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# VARIATION IN THE RESISTANCE OF SOME FABA BEAN GENOTYPES TO *OROBANCHE CRENATA*

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**Abstract**

Four faba bean (*Vicia faba* L.) genotypes were tested for their reaction to *Orobanche crenata* Forsk., infestation. Evaluation was carried out for two cropping seasons at the Ariana research station, Tunisia in a field naturally infested with *O. crenata* and in pot experiments. At maturity, the genotypes Baraca, Giza429 and the breeding line Bader carried 2-6 times less of number of emerged parasites and 3-7 less of dry weight of emerged parasites than the susceptible cv. Badï. The average yield observed for the three resistant genotypes was two to four-fold higher than the one observed for the susceptible genotype. These resistant genotypes seemed to flower earlier and to show late orobanche establishment which gave them an advantage over the parasite. The genotype Bader, which was selected for its resistance to *O. foetida*, was resistant to *O. crenata*, showing that selecting for *O. foetida* resistance can protect against *O. crenata* infection. Besides, the two genotypes Baraca and Giza429 selected for their resistance to *O. crenata* in Spain and Egypt respectively, do not present tubercle necrosis on their roots, showing that they do not respond similarly to the Tunisian population of *O. crenata*. These partially resistant genotypes may provide breeders with additional sources of resistance to *O. crenata*, and can form appropriate material for an integrated control package.

## Introduction

*Orobanche crenata* Forsk., is a serious problem to legume cultivation in the Mediterranean region. This weed parasite constitutes a major constraint on faba bean, pea, chickpea, lentil and other grain and forage legumes. In some cases, the diminution of yield is so great that cultivation is abandoned. In Tunisia, two species of orobanche cause serious damage to crops, *Orobanche crenata* Forsk. in the eastern coastal parts of the country and *O. foetida* Poir. in the Beja region of the north west (Kharrat & Halila, 2004). The total areas infested in Tunisia have been estimated to vary in extent from 5000 to 10000 ha (Kharrat & Souissi, 2004). Kharrat & Souissi (2004) indicated that in highly infested areas, farmers generally avoid growing faba bean or other susceptible crops, resulting in substantial reductions to both the extent of cultivated areas and to food legume production.

Many methods like cultural, chemical and biological have been devised to solve the orobanche problem, but the majority are either too expensive or only partly effective (Parker & Riches, 1993). The most common means of pest control is by chemical pesticides; however, the timing of pesticide application has to be very precise and there is only a narrow margin between destroying the parasite alone and damaging the host as well. Therefore, breeding for resistance is considered the best form of control against orobanche. Many programs in the region (Spain, Egypt, ICARDA, Morocco, Tunisia…) have set up faba bean breeding programs to select broomrape-resistant varieties. As a consequence, a large number of highly tolerant genotypes with higher yield have been identified eg., Giza 402 (Nassib *et al.*, 1978), Baraca (Nadal *et al.*, 2004), Giza 429, Misr 1 and Misr 2 (El-Shirbini & Mamdouh, 2004) and Najeh (XBJ90.03-16-1-1-1) (Abbes *et al.*, 2007a, b, Kharrat *et al.*, 2010).

The aim of the present study was to present and discuss the behavior of some faba bean genotypes, selected for their resistance to different Orobanche species (*O. crenata* and *O. foetida*), in the presence of a Tunisian population of *O. crenata*, in field and greenhouse conditions at Ariana, Tunisia.

## Material and Methods

**Plant material:** Four faba bean genotypes were used in this experiment: The variety Baraca, selected for its resistance to *O. crenata* in Spain (Nadal *et al.*, 2004), the variety Giza429, selected for its resistance to *O. crenata* in Egypt (El-Shirbini and Mamdouh, 2004), the Tunisian cv. Badï recently registered in Tunisia under the INRAT/ICARDA collaborative program (Anonymous, 2004) and known to be susceptible to both *O. crenata* and *O. foetida* and the breeding line Bader, selected by the INRAT breeding program of Tunisia for its resistance to *O. foetida* (Abbes *et al.*, 2007a). These genotypes were obtained from the parent Giza 402 resistant to *O. crenata* (Nassib *et al*., 1978).

