MAPPING QTLS CONTROLLING SUNFLOWER RESISTANCE TO BROOMRAPE (*OROBANCHE CUMANA*)

Begoña Pérez-Vich, Bassou Akhtouch, Leonardo Velasco, and **Jose M. Fernández-Martínez,** Instituto de Agricultura Sostenible (CSIC), Apartado 4084, E-14080 Córdoba, Spain
E-mail: bperez@cica.es,
E-mail: bassou@cica.es,
E-mail: ia2veval@uco.es,
E-mail: cs9femaj@uco.es

Steven J. Knapp, Department of Crop and Soil Science, Oregon State University, Corvallis, Oregon 97331, USA
E-mail: Steven.J.Knapp@orst.edu

Alberto J. Leon, Advanta Semillas, Ruta 226, Km 60, 7620 Balcarce, Buenos Aires, Argentina
E-mail: alberto.leon@ar.advantaseeds.com

Simon T. Berry, Advanta Biotechnology Laboratory, SES-Europe, B-3300 Tienen, Belgium. *Present address*: Advanta Seeds UK Ltd., Station Road, Docking, UK
E-mail: simon.berry@uk.advantaseeds.com

Abstract

Sunflower resistance to races E and F of broomrape (*Orobanche cumana* Wallr.) was studied using a genomic approach. A genetic linkage map using RFLP and SSR markers was constructed from a sunflower population segregating for both race E and race F resistance. The number, position, and nature of QTLs associated with resistance to these races of broomrape were determined. The use of molecular markers linked to such resistance loci in breeding programs for the development of sunflower resistant cultivars is discussed.

Introduction

Sunflower broomrape (*Orobanche cumana* Wallr.) is an obligate, holoparasitic angiosperm that lives attached to the roots of sunflower (*Helianthus annuus* L.), and it is presently regarded as one of the most important constraints of sunflower production in the Mediterranean area. Breeding for resistance is considered the most effective method of controlling sunflower broomrape. Races A to E of broomrape have been described 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). This monogenic and dominant

inheritance of resistance to races A to E has been found in most genetic studies (Ish-Shalom-Gordon et al., 1993; Sukno et al., 1999). The first racial studies in Spain identified races overcoming *Or1*, *Or3*, and *Or4*, but not *Or2* nor *Or5*. Recent studies have shown the presence of a new race, named race F, which overcomes all the known resistance genes. Resistance to this new race has been found in cultivated sunflower (Fernández-Martínez et al., 2004), and is inherited in a digenic and recessive way in this germplasm (Akhtouch et al., 2002).

DNA marker studies for broomrape resistance in sunflower focused on the identification of molecular markers linked to the *Or5* gene, which confers resistance to race E. Lu et al. (2000) mapped *Or5* on top of LG17 of the Cartisol RFLP map (Lu et al., 1999). Recently, Tang et al. (2003) placed the *Or5* gene in a telomeric region of LG3 of the public simple sequence repeat (SSR) map of sunflower. The objective of the present research was to identify and characterize QTLs linked to genes for resistance to broomrape races E and F in cultivated sunflower germplasm.

Materials and Methods

Plant Materials and Broomrape Populations. The mapping population was obtained from a cross between P-96, an inbred line resistant to races E and F of broomrape (Fernández-Martínez et al., 2004), and P-21, a genetic male sterile line highly susceptible to broomrape. F1, F2, and F3 generations from this cross were obtained. Two different race-E broomrape populations were used: SE-194 (Sukno et al., 1999), and CU-796. The race-F broomrape population used was SE-296 (Akhtouch et al., 2002).

Phenotypic Evaluation, Molecular Data Collection, and Linkage Map Construction. Four different experiments were conducted in this study. Experiment 1 evaluated 80 F3 families artificially inoculated with broomrape population SE-194 (race E), conducted in pots in the greenhouse in winter 2000/2001. Experiment 2 evaluated 60 F3 families artificially inoculated with broomrape population CU-796 (race E), conducted in pots in a mesh-cage in spring 2001. Experiment 3 evaluated 113 F2 plants artificially inoculated with broomrape population SE-296 (race F), conducted in the field in spring 2000. Experiment 4 evaluated 52 F3 families artificially inoculated with broomrape population SE-296 (race F), conducted in the field in spring 2001. Plants of the parents as well as F1 plants were also tested. All the experiments were carried out with broomrape artificial inoculation as described in Akhtouch et al. (2002). Three phenotypic parameters for broomrape resistance were evaluated: R= resistance or S=susceptible; NBr= number of broomrapes per F2 plant or F3 family averaged; and PR= Proportion of resistant plants for each F3 family (=number of resistant plants per F3 family/total number of plants evaluated per F3 family). The molecular data collection and linkage map construction follows Pérez-Vich et al. (2004).

