VIRULENCE AND AGGRESSIVENESS OF SUNFLOWER BROOMRAPE (*OROBANCHE CUMANA*) POPULATIONS OVERCOMING THE *OR5* GENE

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Abstract

The inoculation of eight sunflower lines resistant to broomrape with four populations of *O. cumana* (from Central and from Southern Spain, collected in 2000 and 2001) showed that only one of the lines was resistant to the most highly virulent population of broomrape (C-4). In addition, line P96 had full resistance to all the other populations except to C-4, which showed a moderate virulence. In a set of three other inoculation experiments, resistance in line NR5 (with the *Or5* gene) was shown to be overcome by 90% of the broomrape populations tested, including all those collected from Southern Spain. The rest of them were also pathogenic to L86 and P96. This suggests the occurrence of populations in Central Spain that could not be ascribed to race F to which line P96 is resistant, but to a new race G.

Introduction

Attacks of broomrape (*Orobanche cumana* Wallr.) on confectionery sunflower were first observed in Spain in the middle of the past century. Oilseed cultivars were introduced into the country in the 1960s, but since they carried the gene of resistance to race A of the parasite (*Or1*) (Pustovoit, 1966) the disease was not observed on them for more than one decade. The overcoming of this resistance occurred in the 1970s and the occurrence of races A, B and C of *O. cumana* was reported (González-Torres et al., 1982). Single major genes (*Or1* to *Or5*) have been reported to control races A to E of *O. cumana*, each of them conferring resistance to the previously described races in Romania (Vrânceanu et al., 1986). Later studies showed that a wide range of races were present in Spain (Melero-Vara et al., 1989). Also, differences of virulence on resistant lines of sunflower used as race differentials as compared to those referred in Romania (Vrânceanu et al., 1986) were observed (Melero-Vara et al., 1989). However, Record (*Or3*) was susceptible to populations from Spain, while Jdanov-8281 (*Or2*) and P-1380-2 (*Or5*) were resistant to populations sampled between 1989 and 1991 (Saavedra et al., 1994).

The use of resistant sunflower lines carrying the *Or5* gene has become a usual practice in all the areas of the country where broomrape threatens the production (Melero-Vara et al., 2000). The inefficiency of genes of resistance *Or2* and *Or5* began to be observed in the 1990s and the disease on resistant hybrids became very common during the last few years. The frequent appearance of more virulent populations of *O. cumana* causing disease on resistant hybrids triggered the evaluation of broomrape populations overcoming the *Or5* gene. The reaction of several resistant genotypes of sunflower was also tested.

Materials and Methods

Reaction of Resistant Genotypes to Virulent Populations of Broomrape. Four populations of *O. cumana* collected in 2000 and 2001 in Central and Southern Spain (C-2 and C-4, and S-4 and S-18, respectively) were inoculated on eight sunflower lines carrying different sources of resistance to the disease in the spring of 2002. Lines 2 to 5 were kindly provided by Dr. José M. Fernández-Martínez (Institute of Sustainable Agriculture, C.S.I.C., Córdoba, Spain). For every population except for C-4, which was not inoculated onto line 4, seven plants of each of the genotypes were tested. Two-day old sunflower seedlings were transplanted into small pots with 250 g of soil mixture (sand:silt, 1:1, v) uniformly infested with 25 mg of broomrape seed (Sukno et al., 2001) and grown for two weeks under controlled conditions of 25C and 14 h photoperiod. Plants were then transplanted into 2L pots and kept in the greenhouse until the end of the experiment. The first observation of emerged broomrapes was made on six-week old plants, and three additional evaluations were made every two weeks. Thereafter, plants were uprooted and the number of broomrapes attached to the roots was recorded. The experiment had a completely randomized factorial design. The degree of broomrape attack (DBA) was calculated from the number of emerged broomrapes in each plant up to a maximum of 30 broomrapes. Analyses of variance were performed with the values of the area under the disease progress curve (AUDPC) obtained from the accumulated number of emerged broomrapes. Least Significant Difference (LSD) (p=0.05) tests and orthogonal contrasts were used for the comparisons of DBA.