### Experimental conditions

**Field experiment:** The four faba bean genotypes were grown in a field naturally infested with *O. crenata* at the Ariana experimental station, Tunisia (36°50'N, 10°11'E), during the 2004-05 and 2005-06 cropping seasons. Sowing was done during the first week of December each year and no fertilizer or other chemical treatments were applied. Weeds were removed by hand. The experimental design was a complete randomized block with two replications. Each genotype was sown in rows 4 m long, with 0.5 m inter-row spacing. Twenty-five seeds were sown at equidistant intervals in each row. Broomrape infestation was determined at crop maturity by counting the number of emerged orobanche spikes per host plant (NEO/P). The dry weight of emerged orobanche spikes per host plant (DWEO/P), their incidence (percentage of host plants showing emerged spikes using a 0 to 100% scale, Abbes *et al*., 2007b), their severity (according to a 9-point scale, Abbes *et al*., 2007a), the seed yield per faba bean plant (SY/P) and the 100-seeds weight (HSW) were also determined. Crop development was assessed by determining the number of days from sowing to the point when 50% of the plants had started flowering (DF) and by plant height (PH).

During the 2004-05 cropping season, three faba bean plants per replicate were gently dug up from the soil at the early pod setting stage and before broomrape emergence.

The number of underground non-emerged orobanche attachments per plant (NNEO/P) was recorded. Their developmental stage was scored on the scale of Labrousse *et al.* (2001), where S1 represents attachment of the haustorium to the host root; S2 small tubercles without root formation; S3 tubercles with crown root but without shoots; S4 shoot formation remaining underground; S5 shoot emergence. In addition, faba bean shoot dry weight FBSDW/P, orobanche tubercle dry weights (OTDW/P), and the number of ramifications per plant (NR/P) were determined.

**Pot experiment:** Seeds of *O. crenata* were collected in 2001 from a large number of plants over several areas in a faba bean field at Ariana, Tunisia, and stored in the dark at 25°C until used for experimental infestations. Seeds of faba bean and *O. crenata* were surface-sterilized by soaking in calcium hypochlorite (1%) for 15 minutes and were washed twice with sterilized water before use. The four faba bean genotypes were grown in 10-l pots containing sterilized soil and river sand (2:1) artificially inoculated with 10 mg of *O. crenata* seeds per Kg of substrate. Five infested pots were prepared for each genotype. Plants were grown in a glasshouse at 20±3°C and under natural light. The number of emerged orobanche spikes NEO/P and their dry weight DWEO/P (80°C for 48H) per faba bean plant were determined at the flowering and the plant maturity stages.

**Statistical analysis:** Results were analyzed using the SPSS 11.5 software (Windows edition). Mean comparisons were made using Duncan's multiple-range classification test at P = 0.05. The statistical model for pot experiment involved a completely randomized design with five replicates, in which the host genotype was the unique fixed factor.

## Results and Discussion

The aim of this study was to evaluate the behaviour of some faba bean genotypes to a Tunisian population of crenate broomrape in field and greenhouse experiments. There were considerable differences among faba bean genotypes in their response to *O. crenata*. Differences in infection and seed yield among the seasons can be ascribed to variations in weather conditions, which are known to influence both the extent of orobanche infestation and faba bean growth (Rubiales *et al.*, 2003; Abbes *et al*., 2010a). Several criteria have been used by authors to quantify resistance to orobanche infestation, such as: number of orobanche per host plant; dry matter of parasitic plants per host plant; height of the tallest parasitic shoot; number of orobanche per sown surface unit, etc. (Rubiales *et al.*, 2006). According to some authors (Cubero, 1991; Rubiales *et al.*, 2003), the best index is the number of orobanche shoots per host plant, which gives the most reliable estimation of the total level of infestation. However, Sillero *et al.*, (1996), suggested that a screening based only on the number of emerged shoots was misleading, and that the health of the host plant must also be considered.