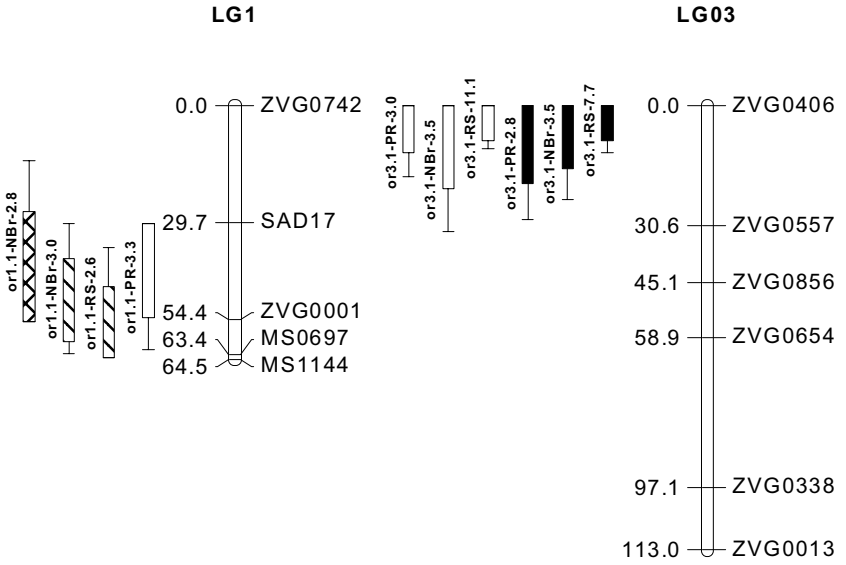
QTL Analyses. For mapping of QTLs we used composite interval mapping (CIM) and the software PLABQTL 1.1 (Utz and Melchinger, 1996). Trait values for the QTL analysis were RS, NBr, and PR. CIM analysis was done with cofactors chosen for each trait by a stepwise regression procedure with the procedure *cov select*. The threshold of the LOD score was 2.6. Estimates of QTL positions, one-LOD support limits for the position of each QTL, the proportion of phenotypic variance explained by each individual QTL (*R2*), and estimates of the additive (*ai*) and dominance (*di*) effects for the *i*th putative QTL were obtained as described in Pérez-Vich et al. (2004).

Results

Phenotypic Segregations. F1 plants from the P-21 x P-96 cross were resistant to both race-E populations and susceptible to the race-F population. Resistance to race E in the F3 followed a 1:2:1 [resistant (R): Segregating (H): susceptible (S)] ratio for broomrape population CU-726 (18R:32H:10S; $\chi 2$=2.4; P=0.30). The observed segregation ratio for the race E SE-194 population (40R:32H:8S) was different from the expected segregation ratio 1:2:1 (20R:40H:20S). Resistance to race F fit a 1:15 (R:S) ratio for the F_2 (10R:103S; $\chi 2$=1.30; P=0.25) and a 1:8:7 ratio for the F3 generation (4R:27H:21S; $\chi 2$=0.35; P=0.84). The number of broomrapes per plant showed continuous distributions for both race E and F populations.

Genetic Map. The parents of the mapping population were screened for RFLPs using 772 probe-enzyme combinations and for SSR polymorphisms using 82 primer pairs. The RFLP-SSR linkage map used for QTL mapping comprised 103 marker loci and spanned a distance of 1144.4 centiMorgans (cM; Haldane) with an average marker interval of 13.3 cM. There were 17 linkage groups. The codominant phenotypic score in F3 families of race E for both the SE-194 and the CU-796 broomrape populations was mapped on top of LG3. The phenotypic scores for race F were not linked to any of the 17 linkage groups.

QTL Analysis. Several QTLs associated with resistance to race E and/or race F were identified. The number and magnitude of effect (LOD values) of QTLs identified are summarised in Figure 1. In total, five QTLs (*or1.1*, *or3.1*, *or7.1 or13.1*, *or13.2*) for resistance to race E, and six QTLs (*or1.1*, *or4.1*, *or5.1*, *or13.1*, *or13.2*, and *or16.1*) for resistance to race F of broomrape were detected on seven of the 17 linkage groups. Phenotypic variance for race



