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The dynamics of faba bean (*Vicia faba* L.) parasitism by *Orobanche foetida*

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Summary. The dynamics of *Orobanche foetida* parasitizing faba bean are examined using Petri dish experiments. Rates of broomrape seed germination and seedling attachment to the host roots were quantified on three resistant genotypes (the Egyptian line Giza 429, the Spanish cultivar Baraca, and the Tunisian cultivar Najeh [XBJ90.03-16-1-1-1]) and the susceptible cv. Bachaar. The percentage of *O. foetida* seed germination (11 to 38%) was lower near the roots of resistant host plants than it was near the roots of 'Bachaar' (67%). *O. foetida* parasitism was followed using three parametric logistic functions. In this way some major parameters of the infection process were quantified: the maximal number (N_{max}) and the maximal rate (R_{max}) of broomrape attachments to the host roots, the median time required for attachment (T_{50}), the maximal percentage of established tubercles reaching the final growth stage at 70 days after inoculation (DAI) ($\%_{max}$), and the maximal rate of established tubercle growth (R'_{max}). Broomrape attachment was lower and slower in resistant plants, as indicated by low N_{max} and R_{max} values combined with high T_{50} values. Furthermore the precocity of the resistant genotypes was correlated with low attachment. The parameters $\%_{max}$ and R'_{max} did not discriminate the susceptible cultivar Bachaar from Giza 429 or Baraca. On the other hand, the $\%_{max}$ and the R'_{max} were lower in the 'Najeh' plants. The findings indicated that both low attachment and limited growth of established tubercles contributed to resistance in the Najeh cultivar.

Key words: parasitic weed, germination, resistance, tubercle.

Introduction

Some *Orobanche* and *Phelipanche* species are serious parasitic weeds causing considerable losses in many major crops including faba bean (*Vicia faba* L.), pea (*Pisum sativum* L.), tomato (*Lycopersicon esculentum* Mill.), tobacco (*Nicotiana tabacum* L.), potato (*Solanum tuberosum* L.), winter oilseed rape (*Brassica napus* L.) and sunflower (*Helianthus annuus* L.) (Parker, 2009). Infestations with these species are widespread all over the world, notably in the Mediterranean basin and in Asia Minor where all the harmful *Orobanche* and *Phelipanche* species (*O. crenata* Forsk., *O. cumana* Wallr., *P. ramosa* L., and *P. aegyptiaca* Pers.) benefit from favorable conditions for growth (Parker, 2009). Broomrape is difficult to control mainly on account of its fecundity and the long-term viability of the seed in the soil. Integrated control methods have been suggested, exploiting cultural, chemical and biological approaches, but with only limited success (Rispail *et al.*, 2007). *Orobanche* has a number of growth

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stages (Labrousse *et al.*, 2001): 1, germination, elicited by stimulants that are secreted by the roots of nearby host plants; 2, attachment to the host vascular system by means of a haustorium, which serves as both an attaching organ and a bridge for water and nutrient transfer from the host; 3, tubercle establishment, with the formation of adventitious roots; 4, development of a subterranean shoot, and 5, emergence from the soil and development of flowering spikes.

Two *Orobanche* species, *O. crenata* Forsk. and *O. foetida* Poir. cause serious damage to crops, and especially faba bean, in Tunisia. *O. crenata* is restricted to eastern Tunisia, and *O. foetida* to the western and north-central parts of the country (Kharrat and Halila, 1994). *O. foetida* is widespread in natural habitats in the western Mediterranean (Portugal, Spain, Morocco and Algeria, as well as Tunisia) parasitizing wild herbaceous and leguminous plants (Pujadas-Salva, 2002). *O. foetida* is an important emergent agricultural parasite in faba bean in Tunisia, causing yield losses of up to 90% in the Beja region (Kharrat *et al.*, 1992; Abbes *et al.*, 2007a). The species seems to be more pathogenic on faba bean and common vetch (*V. sativa*) than on other legumes (Abbes *et al.*, 2008). Moreover, *O. foetida* var. *broteri* was first reported in Morocco only recently, on a common vetch crop, indicating that this parasitic weed is very pathogenic (Rubiales *et al.*, 2005). *O. foetida* displays rapid host-differentiation, shifting hosts from wild legumes to vetch (Roman *et al.*, 2007; Vaz Patto *et al.*, 2008).