In our study, the main parameters used to test orobanche infestation of the faba bean genotypes were the severity, the incidence, the number and dry weight of orobanche spikes per faba bean plant and the seed yield per faba bean plant. All these parameters allowed separating the tested faba bean genotypes into two groups: resistant genotypes (Giza429, Bader and Baraca) and susceptible genotype (Badï). The cultivar Badï was most strongly affected by *O. crenata*, showing the highest incidence and severity (Table 1), the highest orobanche number and dry weight (Table 2), and the lowest grain yield (Table 3), despite its high yield potential when not infested (Anon., 2004). On the other hand, Giza429, Bader and Baraca were less susceptible than Badï and may carry some genes rendering them resistant to orobanche. Indeed, the number and the dry weight of the orobanche spikes on these genotypes were much lower than on the susceptible cv. Badï (Table 2). The seed yield and the 100 seed dry weight per host plant were also considerable and were significantly higher than it was in the susceptible control (Table 3). These results confirm the resistance of Giza429 and Baraca to different *O. crenata* populations and under various environmental conditions. These genotypes showed a good adaptation to Tunisian environmental conditions. In addition, the cv. Bader, which was selected to its resistance to *O. foetida* (Abbes *et al.*, 2007a), demonstrated good level of resistance to *O. crenata*, showing thus the agronomic potential of this genotype.

The examination of the underground infestation by uprooting the host plant when no emerged Orobanche were yet observed and by counting the number of underground parasite attachments allowed us to confirm the behavior of each genotype. During the cropping season 2004-05, roots of Giza429 and Baraca genotypes, followed by Bader, had a low number and low dry weight of attached broomrapes per plant; the tubercles arose essentially in stages 2 and 3 (Table 4). No necroses were observed on the attached parasites, regardless of the genotype. At these stages, Giza429 had the highest shoot dry weights per plant at 25.57 g (Table 4).

**Table 1. Severity and Incidence of orobanche infestation on faba bean genotypes in the 2004-05 and 2005-06 growing seasons.**

| Genotypes | Severity | | Mean | Incidence | | Mean |
|---|---|---|---|---|---|---|
| | 2004-05 | 2005-06 | | 2004-05 | 2005-06 | |
| Bader | 2.50a[a] | 3.00ab | 2.75a | 60.00b | 75.00b | 67.50b |
| Giza429 | 2.00a | 2.00a | 2.00a | 35.00ab | 25.00a | 30.00a |
| Baraca | 1.50a | 3.00ab | 2.25a | 25.00a | 30.00a | 27.50a |
| Badï | 3.43b | 5.00b | 4.21b | 92.50c | 89.17b | 90.83c |

[a]: For each year, within a column, values followed by the same letter are not significantly different (p=0.05) based on Duncan's multiple range test.

**Table 2. Number of emerged orobanche spikes (NEO/P) and their dry weight (DWEO/P) in an *O. crenata* infested field in the 2004-05 and 2005-06 growing seasons.**

| Genotypes | NEO/P | | Mean | DWEO/P (g) | | Mean |
|---|---|---|---|---|---|---|
| | 2004-05 | 2005-06 | | 2004-05 | 2005-06 | |
| Bader | 2.03a[a] | 1.66a | 1.85a | 1.84a | 1.34a | 1.59a |
| Giza429 | 1.08a | 0.66a | 0.87a | 1.11a | 0.53a | 0.82a |
| Baraca | 0.67a | 0.76a | 0.72a | 0.62a | 0.65a | 0.63a |
| Badï | 5.07b | 3.22b | 4.14b | 5.37b | 3.08b | 4.22b |

[a]: For each year, within a column, values followed by the same letter are not significantly different (p=0.05) based on Duncan's multiple range test.