Virulence of Broomrape Populations Overcoming the Or5 Gene. The evaluation of 20 populations of *O. cumana* collected in Central and Southern Spain (C-1 to C-3 and S-1 to S-17, respectively) from 1997 to 2003 was conducted in three experiments (I to III). Broomrape populations C-1 to C-3 were collected in Central Spain, while S-1 to S-17 populations were sampled in the South. Experiments I and II were carried out in a shade house during spring-summer of 2003. Plants of experiment III were grown under greenhouse conditions from September to December, 2003. Genotype NR5 (*Or5* gene) was used as a differential susceptible to race F. In addition, all the populations were inoculated onto genotypes L86 and P96, both of them resistant to race F of broomrape. For every population, eight to ten plants (replications) of each of the genotypes were artificially inoculated with broomrape seed and grown as previously described. The three experiments had a completely randomized factorial design. Disease incidence (DI) was expressed as the percentage of plants with emerged broomrapes, and DBA was recorded at weekly intervals until senescence of the sunflower plants. Analyses of variance of the AUDPC were performed and LSD (p=0.05) tests and orthogonal contrasts were used for the comparisons of DBA.

Results

Reaction of Resistant Genotypes to Virulent Populations of Broomrape. Lines 2 to 5 and P96 were resistant to populations C-2, S-4 and S-18, with mean DBA values from 0 to 1.1 broomrapes/plant for P96 and line 5, respectively. Also, NR5 was resistant (DBA=0) to the C-2 population. Similar results were obtained from the analyses of the four broomrape populations on seven of the genotypes except for population C-4, which showed a higher virulence. Thus, line 3 was the only genotype resistant to population C-4 (DBA=0), while the rest of the genotypes had final DBA values between 7.4 (P96) and 30 (NR5 and line 1) (Figure 1).

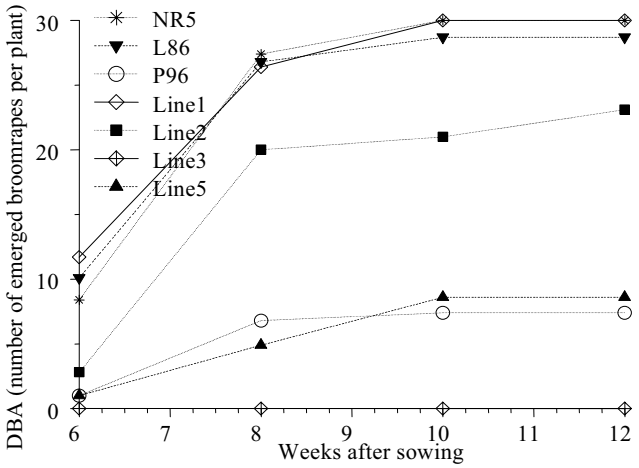

Fig. 1. Virulence of broomrape population C-4 on seven genotypes of sunflower with resistance to the parasite expressed as Degree of Broomrape Attack (DBA).

Virulence of Broomrape Populations Overcoming the Or5 Gene. Eighteen out of the 20 broomrape populations tested were shown to be highly virulent on NR5, DBA ranging from 10 to 38, 22 to 33, and 15 to 38 at the end of experiments I to III respectively. Figure 2 shows the aggressiveness of 10 of these populations on NR5. Populations C-1 and C-2 did not cause disease on NR5. This reaction was confirmed for population C-1 in experiments II and III, whereas both populations were able to infect L86 (DI of 83 and 85% respectively). This genotype had a DBA of 8 broomrapes/plant when inoculated with C-2, while a mean of 6.4 broomrapes/plant were observed when C-1 was used. Another broomrape population, C-3, infected not only NR5 but also L86 and P96. The reaction, observed in experiment I, was confirmed in exp. III. Mean DI of 69.5 and 65% were observed in L86 and P96 respectively. Values of DBA reached 5.4 and 3.6 for genotypes L86 and P96 respectively.

Discussion

Genotypes P96 and line 3 resulted in a more effective resistance to broomrape than the rest of the sources tested. The origin of the resistance in line 3 in unknown, but P96 seems to carry a major gene and also some minor genes responsible for quantitative resistance and whose effect could be added to the qualitative race-specific resistance as recently suggested by Pérez-Vich et al. (2004). In this sense, resistance to race F of *O. cumana* has been reported to be recessive and controlled by alleles at two loci in germplasm derived from cultivated sunflower (Akhtouch et al., 2002).