By definition, host plants are resistant to broomrape when they do not support the entire life cycle of the parasite. Such complete resistance is rare, but degrees in resistance are common. The genetic and molecular components of resistance are now being studied, mainly in legumes challenged with *O. crenata* and in sunflower challenged with *O. cumana* (Rispail *et al.*, 2007; Pérez-de-Luque *et al.*, 2009). Faba bean growers have commonly used *Orobanche*-resistant germplasm accessions from Egypt as the source of resistance. The resulting breeding lines displayed polygenic resistance to *O. crenata,* with components ranging from low broomrape seed germination to the formation of mechanical barriers in the attachment zone and necrosis of established tubercles (Nassib *et al.*, 1978, 1984; Zaitoun *et al.*, 1991; Roman *et al.*, 2002). Some faba bean lines also displayed high resistance to *O. foetida* in the field (Abbes *et al.*, 2007a, 2010). Nonetheless, information concerning the mechanisms involved in resistance to *O. foetida* remains scanty (Abbes *et al.*, 2007a, 2007b; Diaz-Ruiz *et al.*, 2009).

The aim of this study was to further understanding of the relationship between resistant and susceptible faba bean on the one hand, and *O. foetida* on the other. The host-parasite interaction was studied in Petri dish experiments in order to distinguish between real resistance and escape mechanisms due to the precocity, vigor or root architecture of the host, and to determine the components into which resistance is divided. The interest of this approach has been recognized in the literature (Labrousse *et al.*, 2001; Pérez-de-Luque *et al.*, 2005a; Mabrouk *et al.*, 2007; Fernandez-Aparicio *et al.*, 2008). It is an approach that also enables the kinetics of broomrape attachment and the growth of the established tubercles on susceptible and resistant faba bean cultivars to be analyzed and compared. As a result, data models were determined and parameters of the growth patterns quantified using graphic and mathematical analysis (Eizenberg *et al.*, 2005a, 2005b, 2007).

Materials and methods

Plant material

Four faba bean genotypes were used in the experiments: the Tunisian *Orobanche*-susceptible cultivar Bachaar (JORT, 2004) and three *Orobanche*-resistant breeding lines: the Egyptian line Giza 429, the Spanish cv. Baraca and the Tunisian cv. Najeh (XBJ90.03-16-1-1-1). The faba bean cv. Baraca was derived from a cross between the broomrape breeding line VF1071 and 'Alameda' (Nadal *et al.*, 2004). Breeding line VF1071 was derived from Egyptian germplasm accession F402 (Giza 402). The line Najeh was selected by the INRAT breeding program on account of its resistance to *O. foetida* and has been registered in the Tunisian catalogue only recently (Abbes *et al.*, 2007a; Kharrat *et al.*, 2010). It came from a cross between a Tunisian small-seeded pure faba bean line and a breeding line provided by ICARDA (Aleppo, Syria) and bearing some resistance to *O. crenata* from the Egyptian

line Giza 402 (Nassib *et al*., 1978, 1984). Seeds of *O. foetida* were collected in 2003 from a faba bean field at Beja (Tunisia) and stored in the dark at 25°C until use. The viability of the *O. foetida* seeds, as determined by TTC (2,3,5-triphenyltetrazolium) staining (Aalders and Pieters, 1985), was 80.6%.

Seed preconditioning and germination

Orobanche foetida seeds were surface-sterilized for 7 min in sodium hypochlorite (3.61% w:v) and rinsed several times with sterile distilled water. They were preconditioned in the dark at 22°C for one week on glass fiber filter paper moistened with 5 ml sterile distilled water in Petri dishes (35×10 mm diam., Greiner, Bio One GmbH, Frickenhausen, Germany). Faba bean seeds were surface-sterilized in sodium hypochlorite (3.61% w:v) solution for 5 min and rinsed several times in sterile distilled water, then germinated in Petri dishes on glass fiber filter paper moistened with water and maintained in the dark at 25°C. Faba beans grow well under these conditions.