**Table 3. Seed yield per faba bean plant (SY/P) and 100 seed weight (HSW) of faba bean genotypes in an *O. crenata* infested field in the 2004-05 and the 2005-06 growing seasons.**

| Genotypes | SY/P (g) | | Mean | HSW (g) | | Mean |
|---|---|---|---|---|---|---|
| | 2004-05 | 2005-06 | | 2004-05 | 2005-06 | |
| Bader | 11.67b[a] | 5.45b | 8.56b | 50.65b | 65.25b | 57.95b |
| Giza429 | 22.07c | 4.44ab | 13.25b | 75.50c | 68.19b | 71.84c |
| Baraca | 19.62c | 4.38ab | 12.00b | 77.45c | 76.52b | 76.99c |
| Badï | 5.65a | 1.91a | 3.78a | 41.31a | 40.24a | 40.77a |

[a]: For each year, within a column, values followed by the same letter are not significantly different (p=0.05) based on Duncan's multiple range test

**Table 4. Faba bean shoots dry weight per plant (FBSDW/P), and number of ramifications (NR/P) per plant and number of non-emerged orobanche spikes per faba bean plant (NNEO/P) and their tubercle dry weight (OTDW/P) development per plant in faba bean plants pulled out of the soil at the early pod setting stage, at Ariana, Tunisia in the 2004-05 growing season.**

| Genotypes | FBSDQ/P | NR/P | NNEO/P | Tubercles (S2+S3)[a] | Tubercles (S4)[a] | OTDW/P |
|---|---|---|---|---|---|---|
| Bader | 9.79a[b] | 2.33a | 3.00a | 2.83a | 0.17a | 0.34a |
| Giza429 | 25.57b | 1.67a | 1.83a | 1.83a | 0.00a | 0.04a |
| Baraca | 11.49a | 1.33a | 2.33a | 2.00a | 0.33a | 0.36a |
| Badï | 9.56a | 1.50a | 6.50b | 5.67b | 0.83a | 0.76a |

[a]: Orobanche stage

[b]: Values followed by the same letter within columns are not significantly different (p=0.05) based on Duncan's multiple range test

The three resistant genotypes used in this study were obtained from the parent Giza 402 which was characterized by a low occurrence of germination stimulants in root exudates, few *O. crenata* attachments on roots, and necrosis of attached tubercles (Nassib *et al.*, 1978). The resistance of Baraca against *O. crenata* was characterized by a low number of parasite attachments per host plant and by necrosis of a large part of the attached tubercles (Zaitoun & ter Borg, 1994; Nadal *et al.*, 2004). In contrast, in our study no necrosis of attached tubercles was observed upon infection by the Tunisian population of *O. crenata* on Baraca roots. Similar resistance levels displaying no tubercle necrosis were also observed for the Tunisian breeding line Bader and Giza429. Consequently, these results show that the two genotypes Baraca and Giza429, which were selected to their resistance to *O. crenata* in Spain and Egypt respectively, do not respond similarly to the Tunisian population of *O. crenata*. This can be explained by the effect of environment conditions but also should be related to the *O. crenata* population. An analysis of genetic variation of *O. crenata* populations from Egypt, Spain and Tunisia is thus recommended. In the same way, a comparison between two *O. crenata* populations from Spain and Israel demonstrated genetic diversity between individuals within populations and between regions. The results clearly divided six populations by region, with the Spanish populations being more similar to each other than the Israeli populations (Roman *et al.*, 2002). On the other hand, the resistance to *O. crenata* observed for the breeding line Bader demonstrated that some traits of the resistance against *O. foetida* happened to be also efficient in the resistance against *O. crenata*, and thus selecting for *O. foetida* resistance can protect against *O. crenata* infection. Similar results were observed for the XBJ90.03-16-1-1-1 genotype (Abbes *et al.*, 2007a, 2007b).

We observed that the three resistant genotypes flowered earlier than Badï ones (Table 5). As suggested by Oswald & Ramson (2004), Rubiales *et al.*, (2005) and Abbes *et al.*, (2007b), this could contribute to the avoidance of Orobanche attack by delaying the life cycle of host plants. In addition, for faba bean plant height (PH), Bader was the shortest, but it had more ramifications, which led to an overall increase in seed yield (Tables 3 and 4).

