|a| = 0.42$).

Discussion

Phenotypic segregation for race E and race F

Phenotypic segregation for broomrape resistance indicated that resistance to race E in the P-96 line is dominant and determined by alleles at one locus, as observed in the CU-796 experiment, whereas resistance to race F in this line is recessive and controlled by alleles at two loci. These results are in agreement with previous reports for race E (Sukno et al. 1999) and for race F (Rodríguez-Ojeda et al. 2001; Aktouch et al. 2002).

The observed segregation ratio in the race E SE-194 experiment was significantly different from that expected for a one-gene segregation. There was a deficiency in the number of both susceptible and segregating F_3 families and a corresponding excess of resistant F_3 families. Previous studies using the same race E broomrape population (SE-194) but infecting a different resistant line (Sukno et al. 1999), or using a different race E broomrape population (CU-796) in the same genetic background (line P-96, this study), indicated a monogenic, dominant inheritance to race E resistance. In addition, none of the RFLP or SSR-marker loci in the P-21×P-96 map deviated significantly from the expected one-gene segregation ratios. In consequence, we concluded that the segregation distortion from a 1:2:1 ratio observed in the SE-194 experiment arose from misclassification of susceptible and segregating F_3 families as segregating and resistant, respectively, caused by some susceptible plants which escaped broomrape infection.

QTL analysis for race E resistance

One major QTL (*or3.1*) affecting race E resistance was detected. This QTL was identified for all the resistance parameters studied, and was stable over the two race E experiments carried out in different environments and with different race E broomrape populations. The dominant gene controlling resistance to race E has been designated *Or5* in previous classical genetic studies in which monogenic inheritance of resistance to race E of broomrape was observed (Vrânceanu et al. 1980; Sukno et al. 1999). This gene was mapped by Lu et al. (2000) and Tang et al. (2003). Tang et al. (2003) placed the *Or5* gene in a telomeric region of LG3 of the public SSR linkage map of sunflower, with the closest SSR marker mapping 6.2 cM proximal to the *Or5* locus. The map position of the QTL *or3.1* detected on top of LG3 corresponds to the region where *Or5* was previously mapped. In fact, the score of race E resistance in both the SE-194 and the CU-796 race E tests mapped distal to the markers in LG3. The closest marker (*ZVG406*) was 4.2 cM downstream from the race E score in the F_3-CU-796 test (Fig. 1). The parental line P-96, which most likely contains the *Or5* gene, contributed the positive allele at *or3.1*. Therefore, we have strong evidence that the major QTL affecting broomrape resistance to race E in the P-21×P-96 population (*or3.1*) represents the known qualitative *Or5* resistance gene.

Three additional QTL affecting resistance to the race E broomrape populations SE-194 and CU-796 were detected on LG7 (*or7.1*) and LG13 (*or13.1* and *or13.2*). These three QTL were associated with the proportion of resistant plants for both race E populations, with the corresponding disease-screening tests being performed in different environmental conditions. This indicated that they are stable across environments. *Or7.1* also affected the total number of broomrape in the SE-194 test, and *or13.2* was associated with the same trait in the CU-796 test. In addition, a fourth QTL, *or1.1*, affecting the proportion of resistant plants was only detected in the CU-796 test. It is evident from these results that there are factors other than *Or5* that influence resistance to race E in the P-96 line.

Whilst the *or3.1* QTL had a major effect on presence or absence of race E broomrape (RS trait), other QTL detected in the present research were associated with the number of broomrape per plant. So far, broomrape resistance to race E in sunflower has been considered a

qualitative trait, as simple inheritance patterns have usually been found with clear resistant and susceptible groups (Sukno et al. 1999; Lu et al. 2000; Tang et al. 2003). Our results suggest that resistance to race E in P-96 is not only the result of a major gene *Or5*, but it is also composed of a quantitative component that influences the number of broomrape per plant. Although the relative weight of both mechanisms of resistance will probably be genotype dependent, the results of the present research revealed that the *Or5* QTL accounted for a greater proportion of the phenotypic variance than the QTL associated with the number of broomrape per plant.