***Orobanche foetida*-faba bean cultures in Petri dishes**

Petri dishes were prepared as described by Labrousse *et al*. (2004). Sprouted faba bean seeds were transferred to large square Petri dishes (245×245×25 mm, Lab-Tek, Nunc Inc., Naperville, IL, USA) when they were 5–7 days old. The Petri dishes were wrapped in aluminium foil and placed vertically in plastic bowls (390×290×230 mm) containing 2.5 L half-strength Coïc medium (Coïc and Lesaint, 1975). The culture medium was replaced three times weekly. The culture bowls were placed in a growth chamber at 22°C with 100 μmole PAR m^{-2} s^{-1} and incubated with a 14 h day and 70% relative humidity. Two weeks later, preconditioned seeds of *O. foetida* (5 mg, about 1250 seeds) were placed homogeneously with a micropipette 1–3 mm away from either side of well-developed faba bean roots at an average density of 50 seeds cm^{-2}.

Twenty days after host root inoculation, percent *Orobanche* germination was assessed under a stereoscopic microscope by determining the mean number of seeds with an emerged radicle. Seeds were measured in eight squares of 1 cm^2 near inoculated faba bean roots. Four hundred broomrape seeds were counted per faba bean plant. The germination percentage was corrected for viability by the TTC method (Rubiales *et al*., 2006; Mabrouk *et al*., 2007; Fernandez-Aparicio *et al*., 2008). In addition, the number of broomrape attachments per host plant was recorded weekly for up to 70 days after inoculation (DAI), at which time the host plants had reached pod maturity. The broomrape growth stages were determined according to the 1–5 scale of Labrousse *et al*. (2001, 2004), slightly modified; for simplicity, all the tubercles bearing a spike were classified as stage 4. Broomrape attachment was followed weekly by counting the attached parasites on each host plant. Tubercle growth was determined weekly by measuring the changes in the percentage of tubercles reaching stage 4 in each host plant for the 70 d duration of the experiment.

Graphic and statistical analysis

Data were collected from five Petri dishes per faba bean genotype. The experiments were repeated twice. Since the results of the two experiments were similar, they were pooled for the mathematical models and statistical analysis (n=10). *O. foetida* growth on the various faba bean genotypes was quantified using a three-parametric logistic function (Equation 1) (Brown and Mayer, 1988; Eizenberg *et al*., 2005a, 2005b):

$$Y = N_{max} / (1+(x/T_{50})^S), \qquad [1]$$

where Y represents the number of attachments; N_{max} the upper (maximum) asymptotic number of attachments; T_{50} the median time from inoculation to 50% of the maximum number of attachments; and S, the slope. R_{max} corresponds to the maximum rate of broomrape attachment (attachment DAI^{-1}) and is calculated as the slope at T_{50}, or $-N_{max} \times S/4T_{50}$. Data were subjected to analysis of variance (ANOVA) with the genotype as the factor. The significance of the mean difference between genotypes was evaluated by the least significant difference test (LSD) at $P<0.05$. Non-linear regressions and statistical analysis were performed with SigmaPlot® 10.0. Changes in the percentage of the tubercles reaching growth stage 4 were analyzed in the same way. Nevertheless the data were sin^{-1} (square root)-transformed to normalize the ratios and they were further analyzed by ANOVA.

Results

Performance of faba bean in Petri dishes

The influence of culture conditions on faba bean growth was genotype-dependent. Faba beans cv. Najeh, Giza 429 and Baraca grew faster and were more resistant than those of cv. Bachaar. The three resistant genotypes flowered 30 days after being transferred to the Petri dishes and reached seed-setting 60 days after transfer. In cv. Bachaar, on the other hand, flowering and seed-setting did not occur until a further 15 days.

Broomrape seed germination

The maximum percentage of broomrape seed germination near the faba bean roots (corrected according to the TTC viability test) was determined twenty days after inoculation, irrespective of the faba bean genotype. The germination percentage ranged from 11 to 67% for the four faba bean genotypes (Table 1). The effect of the genotype was highly significant (ANOVA, $P<0.001$). The lowest germination percentages occurred near the roots of the three resistant genotypes, especially Giza 429 and Najeh. Broomrape germination was much higher when the seeds were placed near the roots of the susceptible cv. Bachaar.

