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Genetic Studies in Sunflower Broomrape

Abstract: Much research has been conducted to identify sources of genetic resistance to sunflower broomrape (*Orobanche cumana* Wallr.) and to study their mode of inheritance. However, studies on the parasite have been scarce. This manuscript reviews three genetic studies in sunflower broomrape. First, the inheritance of the absence of pigmentation in a natural mutant of this species with yellow plant color phenotype was studied. In a first stage, lines from the unpigmented mutant and a normally pigmented population were developed by several generations of self-pollination. Plants of both lines were crossed and the F₁, F₂, and F₃ generations were evaluated. The results indicated that plant pigmentation is controlled by a partially dominant allele at a single locus. Second, the unpigmented mutant was used to evaluate outcrossing potential of the species. Two experiments in which single unpigmented plants were surrounded by normally pigmented plants were conducted under pot and field conditions. The cross-fertilization rate was estimated as the percentage of F₁ hybrids in the progenies of unpigmented plants, which averaged 21.5% in the pot and 28.8% in the field experiment. The results indicated that, under the conditions of this study, the species was not strictly self-pollinated. Finally, the inheritance of avirulence was studied in crosses of plants from

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lines of *O. cumana* races E and F, developed by several generations of self-pollination. The F₁ and F₃ generations were evaluated on the differential line P-1380 carrying the race-E resistance gene *Or*₅. The results suggested that race E avirulence and race F virulence on P-1380 are allelic and controlled by a single locus, which confirmed the gene-for-gene theory for the *O. cumana*–sunflower interaction.

Key words: avirulence, cross-fertilization, genetic studies, hybridization, inheritance, virulence

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Introduction

The *O. cumana*–sunflower parasitic system is characterized by a clear differentiation of physiological races of the parasite and a host–parasite interaction that generally fits the gene-for-gene model. This contrasts with other crop–*Orobanchae* associations, in which the genetic control of the avirulence of the parasite and the resistance of the crop plant are generally under quantitative genetic control (Pérez-Vich *et al.*, 2013). In recent years, increasingly virulent races have appeared and spread into large areas of the main sunflower producing countries in the Old World, jeopardizing sunflower production (Škorić *et al.*, 2010; Antonova *et al.*, 2013; Molinero-Ruiz *et al.*, 2014). Understanding race evolution in *O. cumana* requires basic studies on its mating system and the inheritance of avirulence/virulence mechanisms.

The genus *Orobanche* encompasses species that are largely pollinated by insects and species which are mainly self-pollinated (Musselman *et al.*, 1981). Sunflower broomrape is considered a predominantly autogamous species on account of its flower morphology (Satovic *et al.*, 2009) and population structure, generally characterized by marked among-population differences and low intra-population genetic diversity (Gagne *et al.*, 1998; Pineda-Martos *et al.*, 2013; Molinero-Ruiz *et al.*, 2014).

Even though other forms of genetic control have been reported, the occurrence of resistance mechanisms to broomrape controlled by single dominant genes is common in sunflower (Vrânceanu *et al.*, 1980; Sukno *et al.*, 1999; Velasco *et al.*, 2012). A full demonstration of the occurrence of a gene-for-gene relationship in the sunflower–*O. cumana* interaction requires studying the inheritance of avirulence in the parasite.

This paper summarizes several recent genetic studies on *O. cumana*, in particular the inheritance of a natural mutation resulting in absence of anthocyanin pigmentation, the use of this mutation for estimating the rate of cross-pollination under experimental conditions, and the inheritance of avirulence in crosses between *O. cumana* plants of races E and F.

Materials and methods

Detailed description of the materials and methods used in these genetic studies can be found in the original papers reporting the inheritance of the unpigmented plant trait (Rodríguez-Ojeda *et al.*, 2011), the rate of cross-fertilization (Rodríguez-Ojeda *et al.*, 2013a), and the inheritance of avirulence (Rodríguez-Ojeda *et al.*, 2013b). In short, a spontaneous mutant lacking anthocyanin pigmentation was identified in a population collected in Central Spain (Figure 1). This mutant plant of *O. cumana* (S_0 plant) was isolated before flowering with two microperforated plastic bags 61×8 cm made up of Cryovac® SM570Y film (Sealed Air Corporation, Elmwood Park, NJ, USA) (Rodríguez-Ojeda *et al.*, 2010). S_1 seeds were used to inoculate eight plants of the susceptible sunflower cultivar DMM for confirmation of the mutant phenotype in S_1 plants. The S_1 plants were bagged for self-fertilization as described above and the S_2 seeds were bulked. This procedure was repeated one more generation to obtain S_3 seeds. This $S_{2;3}$ *O. cumana* line was named EK-A1.

The inheritance of the trait was determined by studying the phenotypic segregation of the F_1 , F_2 , and F_3 generations from the cross of plants of the mutant line EK-A1 with normally pigmented plants of the $S_{2;3}$ line EK-12, derived from a population collected in Écija (Spain). Crosses were made by removing with forceps the corolla and the four stamens of the flower of the female plant approximately 2 days before flower opening (Figure 2), followed by rubbing fresh anthers of the male plant onto the stigma of the emasculated flower. In all cases, both female and male plants were bagged before flowering (Figure 3). The mutant trait was also used for estimating the rate of cross-fertilization taking advantage of the intermediate phenotype of F_1 hybrids (Figure 4). Individual mutant plants were surrounded by normally pigmented plants at a minimum distance of around 15 cm under open pollination conditions in both pot and field experiments. The rate of cross-fertilization was estimated as the percentage of hybrid plants in the progenies of mutant plants.