QTL analysis for race F resistance

In contrast to the preponderant role of the *Or5* QTL (*or3.1*) in resistance to race E, no equivalent major QTL was identified for resistance to race F in P-96. The six QTL associated with race F resistance identified in F_2 and/or F_3 evaluations (*or1.1*, *or4.1*, *or5.1*, *or13.1*, *or13.2* and *or16.1*) accounted for similar and moderate percentages of the phenotypic variance for broomrape race F resistance. One possibility is that major QTL do exist, but they were missed due to incomplete genome coverage, or their magnitudes were reduced by increased variation due to environmental effects. However, race F tests were performed in two completely different environments, which gave similar results. In addition, the F_3 score for race F (i.e. considering race F resistance as a major phenotypic locus) failed to map in any of the 17 LGs from the P-21 × P-96 linkage map. Finally, race F resistance was mainly explained by the NBr trait, in contrast to a predominant role of the RS trait in race E resistance. The percentage of the phenotypic variance of individual QTL explained by the RS trait was similar to that explained by the NBr trait for race F, whereas the RS trait gave significantly higher R^2 values for race E resistance. In addition, isolation of the P-96 line for resistance to race F from a germplasm accession required several cycles of selection, which is not consistent with a resistance controlled by a major qualitative locus. It therefore seems likely that race F resistance is controlled by several QTL with a small to moderate effect, despite the existence of a major QTL can not be completely ruled out.

Or1.1, *or5.1*, *or13.1* and *or13.2* were detected in the evaluation of both F_2 and F_3 generations, conducted under different environments, indicating stability across environments. By contrast, *or4.1* and *or16.1* were only detected in one environment. In addition to their environmental stability, o*r1.1*, *or13.1* and *or13.2* were non-race specific, as they were also identified in at least one of the experiments conducted with race E. Such QTL in the same genome region affecting both race E and race F of broomrape may result from linkage or pleiotropy. Linkage would be expected since the majority of plant resistance genes appear to be organized as clusters, conferring resistance to different races of a pathogen (Michelmore and Meyers 1998). On the other hand, the role of pleiotropy should also be investigated. Common QTL affecting resistance to both race E and race F could also result from the same components in the mechanism of resistance to these two races. The existence of QTL determining resistance to different races of a pathogen is well documented, and it has been demonstrated for other parasitic angiosperms-plant interactions such as *Striga gesnerioides* attacking cowpea [*Vigna unguiculata* (L.) Walp.] (Ouédraogo et al. 2001) and a number of plant-pathogens systems (Jeuken and Lindhout 2002; Chen et al. 2003; Udupa and Baum 2003).

Disease resistance in plants can be classified into two major types (Vanderplank 1982). Various terms have been used to describe the two types of resistance, such as vertical versus horizontal resistance, qualitative versus quantitative resistance and complete versus partial resistance. Qualitative or ‘vertical’ resistance is modulated by the interaction between a disease-resistance gene in the host plant and an avirulence gene in the pathogen population (Flor 1971) and is specific to pathogen race. Quantitative or ‘horizontal’ resistance, on the other hand, is associated with numerous genes having smaller effects but presumably acting against a broad spectrum of pathogenic races (Nelson 1972). The results of the present research suggest that broomrape resistance in sunflower is composed of both qualitative and quantitative components. Dominant resistance to race E has a major qualitative component determined by the main race E QTL (*or3.1*), which is associated with presence or absence of broomrape. Conversely, recessive resistance to race F is mainly conferred by QTL that jointly contribute with a similar, small-to-moderate effect in decreasing the number of broomrape. Some of the latter QTL are also associated to the quantitative component of race E resistance. Plant disease resistance to specific pathogens has been reported previously to show both qualitative and quantitative components (Young 1996; Li et al. 2001; Jeuken and Lindhout 2002).

The differences among the resistance QTL detected in this study imply that they might function in different pathways in the sunflower defensive system. The predominant role of *or3.1* in race E resistance, its association mainly with the RS qualitative trait, and its race specificity indicate that it may play a role in an early stage of the plant-pathogen interaction (i.e. pathogen recognition). In contrast, the nature of *or1.1*, *or13.1* and *or13.2* and its lack of race specificity suggest a possible regulatory role in the more downstream mechanisms of the sunflower defensive system. Thus, identifying and mapping candidate genes underlying the QTL associated with broomrape resistance would greatly enhance our understanding of the defence system of sunflower.

In conclusion, this study revealed the complex nature of sunflower resistance to broomrape and demonstrated that identification of QTL involved in this character is possible in cultivated sunflower genotypes. Genetic dissection of broomrape resistance in sunflower provided insight into race-specific resistance and revealed that resistance to broomrape seems to be controlled by a

combination of qualitative race-specific resistance affecting the presence or absence of broomrape and a quantitative, non-race-specific resistance affecting their number. The consistency of the resistance QTL identified in the P-21×P-96 cross will have to be further evaluated over years, locations, new broomrape races, genetic backgrounds, screening conditions and evaluation criteria in order to validate their usefulness for marked assisted selection (MAS). The identification of new resistant loci from other sources is also an objective which is being currently carried out in order to accumulate multiple resistance alleles in a genotype. An additional approach will be to begin identifying candidate genes underlying sunflower broomrape resistance.

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