Broomrape attachments to faba bean roots

Regardless of the host genotype, broomrape attachment during the 70-d culture period followed a sigmoid pattern (Figure 1).

The effect of the host genotype on N_{max} was highly significant ($P<0.001$, Table 1). N_{max} ranged from 3.2 for the resistant 'Giza 429' and 'Najeh', to 14.2 for the susceptible 'Bachaar'. The three resistant genotypes had the lowest N_{max} values, with 'Baraca' occupying an intermediate position.

The rate of broomrape attachment was also genotype-dependent. Although 'Bachaar' plants grew more slowly than plants from the resistant genotypes, the first broomrape attachments to 'Bachaar' roots appeared already 21 DAI (Figure 1) when this genotype had not yet flowered. Attachments to 'Giza 429' and 'Baraca' started one week later, and to 'Najeh' plants after a further two weeks, when these plants had already flowered. The number of attachments to the susceptible cultivar Bachaar increased quickly in the days following (Table 1), with a maximal rate of attachment (R_{max}) significantly higher than that on the resistant 'Giza 429' and 'Najeh' plants. 'Baraca' still occupied an intermediate position for this parameter. The early attachment to the roots of the host not only led to a high rate of broomrape

Table 1. *Orobanche foetida* seed germination near the roots of various faba bean genotypes and coefficients of the three-parameter sigmoid non-linear regression ($Y=N_{max}/(1+(x/T_{50})^S)$) between the number of broomrape attachments to the faba bean roots and the number of days after inoculation (DAI). The maximum rate of broomrape attachment (R_{max}, attachment number DAI^{-1}) was calculated from the derivative value of T_{50} and was equal to $-N_{max}.S/4T_{50}$.

Faba bean genotype	*O. foetida* seed germination (% viable seeds)[a]	Coefficient			Regression		
		Nmax	*S*	T_{50} *(DAI)*	R_{max}	R^2	*P*
Bachaar	67.01	14.151	-3.617	30.021	0.426	0.988	0.0003
Baraca	38.11	8.372	-5.378	41.169	0.273	0.996	< 0.0001
Giza 429	11.34	3.161	-4.816	41.165	0.093	0.994	< 0.0001
Najeh	15.21	3.582	-9.337	46.126	0.181	0.991	0.0002
LSD[b]	12.34	1.251	2.494	3.544	0.154		

[a] Germination percentage was determined 20 days after *O. foetida* inoculation by counting the number of seeds with an emerged radicle. Germination percentage was corrected according to the TTC viability test.
[b] LSD, least significant difference.

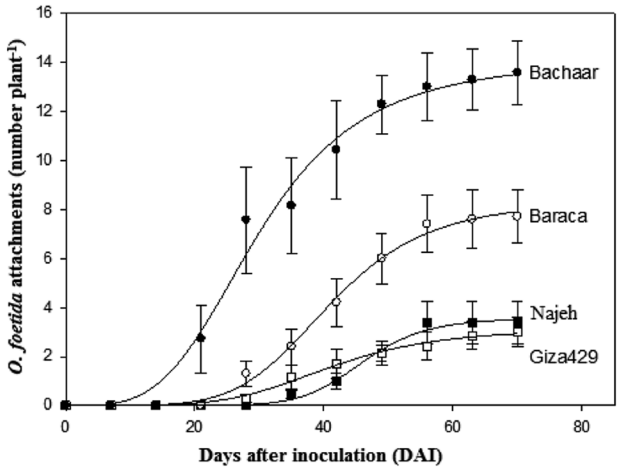

Figure 1. Time course of *Orobanche foetida* attachments on the roots of susceptible and resistant faba bean genotypes in Petri dishes. Bars represent the SE of ten measurements (●, Bachaar; ○, Baraca; ■, Najeh; □, Giza 429).

