

The inheritance of resistance to *Orobanche cumana* in sunflower.

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Summary:

Broomrape (*Orobanche cumana* Wallr.) parasites sunflower roots and causes economic losses in several European countries. The parasite exists as physiological races but cultivated and wild sunflowers show resistance. The genetics of resistance are not yet clear and some results reported are contradictory. This study presents analysis of reaction to three broomrape isolates before localisation of the resistance genes involved. The F3 progenies from two crosses were tested with, in one case the weakly virulent Spanish isolate « Pipa Blanca » and in the other case, both a highly virulent Spanish isolate « Mencia » and a modern Bulgarian isolate « Bul 1 ». Resistance to the weakly virulent isolate appears to be controlled by a single gene. For the other resistance source, the same may be true for the proportion of entirely susceptible progenies which agrees with this hypothesis, but the proportion of entirely resistant progenies is much smaller than that expected. The possible explanations for these results are discussed. The reaction of more than half the F3 progenies differed for the two broomrape isolates, so there is no evidence that these resistances are linked.

Résumé :

L'orobanche (*Orobanche cumana* Wallr.), parasite des racines du tournesol (*Helianthus annuus* L.) et originaire du pourtour méditerranéen, entraîne d'importants dégâts dans certains pays producteurs. Il existe des races physiologiques d'orobanche et des résistances naturelles chez le tournesol. La génétique de la résistance à l'orobanche chez le tournesol n'est pas encore bien comprise et les résultats obtenus sont parfois contradictoires. L'objectif de ce travail a été d'étudier la génétique de la résistance à trois populations d'orobanche, avant de localiser les gènes de résistance sur la carte génétique du tournesol. Les familles F3 de deux croisements ont été testées pour l'un par une population espagnole peu virulente « Pipa Blanca », pour l'autre par une population espagnole très virulente « Mencia » et par une population bulgare « Bul 1 ». La résistance à la population espagnole la moins virulente semble contrôlée par un gène de résistance. Il pourrait de même pour les résistances aux deux autres populations mais, si la proportion de familles F3 entièrement sensibles correspond à cette hypothèse, la proportion de familles entièrement résistantes est largement en dessous de celle attendue. Les raisons possibles pour ces observations sont discutées. Les réactions aux deux isolats de plus de la moitié de familles F3 sont différentes, ainsi, il n'y a pas de raison de penser que les gènes impliqués dans les deux résistances soient liés.

Introduction

Broomrape (*Orobanche cumana*) is an important parasite of sunflower. The genus is present in 16 million hectares in Mediterranean regions and Southwest Asia, and the most important species, *O. cumana*, has become a limiting factor for the crop in Eastern Europe and in Spain. In central and Southern Spain, at least 350 000 ha of sunflowers can be infected (Casteljon-Munoz *et al.*, 1991). This species shows physiological races, which, in Rumania were denoted A, B, C, D and E (Vranceanu *et al.*, 1986).

Broomrape is difficult to control because it produces a very large number of seed which remain viable in the soil for many years. However, there exists genetical resistance within both cultivated sunflowers and wild species of the genus *Helianthus* (Sukno *et al.*, 1998) and this resistance is at present the best method to control the disease. Many resistance sources have been identified but the genetics of resistance are still not well understood. The first studies by Pustovoit (1966) suggested that resistance was quantitative, but other authors concluded that it was monogenic dominant (Ish-Shalom-Gordon *et al.*, 1993, Sukno *et al.*; 1999), monogenic recessive (Ramaiah, 1987) or controlled by two independent dominant genes (Dominguez, 1996). The resistance to the 5 races reported in Rumania, were found by Vranceanu *et al.* (1986) to be controlled by dominant genes, called *Or*: *Or1* giving resistance to race A, *Or2* resistance to races A and B, *Or3* to races A, B and C, *Or4* to A, B C and D and *Or5* to all five races. It was suggested that these genes are independent.

The equivalence of Spanish races and those reported in Rumania has not been established with certainty and in Bulgaria there appear to be mixtures of races which may or may not be the same as the Rumanian races. A new race was reported in Spain in 1996 by Alonso *et al.*, to which *Or5* did not give resistance. Therefore, it appeared essential to determine the heredity of resistance to races at present important, in order for breeding programmes to use the best genes. The objective of this study was to analyse the segregation of F₂ progenies from two crosses, infected with 3 broomrape isolates that had been characterised by molecular studies (Gagne *et al.*, 1998), to determine the number of genes involved and to prepare for localisation of these genes on a genetic map of sunflower.