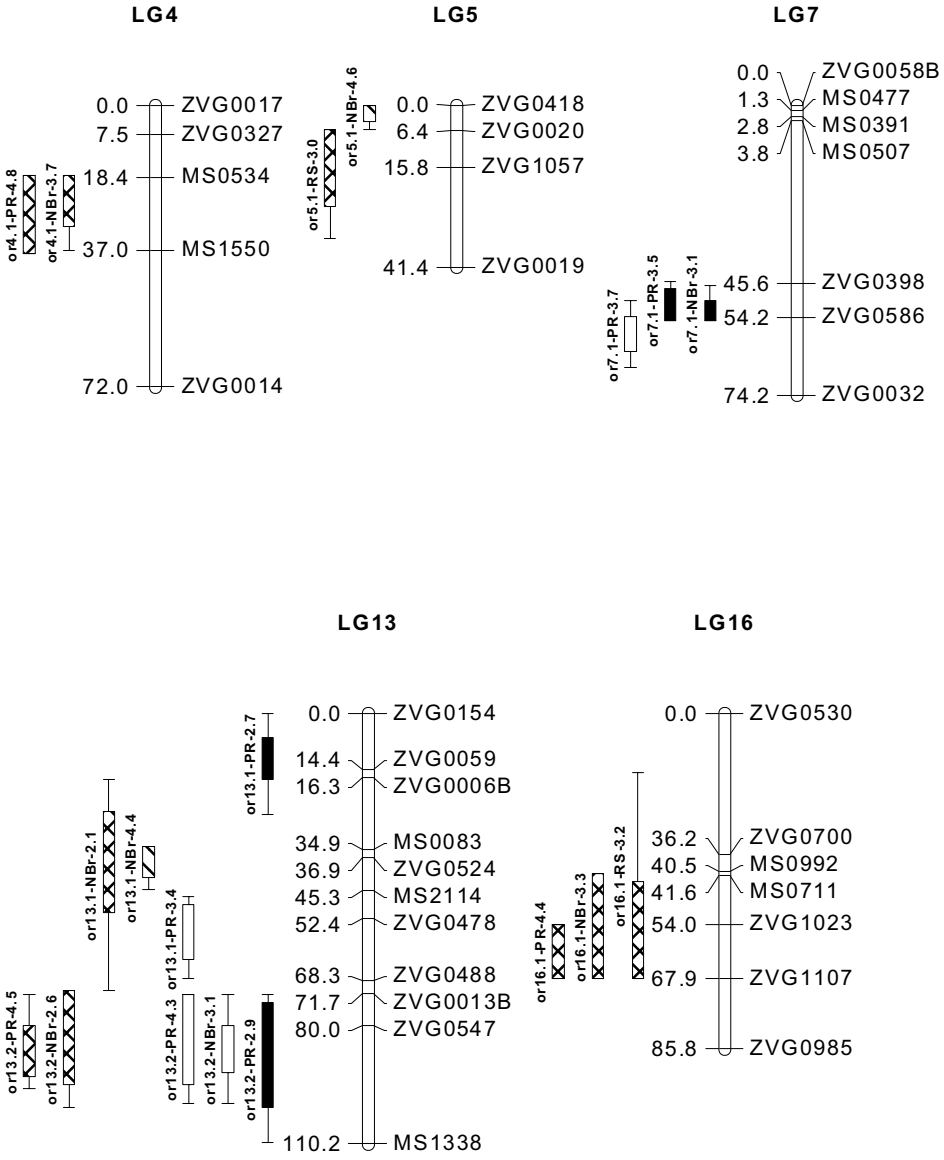


Figure 1. Genomic location for QTLs affecting broomrape resistance in P-21 x P-96. QTLs are indicated at the left of each linkage group (LG) as follows: QTL name-resistance criteria (RS=Resistance or susceptibility; NBr= Number of broomrapes; PR= Proportion of resistant plants)-QTL LOD peak. For each QTL an outer (two LOD) and an inner (one LOD) interval is specified. Four different experiments are indicated through QTL fill style: Black: F3 race E evaluation (SE-194); White: F3 race E evaluation (CU-796); Diagonal bars: F2 race F evaluation (SE-296); Crossed bars: F3 race F evaluation (SE-296).

E resistance was mainly explained by the major QTL *or3.1* on LG3 associated to the resistance or susceptibility character (LOD=11; *R2*=59%), while race F resistance was explained by QTLs with a small to moderate effect (*R2* from 15.0% to 38.7%) mainly associated with the number of broomrapes per plant. *Or3.1* was race E-specific, while *or1.1*, *or13.1* and *or13.2* 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 resistant-enhancing alleles at each QTL came from the resistant parental line P-96, with the exception of the QTL on LG5, *or5.1*.

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 is 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; Akhtouch et al., 2002).

QTL Analysis. The major QTL affecting broomrape resistance to race E in the P-21 x P-96 population (*or3.1*) represents the known qualitative *Or5* resistance gene, since it was mapped at the same position as that previously reported by Lu et al. (2000), and Tang et al. (2003). The results presented also indicate that there are factors other than *Or5* controlling broomrape resistance to race E in the P-96 line. Some of these factors were mainly associated with the number of broomrapes per plant (NBr trait). 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 broomrapes per plant.