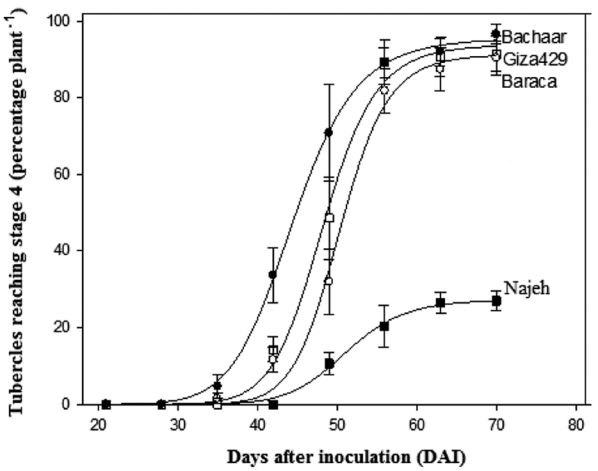

Figure 2. Dynamics of growth of established tubercles of *Orobanche foetida* in susceptible and resistant faba bean genotypes in Petri dishes. Bars represent the SE of ten measurements (●, Bachaar; ○, Baraca; ■, Najeh; □, Giza 429).

attachment, but also resulted in a low T_{50} for the susceptible 'Bachaar' (Table 1), as compared with the resistant 'Najeh' and 'Giza 429'.

Growth of established tubercles

Changes in the percentage of tubercles reaching stage 4 during the 70-d culture period were examined as an index of the growth of established tubercles. A sigmoid equation fitted the experimental data for the four genotypes (Figure 2). First, this percentage increased from 28–35 DAI on 'Bachaar', 'Baraca' and 'Giza 429' roots, to reach a high $\%_{max}$ value 56–63 DAI (91–96%, Table 2). Almost all the attached parasites therefore reached growth stage 4 70 DAI. By contrast, the $\%_{max}$ was much lower (less than 30%) on the resistant 'Na-

Table 2. Coefficients of the three-parameter sigmoid non linear regression ($Y=\%_{max}/(1+(x/T_{50})^S)$) between the percentage of *O. foetida*-at growth stage 4 and the number of days after inoculation (DAI). The maximum rate of growth of attached *O. foetida* (R'_{max}, % tubercles reaching stage 4 DAI^{-1}) was calculated from the derivative value at T_{50} and was equal to $-\ \%_{max}\ .S/4T_{50}$.

Faba bean genotype	Coefficient			Regression		
	%max	*S*	T_{50} *(DAI)*	R'_{max}	R^2	P
Bachaar	95.577	-11.205	44.498	6.017	0.998	<0.0001
Baraca	91.303	-16.802	50.391	7.611	0.994	0.0002
Giza 429	93.837	-14.655	48.308	7.117	0.996	< 0.0001
Najeh	27.383	-13.874	51.191	1.855	0.996	< 0.0001
LSD[a]	24.517	3.549	1.144	3.930		

[a] LSD, least significant difference.

jeh' plants. The effect of genotype on the maximal rate of tubercle growth (R'_{max}) was also significant (Table 2). The tubercles fixed to 'Baraca' and 'Giza 429' plants grew as rapidly as the tubercles attached to the susceptible cv. Bachaar. On the other hand, the broomrape attachments to 'Najeh' plants had a low R'_{max}.

Discussion

Recent studies report that the faba bean lines Najeh, Baraca (Abbes *et al.*, 2007a) and Giza 429 (M. Kharrat personal communication) grow well in fields highly infested with *O. foetida*. Compared with susceptible cultivars, these resistant lines have fewer broomrape attachments and suffer less yield loss when they are grown in broomrape-infected conditions, especially the line Najeh, selectively bred by INRAT. This line is characterized by escape mechanisms (deeper rooting) combined with resistance mechanisms that have remained undetermined (Abbes *et al.*, 2007a, 2007b).

The percentage of *O. foetida* seed germination (67%) was highest near roots of the susceptible faba bean cv. Bachaar. Because *O. foetida* requires stimulants to germinate, any low germination probably derives from a low production of stimulants (Yoneyama *et al.*, 2008). A low level of stimulants is essential for the resistance of faba bean to *O. crenata* (Nassib *et al.*, 1978; Wegmann *et al.*, 1991) and in other legumes, including *Vicia* spp. (Sillero *et al.*, 1999), *Lathyrus* spp. (Sillero *et al.*, 2001), *Cicer* spp. (Rubiales *et al.*, 2003b) and *Pisum sativum* (Rubiales *et al.*, 2003a). Nevertheless, since the present study did not attempt to determine whether resistant plants produce low levels of strigolactones, the possibility cannot be excluded that faba bean roots produce inhibitors of broomrape seed germination or phytoalexins causing resistance to *O. foetida* (Echevarria-Zomeno *et al.*, 2006; El-Halmouch *et al.*, 2006).