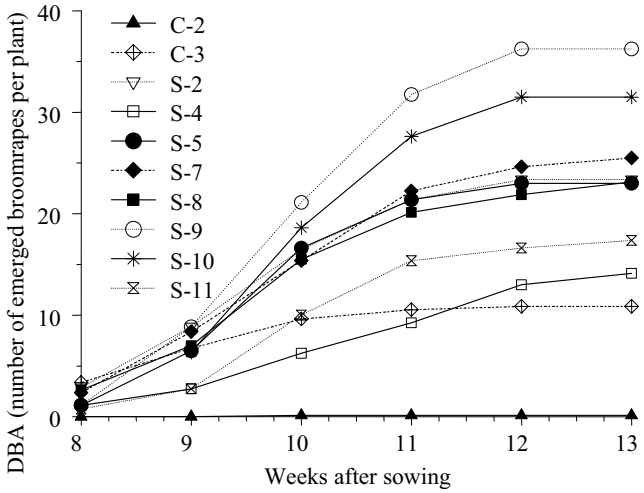

Fig. 2. Agressivenness of ten broomrape populations from different geographical origin on genotype NR5 of sunflower, expressed as Degree of Broomrape Attack (DBA). Experiment I.

The effectiveness of the resistance of P96 was also observed with most broomrape populations tested in experiments I to III. Nevertheless, the results of these three experiments also pointed out that some populations of broomrape are virulent even on P96. This observation is the first report of the existence in the parasite of virulence higher than F that could be tentatively termed race G. The virulence on P96 was observed on a broomrape population from Central Spain. The present paper shows consistent information that pathogenic characteristics of populations of *O. cumana* from this area differ from those of populations from the South. Populations from Central Spain are virulent on NR5 but their aggressiveness is lower than that of populations from the South. On the contrary, broomrape populations from Central Spain show a higher aggressiveness on L86 than those from the South. Therefore, an exhaustive prospecting of sunflower broomrape populations in areas from Central Spain would allow a better knowledge of the frequency of populations of race G in the area. On the other hand, molecular studies of different populations of broomrape are in progress and will probably help us better understand the pathogenic diversity of *O. cumana*.

Acknowledgements

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References

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

González-Torres, R., Jiménez-Díaz, R.M. and Melero-Vara, J.M. 1982. Distribution and virulence of *Orobanche cernua* in sunflower crops in Spain. Phytopath. Z. 104:8-89.

Melero-Vara, J.M., Domínguez, J. and J.M. Fernández-Martínez. 1989. Evaluation of differential lines and a collection of sunflower parental lines for resistance to broomrape (*Orobanche cernua*) in Spain. Plant Breeding. 102:322-326.

Melero-Vara, J.M., Domínguez, J. and J.M. Fernández-Martínez. 2000. Update on sunflower broomrape situation in Spain: racial status and sunflower breeding for resistance. Helia. 23(33):45-56.

Pérez-Vich, B., Akhtouch, B., Knapp, S.J., Leon, A.J., Velasco, L., Fernández-Martínez, J.M. and S.T. Berry. 2004. Quantitative trait loci for broomrape (*Orobanche cumana* Wallr.) resistance in sunflower. Theor. Appl. Genet. (Published online 13 Feb 2004, DOI 10.1007/S00122-004-1599-7).

Pustovoit, V.S. 1966. Selection, seed culture and some agrotechnical problems of sunflower. Translated from Russian in 1976 by Indian National Scientific Documentation Centre, Delhi, India.

Saavedra del Río, M., Fernández-Martínez, J.M. and Melero-Vara, J.M. 1994. Virulence of populations of *Orobanche cernua* Loefl. attacking sunflower in Spain. In: H. Pieterse, J.A.C. Verkleij and S.J. Ter Borg (eds.). Biology and Management of *Orobanche*. Proc. 3rd Intr.. Workshop on *Orobanche* and related *Striga* Research. Amsterdam, The Netherlands, Royal Tropical Institute, Amsterdam, The Netherlands. p. 139-141.

Sukno, S., Fernández-Martínez, J.M. and J.M. Melero-Vara. 2001. Temperature effects on the disease reactions of sunflower to infection by *Orobanche cumana*. Plant Dis. 85:553-556.

Vrânceanu, A.V., Pirvu, N., Stoenescu, F.M. and M. Pacureanu. 1986. Some aspects of the interaction *Helianthus annuus* L./*Orobanche cumana* Wallr. and its implications in sunflower breeding. In: Proceedings of a Workshop on Biology and Control of Orobanche. S.J. Ter Borg (ed.) LH/VPO, Wageningen, The Netherlands. p. 181-189.