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 QTLs associated with race F resistance identified in F2 and/or F3 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. These results suggested that resistance to race F in P-96 is controlled by several QTLs with a small to moderate effect, despite the fact that the existence of a major QTL cannot be completely ruled out.

Or1.1, *or5.1*, *or13.1* and *or13.2* were detected under different environments, indicating stability. In addition, *or1.1*, *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 QTLs in the same genome region affecting both race E and race F of broomrape may result from linkage or pleiotropy. The role of both mechanisms should be investigated. 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* (Willid.) Vatke attacking cowpea [*Vigna unguiculata* (L.) Walp.] (Ouédraogo et al., 2001).

Disease resistance in plants can be classified into two major types. 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 QTLs that jointly contribute with a similar small to moderate effect in decreasing the number of broomrapes. Some of the latter QTLs are also associated to the quantitative component of race E resistance.

The consistency of the resistance QTLs identified in this study 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 marker assisted selection (MAS). The identification of new resistant loci from other sources is also an objective which is currently being carried out in order to accumulate multiple resistance alleles in a genotype.

References

Akhtouch, B., J. Muñoz-Ruz, J. Melero-Vara, J.M. Fernández-Martínez, and J. Domínguez. 2002. Inheritance of resistance to race F of broomrape in sunflower lines of different origins. Plant Breed. 121:266-268.

Fernández-Martínez, J.M, B. Pérez-Vich, B. Akhtouch, L. Velasco, J. Muñoz-Ruz, J. Melero-Vara, and J. Domínguez. 2004. Registration of four sunflower germplasms resistant to race F of broorape. Crop Sci. 44 (in press).

Flor, H.H. 1971. Current status of gene-for-gene concept. Ann. Rev. Phytopathol. 9:275-296.

Ish-Shalom-Gordon, N., R. Jacobsohn, Y. Cohen. 1993. Inheritance of resistance to *Orobanche cumana* in sunflower. Phytopathology. 83:1250-1252.

Lu, Y.H., G. Gagne, B. Grezes-Besset, and P. Blanchard. 1999. Integration of a molecular linkage group containing the broomrape resistance gene *Or5* into an RFLP map in sunflower. Genome. 42:453-456.

Lu, Y.H., J.M. Melero-Vara, J.A. García-Tejada, and P. Blanchard. 2000. Development of SCAR markers linked to the gene *Or5* conferring resistance to broomrape (*Orobanche cumana* Wallr.) in sunflower. Theor. Appl. Genet. 100:625-632.

Nelson, R.R. 1972. Stabilizing racial populations of plant pathogens by use of resistance genes. J. Environ. Qual. 1:220-227.

Ouédraogo, J.T., V. Maheshwari, D.K. Berner, C.A. St-Pierre, F. Belzile, and M.P. Timko. 2001. Identification of AFLP markers linked to resistance of cowpea (*Vigna unguiculata* L.) to parasitism by *Striga gesnerioides*. Theor. Appl. Genet. 102:1029-1036.

Pérez-Vich, B., S.J. Knapp, A.J. Leon, J.M. Fernández-Martínez, and S.T. Berry. 2004. Mapping minor QTL for increased stearic acid content in sunflower seed oil. Mol Breed. (in press).

Rodríguez-Ojeda, M.I., J. Fernández-Escobar, L.C. Alonso. 2001. Sunflower inbred line (KI-374) carrying two recessive genes for resistance against a highly virulent Spanish population of *Orobanche cernua* Loefl. / *O. cumana* Wallr. race "F." *In*: Proc. 7th Intr. Parasitic Weed Symposium, 5-8 June, Nantes, France. p. 208-211.

Sukno, S., J.M. Melero-Vara, J.M. Fernández-Martínez. 1999. Inheritance of resistance to *Orobanche cernua* Loefl. in six sunflower lines. Crop Sci. 39:674-678.

Tang, S., A. Heesacher, V.K. Kishore, A. Fernández, E.S. Sadik, G. Cole, and S.J. Knapp. 2003. Genetic mapping of the *Or5* gene for resistance to *Orobanche* race E in sunflower. Crop Sci. 43:1021-1028.

Utz, H.F., and A.E. Melchinger. 1996. PLABQTL: A program for composite interval mapping of QTL. J. Quant. Trait Loci. 2(1).

Vrânceanu, A.V., V.A. Tudor, F.M. Stoenescu, N. Pirvu. 1980. Virulence groups of *Orobanche cumana* Wallr., differential hosts and resistance source genes in sunflower. *In*: Proc 9th Intr. Sunflower Conf. 8-9 June, Torremolinos, Spain. p. 74-82.