As was found in earlier studies, host-parasite development followed a sigmoid pattern when plotted over time (Eizenberg *et al.*, 2005a, 2005b, 2007). In the present study, graphic analysis provided a better understanding of broomrape infection by quantifying the major parameters. The total number of attached tubercles per plant (N_{max}) depends on the rate of broomrape seed germination and its success in attaching itself to the host, which correlates with the resistance level of faba bean (see below for the mechanisms involved in resistance). In this way, the N_{max} parameter distinguished the susceptible cv. Bachaar from the three resistant lines, especially Giza 429 and Najeh, which had only a low capacity to bring about broomrape seed germination. The findings showed that low stimulation of broomrape germination was a key factor accounting for the resistance of those faba bean genotypes to *O. foetida*. This in turn confirmed the preliminary data obtained with 'Bachaar' and 'Najeh' plants challenged with *O. foetida* in root chambers (Abbes *et al.*, 2006). Although the R_{max} parameter did not distinguish the susceptible cv. Bachaar from the resistant accession Baraca, it did discriminate Bachaar from the resistant lines Giza 429 and Najeh. Germinated broomrape seeds became attached to 'Giza 429' and 'Najeh' roots 2.5 to 4.5 times more slowly than they did to the 'Bachaar' roots. These faba bean lines also significantly delayed the rate at which broomrape became attached to them, as shown by their high T_{50} value. This suggests that the slow rate of broomrape attachment was due to physical and/or chemical barriers in the roots of these resistant lines. Such delaying barriers also occur in some resistant legume accessions challenged with *O. crenata*: they consist of changes occurring in the root cell walls plus higher levels of toxic compounds (Pérez-de-Luque *et al.*, 2005a, 2006a; Echevarria-Zomeno *et al.*, 2006; Fernandez-Aparicio *et al.*, 2008). In none of the faba bean-*O. foetida* combinations did the parasite die after attachment. By contrast, necrosis of broomrapes that became attached was reported on the parent line Giza 402 as a major component of resistance to *O. crenata,* in addition to low broomrape germination (Nassib *et al.*, 1978). Zaitoun *et al.* (1991) also reported that the late attachment of *O. crenata* to 'Giza 402' roots was due to the growth of corky tissue at the site of penetration. Moreover, 'Giza 402' roots had a slightly thicker epidermis, cortex and xylem and an intact endodermal layer with thick walls, due to reduced secondary growth, when compared with the roots of a susceptible faba bean cultivar (Khalaf and El-Bastawesy, 1989; Attia, 1991). All these findings indicate that the whole genetic stock causing resistance to *O. crenata* in the parent Giza 402 line was not transferred to the derived genotypes Baraca, Giza 429 and Najeh.

The high precocity of the resistant genotypes could also be involved in the delayed growth of broomrape, especially with the Najeh line. Even after flowering, the 'Najeh' plants still did not bear any attached broomrape, although the parasite had already attached itself to the other three faba bean lines. The number of attached broomrapes was already at maximum levels (N_{max}) with 'Bachaar' and 'Giza 429' when these plants flowered. Such a delay in broomrape attachment to 'Najeh' plants has also been reported in field studies (Abbes *et al.*, 2007a), and it certainly gives these resistant lines a significant advantage over the parasite during their reproductive stage, as demonstrated by the low impact of *O. foetida* on the seed yield of 'Najeh' as compared with that of the other resistant faba bean lines.