In order to confirm the comparative susceptibility of the four genotypes to *O. crenata*, artificial infestation experiments were carried out in pots in a greenhouse. During these experiments, no orobanche establishment was observed on Baraca and Bader at the first harvesting date (Flowering stage), showing late in orobanche formation on these genotypes which form a disadvantage to *O. crenata* (Table 6). By the end of the experiment, differences were more pronounced and 2 groups for NEO/P and DWEO/P were identified; one consisted of Baraca, Bader and Giza429 and one containing only cv. Badï (Table 6). No tubercle necrosis was observed on all genotypes confirming thus the field results.

**Table 5. Days to flowering (DF) and plant height (PH) of faba bean genotypes in an *O. crenata* infested field in the 2004-05 and the 2005-06 growing seasons.**

| Genotypes | D F | | Mean | P H (cm) | | Mean |
|---|---|---|---|---|---|---|
| | 2004-05 | 2005-06 | | 2004-05 | 2005-06 | |
| Bader | 75.00a[a] | 81.00b | 78.00b | 62.17a | 67.00a | 64.58a |
| Giza429 | 74.00a | 75.00a | 74.50a | 80.00b | 68.25a | 74.12b |
| Baraca | 74.00a | 75.00a | 74.50a | 75.33b | 64.75a | 70.04ab |
| Badï | 77.63b | 82.67c | 80.15c | 79.92b | 65.28a | 72.60b |

[a]: For each year, within a column, values followed by the same letter are not significantly different (p= 0.05) based on Duncan's multiple range test.

**Table 6. Number of non-emerged orobanche spikes (NNEO/P) and emerged orobanche spikes per faba bean plant (NEO/P) and their tubercle dry weight (OTDW/P) per plant in faba plants in pot experiment. D1: Flowering stage; D2: Maturity stage.**

| Genotypes | Total orobanche number / plant | NNEO/P | NEO/P | OTDW/P |
|---|---|---|---|---|
| **Date 1:** | | | | |
| Bader | 0.00a[a] | 0.00a | 0.00a | 0.00a |
| Giza429 | 0.50a | 0.50a | 0.00a | 0.10a |
| Baraca | 0.00a | 0.00a | 0.00a | 0.00a |
| Badï | 1.33a | 1.33a | 0.00a | 0.25a |
| **Date 2:** | | | | |
| Bader | 1.33a | 0.00a | 1.33a | 1.03a |
| Giza429 | 0.67a | 0.33a | 0.33a | 0.10a |
| Baraca | 0.67a | 0.33a | 0.33a | 0.13a |
| Badï | 6.67b | 4.00b | 2.67b | 2.17b |

[a]: Values followed by the same letter within a column are not significantly different (p=0.05) based on Duncan's multiple range test.

Both the earliness of flowering and the late of orobanche attachment significantly reduced the dry matter of the parasite, and in consequence led to a better growth of the host plant. This was also observed in cv. Najeh resistant to both *O. crenata* and *O. foetida* (Abbes *et al.*, 2007a, b). Its resistance was mainly due to reduced orobanche seed germination, a deeper root system and a delay in both parasite attachments to the host roots and growth of the attached parasites (Abbes *et al.*, 2006, 2007b, 2010b). The limited growth of parasites on this genotype was explained by the low soluble invertase activity, the low osmotic potential of the infected roots and the organic nitrogen deficiency of the host phloem sap (Abbes *et al.*, 2009a, 2009b). Several other mechanisms like the development of mechanical and physiological barriers to infection (Nassib *et al*., 1984; Wegmann, 1986; Perez-de-Luque *et al.*, 2005; Khan *et al.*, 2009) were involved in the resistance of faba bean to orobanche. More detailed studies on the relationships between the three resistant lines used in this study (especially Bader) and *O. crenata* could be useful in order to determine the mechanisms implied in this resistance.

In conclusion, the results of this study will be used to help farmers to choose the best genotypes for areas that are infested by *O. crenata* and then form cost of a project on an integrated *Orobanche* control package.

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