Materials and Methods

Sunflower genotypes:

The two crosses were made between an entirely susceptible inbred from IWS, Bulgaria, BG2256 and firstly an INRA line FN, bred from the Russian population Peredovik, resistant to some races of *O. cumana* and secondly a line from Rustica Prograin Génétique, ORB3, resistant to most of the Spanish and Bulgarian isolates. The F₂ and then F₃ progenies were obtained by selfing. For each F₃ family, about 20 plants were tested.

O. cumana isolates:

The F₃ progenies FN was tested with the Spanish race known as “Pipa Blanca » and the other cross with two isolates, “Mencia” from Spain, which has similar virulence to the race reported by Alonso *et al.* (1996), which has similar virulence to the newmay and isolate “BUL1” from Bulgaria (Gagne *et al.*, 1998).

Resistance tests:

These were according to the method described by Lu *et al.*, 1999, under controlled conditions in the greenhouse. Sunflower seeds were sown in tubes containing a mixture of compost and broomrape seeds. Growth conditions were 23°C±2° by day and 20°C±2° at night. Photoperiod was 16h/24h. Susceptible controls were infected at each test. Observations were made 4 to 5 weeks after sowing, of the presence or absence of tubercles on roots. Five classes were distinguished for the cross BG2256 x FN but only 4 for the cross BG2256 x ORB3: 1 no tubercles; 2 and 3 some tubercles; 4 and /or 5: many tubercles, symptoms equivalent to those on the susceptible controls.

Results

The two F1 progenies were tested previously and BG2256 x FN was reported as susceptible, although with no details of the numbers of broomrape tubercles which appeared. This result suggests that the resistance of FN was recessive. The BG2256 x ORB3 cross was only tested with the Mencia isolate, and in this case also, resistance was not complete, only 3 of 14 plants tested developed no tubercles. In the tests of the F3 progeny, the susceptible line was infected as a control for each test. In the two series of tests with «Pipa Blanca», the susceptible controls showed 73% and 80% of plants with broomrape symptoms. Thus, the numbers of resistant plants in the segregating generation were, if anything overestimated. For both the tests with the second cross, the susceptible control was 100% infected.

Table 1. Segregations of reaction to broomrape of F3 progenies from two crosses between sunflower lines, using 3 isolates: numbers of F3 progenies showing different classes of symptoms (class 1: resistant, classes 2/3: some symptoms, classes 4/5: very susceptible).

Cross	Broomrape isolate	Class 1 only	Class 1+2+3	Class 1+2+3+4/5	Class 2+3+4/5	Total
BG2256 x FN	Pipa Blanca	31	0	74	34	139
BG2256 x ORB3	Bul 1	2	13	69	33	117
BG2256 x ORB3	Mencia	0	9	71	39	119

Table 1 presents the results of the broomrape tests on the F3 progenies. The progenies showing plants only in class 1 can be classified as resistant without any doubt, whereas all the other families are more difficult. For the cross BG2256 x FN, the 9 progenies with only one plant, out of 20, not in class 1, were considered as resistant. The other progenies either showed plants in classes 2-5 or plants in all classes, so it was considered most reasonable to consider the former as entirely susceptible, since all F3 plants showed some nodules, and the latter as segregating since some plants in each progeny appeared to be resistant. This classification gave a segregation of 31R: 74 segregating: 34 susceptible, which agrees with a theoretical segregation of one gene: 1R: 2Seg: 1S. However, it was not possible to conclude

about the dominance or recessiveness of this resistance gene, the susceptible F1 had suggested that it was recessive, but of the segregating F3 progenies, 35 showed more resistant (class 1) than susceptible (classes 2+3+4+5), 4 progenies had equal numbers of plants in each category, and 35 more susceptible than recessive.