The growth of established tubercles was also analyzed graphically. Although tubercles grew on the roots of the susceptible cultivar as rapidly as they did on the roots of the resistant 'Baraca' and 'Giza 429' plants, as shown by their similar R'_{max} values, 'Najeh' plants had a much lower R'_{max} value. This suggested that 'Najeh' plants did not favor the growth of established tubercles. Consequently, only a small percentage of established tubercles tested with this genotype reached growth stage 4 in the Petri dish experiments, unlike the tubercles tested with the other genotypes. The poor performance of the broomrapes attached to 'Najeh' plants can be explained in various ways: (i) obstructing compounds became deposited in the root xylem vessels of the host, reducing water and nutrient flow to the attached parasites. Such obstructing compounds also occur with other plants: when resistant sorghum is challenged with *Striga hermonthica*, crystalline structures are deposited (Arnaud *et al.*, 1999); in sunflower resistant to *O. cumana,* apposition to the cell wall occurs (Labrousse *et al.*, 2001); in resistant pea challenged with *O. crenata,* there is carbohydrate deposition (Pérez-de-Luque *et al.*, 2005b), when resistant lentil accessions are challenged with *O. crenata,* mucilage levels go up (Pérez-de-Luque *et al.*, 2006b); and in faba bean resistant to *O. crenata* the cell walls are reinforced by callose deposition and lignification of the endodermal cells (Pérez-de-Luque *et al.*, 2007). More studies are needed to determine what happens when faba bean is challenged with *O. foetida*. (ii) The roots of 'Najeh' plants have a lower water potential than those of 'Bachaar', 'Giza 429' and 'Baraca' (Abbes *et al.*, 2009b), and this may lower the water flow towards the established tubercles, as was suggested for the parent line Giza 402 to *O. crenata* by Wegmann *et al.* (1991). (iii) The leaf phloem exudates collected from 'Najeh' plants when the *O. foetida* tubercles reach growth stage 4 are deficient in free amino acids as compared with the phloem exudates from the susceptible 'Bachaar' (Abbes *et al.*, 2009a). Since the amino acid composition of the phloem is one important factor determining the nutritional quality of plants for aphids (Karley *et al.*, 2002), the low nutritional quality of the phloem of 'Najeh' plants could explain at least in part the poor growth of *O. foetida*, which depends almost entirely on the host phloem for its carbon and nitrogen requirements. Furthermore, compared with the rapidly growing tubercles on the roots of the susceptible 'Bachaar', the much more slowly growing tubercles on the 'Najeh' roots had a low invertase activity and thus probably a low capacity to exploit the carbohydrates from the host (Abbes *et al.*, 2009a). Indeed the rapid uptake of sucrose by broomrape and its immediate cleavage into glucose and fructose doubles the osmotic value of the sugar component, and osmosis is known to be a major process involved in the water flow from faba bean to broomrape (Whitney, 1972; Wegmann *et al.*, 1991).

Legumes have only moderate to low levels of incomplete resistance or tolerance to *O. crenata* (Rubiales *et al.*, 2006). Similarly, the resistance of the tested faba bean genotypes to *O. foetida* is mediated by multiple components derived from the Egyptian line Giza 402. These components include: low parasite seed germination, a low rate of parasite attachment to the host roots, and a low growth rate of the established tubercles, especially with the breeding line Najeh. Delayed growth of established tubercles has been reported in previous studies on resistant legume accessions challenged with *O. crenata* but no attempt was there made to quantify the components of resistance (Pérez-de-Luque *et al.*, 2005b; Sillero *et al.*, 2005; Fernandez-Aparicio *et al.*, 2008). We here propose a method of quantification that should help in understanding the genetic and molecular bases (quantitative trait loci, or loci) of this resistance, and the heritability of these bases. As recently reported by Roman *et al.* (2007), the Tunisian

O. foetida populations have considerable genetic variability and this is related to the rapid differentiation of the host species and is accompanied by variations in broomrape pathogenicity. In the present study, broomrape seeds were sourced from a single site usually used for faba bean breeding, suggesting that high selection pressure from the host contributed to the low genetic diversity of the *O. foetida* seed bank in this area. Given the potentially high variation in the pathogenicity of this species, the use of new resistant faba bean cultivars, such as the Tunisian 'Najeh' line, showing multiple components of resistance combined with escape mechanisms, is strongly recommended to avoid a breakdown in resistance due to new populations of the parasite.

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