For the study of avirulence, lines of *O. cumana* races E and F were developed by selfing for three generations single plants parasitizing sunflower cultivars



Figure 1: Mutant plant of *Orobanche cumana* lacking anthocyanin pigmentation (left) together with a normally pigmented plant (right)

resistant to races A through D (for race E *O. cumana* lines) or A through E (for race F *O. cumana* lines). Confirmatory experiments showed that seeds of race E lines showed virulence on plants of sunflower cultivars Ritmo and S-1358, resistant to races A through D, and avirulence on plants of sunflower cultivar P-1380, resistant to race E (Vrânceanu *et al.*, 1980). Similarly, seeds of race F lines showed virulence on plants of cultivar P-1380 and avirulence on plants of cultivars I371B and P96, resistant to race F (Fernández-Martínez *et al.*, 2004). Crosses between plants of race E and race F lines were made as described above.



Figure 2: Manual emasculating of *Orobanche cumana*



Figure 3: Isolation of *Orobanche cumana* plants with microperforated plastic bags

The study was based on the avirulence/virulence reactions observed for the *O. cumana* F₁ and F₃ generations on the race E-resistant differential sunflower line P-1380 (Vrânceanu *et al.*, 1980).

Results and discussion

In the study of the inheritance of the unpigmented plant trait, F₁ plants from crosses between unpigmented and normally pigmented plants showed an intermediate phenotype characterized by a greenish stem color, compared to a yellowish stem color in unpigmented plants and a bluish-violet stem color in normally pigmented plants. F₂ plants segregated as 1 bluish violet:2 greenish:1 yellowish stem color, suggesting that pigmentation of *O. cumana* plants is controlled by partially dominant alleles at a single locus. This monogenic inheritance was confirmed by studying the stem color of the F₃ generation (Rodríguez-Ojeda *et al.*, 2011).

The intermediate phenotype of the F₁ hybrid (Figure 4) allowed the use of the mutant trait as a morphological marker for the study of the rate of cross-fertilization in *O. cumana*. The results showed that 21.5% of the plants derived from seeds produced by unpigmented plants in the pot experiment and 28.8% of the plants derived from seeds produced by unpigmented plants in the field experiment had an intermediate phenotype, indicating the existence of cross-pollination (Rodríguez-Ojeda *et al.*, 2013a). It is important to note that these figures were obtained under experimental conditions using individual mutant plants surrounded by many normally pigmented plants, but they do not necessarily reflect the actual cross-fertilization rate under specific natural conditions, which should be determined on a case-by-case basis. In any case, the results demonstrated that the species is not strictly self-pollinated and some extent of cross-pollination can be expected, which has important implications for understanding population structure in *O. cumana*. We observed that cross-pollination was mainly conducted by small insects of the Hymenoptera, with a length from around 3 to 9 mm. Some of them were identified as belonging to the Halictidae family (Rodríguez-Ojeda *et al.*, 2013a).

The study of the inheritance of avirulence in crosses between plants of *O. cumana* lines of races E and F was conducted by evaluating the virulence reaction of the F₁ and F₃ generations on the sunflower differential line P-1380 resistant to *O. cumana* race E but susceptible to race F. In all cases, *O. cumana* F₁ seeds did not parasitize sunflower P-1380 plants, which suggested dominance of race E avirulence over race F virulence. The evaluation of 387 F_{2,3} *O. cumana*



Figure 4: F_1 *Orobanche cumana* plant with intermediate pigmentation identified in the progenies of a mutant plant under open pollination conditions

families from the above-mentioned crosses showed complete absence of parasitism on sunflower line P-1380 in one fourth of the $F_{2:3}$ families, whereas the remaining families were able to parasitize P-1380 plants. These results suggested that race E avirulence and race F virulence on P-1380 are allelic and controlled by a single locus, which confirmed the gene-for-gene theory for the *O. cumana*–sunflower interaction.

Monogenic inheritance of avirulence/virulence in *O. cumana* is important for understanding race evolution in this parasitic weed, since new races may appear as the result of single mutation events. This is particularly relevant for an species in which single plants produce thousands of seeds. Recently, Pineda-Martos *et al.* (2013) and Molinero-Ruiz *et al.* (2014) identified two distinct gene

pools of *O. cumana* in Spain, both of them containing low genetic diversity but populations classified as either race E or race F, suggesting that race F mutations occurred independently in both gene pools.

Because of the simple inheritance of avirulence/virulence and the existence of a relatively high rate of cross-fertilization in *O. cumana*, recombination of different virulence genes may play an important role in race evolution if populations with different virulence genes get into contact. In this sense, Pineda-Martos *et al.* (2013) documented the presence of heterozygotes in a few Spanish populations of *O. cumana* in which individuals of the two predominant gene pools were present.

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