The tests on the cross BG2256 x ORB3 were carried out independently, in Bulgaria and in Spain. In both cases, the numbers of F3 progeny which showed no plants with symptoms was very small, 2 with isolate Bul 1 and none with Mencia. The same groups of reactions as for the FN cross were made, accepting that one plant out of 20 could be badly classed. The numbers of F3 progeny with no resistant plants was 33/117 for Bul 1 and 39/119 for Mencia. These proportions are not significantly different from $\frac{1}{4}$, which would be expected from the segregation of a single gene. However, in both case, the numbers of progenies which can perhaps be classed as resistant (no, or only 1 plant, in class 4) were much less than $\frac{1}{4}$ (15/117 and 9/119 respectively). Thus, these segregations do not agree with 1:2:1 segregations for a single gene (X^2 9.30 and 19.57 for Bul 1 and Mencia respectively, limit for $P < 0.05$: 5.99) and only just, for Mencia only, with a 1R: 8Seg: 7S as would be expected for two complementary genes, whether dominant or recessive (X^2 : 15.65 and 5.82 respectively). Concerning dominance, the two isolates gave rather different results: with Bul 1, 20 segregating F3 progenies showed more resistant than susceptible plants, 4 had the same numbers and 45 more susceptible than resistant, whereas with Mencia, no segregating progenies showed more resistant than susceptible, 2 the same numbers and the other 69, more susceptible than resistant. Nevertheless, both series of results lead to the conclusion that resistance, as it is read in the seedling tests, is recessive.

Comparison of the results of the two tests shows that, for the 117 families tested with both isolates, only 55 showed the same reactions (3 resistant, 40 segregating and 12 susceptible). The other progenies showed differences in reactions, with similar numbers for each type of recombinant (6 R Mencia - seg. Bul 1, 9 seg. Mencia - R Bul 1; 21 seg. Mencia - S Bul 1, 24 S Mencia - seg. Bul 1; 0 R Mencia - S Bul 1, 2 S. Mencia - R Bul 1). Thus, with more than 50% recombination, there is no evidence that the resistances to Mencia and Bul 1 are linked.

Discussion

These analyses of segregation of broomrape resistance were carried out as a preliminary to localisation of the resistance genes on the molecular map of sunflower. The resistance of the inbred FN to the isolate « Pipa Blanca » agrees with the hypothesis for one gene. It is thought that Pipa Blanca could be equivalent to races A and or B of Vranceanu *et al.* (1986) so this could be *Or1* or *Or2*.

The resistance of ORB3 to most, if not all known races of broomrape suggests that it could contain an « *Or6* » or equivalent. It was therefore thought possible that, like the downy mildew resistance genes giving resistance to many races of *Plasmopara halstedii* (P15-P18) (Vear *et al.*, 1997), this gene could in fact be a group of several genes each giving resistance to one race. However, the large reduction in the proportion of resistant F3 families that was observed in the present study cannot be easily explained by segregation between such genes. It is possible to determine Mendelian segregations of 3 or 4 complimentary genes, some recessive, some dominant, whose theoretical segregation would agree with the observed results, but it appears more likely that genetical control is basically simple, as reported

elsewhere. The gradation in severity of symptoms and lack of agreement of observations with theoretical segregations for one or two genes requires some explanation concerning tests or genetic backgrounds.

It is possible that the greenhouse tests on seedlings permitted some limited development of broomrape tubercles on the sunflower genotypes studied. This would be comparable with downy mildew, where resistance is also monogenic. In seedling resistance tests, some genotypes show some downy mildew symptoms, described as “cotyledon limited sporulation” (Vear, 1978) or type II resistance (Mouzeyar *et al.*, 1993). It is thought that the resistance mechanisms take some time to develop and in the very favourable conditions in growth chambers, downy mildew is able to spread to the cotyledons before being isolated and eliminated by the plant. For the broomrape resistance tests, a similar phenomenon could exist.

It is also possible that the susceptible line BG2256 had some effect on symptom appearance, with the broomrape isolates used, since, in crosses with other susceptible lines ORB3 has been observed to show normal segregations to other isolates (Grezes-Besset, personal communication). If this line not only lacks the resistance genes but also has deficient defence genes, it is possible that the defence mechanisms activated by the resistance gene in ORB3 may not be efficient. The question of whether the inbreds providing resistance were, in fact, homozygous for the resistance genes, *Or6* in particular. This line has been shown to be homozygous for *Or5*, but since it was not bred for resistance to Mencia, it is possible that some residual heterozygosity remained. Although unlikely, this will have to be checked.

It is also possible that the gene or genes in ORB3 do not give complete resistance, although this has not been found in the field. What is striking is that the same lack of completely resistant families occurs in two series of tests, carried out in different countries and with different *O. cumana* isolates. It would seem that the cross (BG2256 x ORB3) has overall similar reaction to seedling tests with both isolates. However, since the reactions of more than 50% of the individual F3 progenies differ between the two tests, there is no evidence that ORB3 contains a cluster of genes giving resistance to all races. Resistance to Mencia and to Bul 1 may be independent. Further studies of segregation and, more particularly of localisation by linkage with molecular markers, will be necessary to determine what genes are present in ORB3 and on which linkage groups they are placed.

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