Study of some resistance mechanisms to *Orobanche* spp. infestation in faba bean (*Vicia faba* L.) breeding lines in Tunisia

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ABSTRACT

The behavior of seven faba bean breeding lines toward *Orobanche foetida* and *Orobanche crenata* infestation was examined under field, pots, and *in vitro* conditions and compared to reference cultivars. The breeding lines presented resistance reaction to *Orobanche* spp. in different experiment conditions. In infested field by *O. foetida*, the grain yield reduction ranged from 55.7 to 83% for the breeding lines compared to 97% for the susceptible cultivar Badi. Lines L6 and L7 were the less affected by *Orobanche* parasitism considering severity, number of emerged *Orobanche*, and yield. In pots, the number of attachments varied from 0.6 to 3.4 and from 1.4 to 6.4 for the breeding lines against 10.4 and 12.3 for Badi inoculated, respectively, by *O. foetida* and *O. crenata*. In Petri dish experiment, *Orobanche* germination reached the highest rates; 69.9 and 59.7%, respectively, with *O. crenata* and *O. foetida* for Badi. For the breeding lines, it ranged from 6.3 to 44.9% for *O. crenata* and from 4.8 to 40.8% for *O. foetida*. Moreover, all breeding lines showed low tubercles number and delay in *Orobanche* attachments as compared to Badi. All breeding lines, except L5, maintained an acceptable level of resistance to *Orobanche* species manifested by a reduced *Orobanche* germination rate, low *Orobanche* number and dry weight, delay of attachments, and higher grain production compared to Badi. L5 seems to be less resistant even it behaves better than Badi in different culture conditions. The studied breeding lines could be recommended as resistance sources or candidates for varieties registrations.

Grain legume such as faba bean (*Vicia faba* L.) is an important crop cultivated worldwide as the primary source of protein for human food and animal feed (Abang et al., 2007). In East Africa, the Middle East and Mediterranean region, the development of this crop is facing many biotic stresses (Abang et al., 2007). The root parasitic weeds (*Orobanche* spp.) are the most damaging pathogens on the crop. In Tunisia, broomrapes *Orobanche foetida* and *Orobanche crenata* are known to be detrimental on faba bean. *O. foetida* has been reported to damage faba bean only in Tunisia (Kharrat et al., 1992). The considerable grain losses can reach more than 95% in highly infested fields (Abbes, Kharrat, & Delavault et al., 2007; Kharrat et al., 2010) depending on host susceptibility, level of infestation, and environmental conditions.

*Orobanche* seeds germination occurs after a preconditioning period (moist and suitable temperatures for several days) and exposure to germination stimulants exuded by host roots (Sato et al., 2003). After several weeks of underground development, the parasite emerges above the soil surface and develops flowering stems which produce seeds within a short period of time. Most of the seeds in the soil will not be affected by the stimulant, forming a seed bank for the next cropping seasons. They can remain viable in the soil for more than 10 years, thus, if host crops are frequently cultivated, the seed bank in the soil increases tremendously leading to the failure of cultivating host crops. These characteristics limit the development of successful control measures which can be accepted and applied. However, several methods of control were developed in different countries in the Mediterranean region including cultural, mechanical, physical, chemical, biological, germination stimulants resistant varieties, and other innovative techniques were suggested (Abbes et al., 2014; Bouraoui et al., 2012, 2016; Fernández-Aparicio et al., 2011). However, no one single method can give satisfactory control; they only allow the reduction of infestations. Breeding for *Orobanche*-resistant crop plants as a long-term measure for *Orobanche* control seems to be more suitable than costly and doubtful chemical or physical control procedures to reduce the infestation and to improve the yield of faba bean in infested fields. However, breeding...
for *Orobanche* resistance is difficult due to limited sources of resistance, complex nature of the resistance mechanism, and low heritability (Pérez-de Luque et al., 2010).

Breeding for resistance to broomrapes in faba bean against *O. crenata* (Rubiales et al., 2014; Trabelsi et al., 2015) was well studied. Rubiales et al. (2014) summarized the work conducted in legume breeding for broomrape resistance. It showed various degrees of susceptibility/resistance in faba bean to broomrape. Only one broad bean variety with resistance to *O. crenata*, F-402 that was identified in Egypt, has been successfully used in breeding programs (Nassib et al., 1984). Recently Baraca (Spanish cultivar) was also selected for its resistance to *O. crenata* (Nadal et al., 2004). In Tunisia, two new faba bean small seed varieties were developed by the national breeding program: ‘Najeh’ and ‘Chourouk’. Both varieties are registered as partial resistant varieties to *O. foetida* and faba bean small seeds breeding lines selected by INRA Rennes (France).

| Breeding lines/varieties/Pedigree | Origin/characteristics |
|----------------------------------|------------------------|
| L1: XAR-VF00.12–12-3–1-3–1-3     | Cross performed in Ariana (Tunisia) in 2000, between Tunisian breeding line resistant to *O. foetida* and large seeds population Malti |
| L2: XAR-VF00.13–8-3–1-1-1-1      | Cross performed in Ariana (Tunisia) in 2000, between Tunisian breeding line resistant to *O. foetida* and faba bean small seeds breeding lines selected by INRA Rennes (France) |
| L3: XAR-VF00.13–89–2–1-1-1-1-1-1 | Cross performed in Ariana (Tunisia) in 2000, between Tunisian breeding line resistant to *O. foetida* and faba bean small seeds breeding lines selected by INRA Rennes (France) |
| L4: XBJ92.10–27-1-1-1-1-1-1      | Cross performed in Beja (Tunisia) in 1992 between faba bean breeding line selected for resistance to *O. crenata* by ICARDA and faba bean small seeds selected by INRAT |
| L5: XBJ92–10–46–1–3–1–1–1–1–1–1–6–A | Cross performed in Beja (Tunisia) in 1992 between faba bean breeding line selected for resistance to *O. crenata* by ICARDA and faba bean small seeds selected by INRAT |
| L6: XBJ90.04–6–2–1-1–1-4-C       | Cross performed in Beja (Tunisia) in 1990 faba bean breeding line selected for resistance to *O. crenata* by ICARDA and faba bean small seeds local population |
| L7: XBJ90.04–2–3–1-1–1-1-2-A     | Cross performed in Beja (Tunisia) in 1990 faba bean breeding line selected for resistance to *O. crenata* by ICARDA and faba bean small seeds local population |
| Baraca                           | Spain / Partial resistant variety to *O. crenata* |
| Najeh                            | Small seeded variety released in 2009 / partial resistant variety to *O. foetida* and *O. crenata* |
| Badi                             | Small seeded variety released in 2004 / susceptible to *O. foetida* and *O. crenata* |

1. Material and methods

1.1. Plant material

Ten faba bean entries were used in this study (Table 1); the susceptible control: cv. Badi, the resistant cultivar to *O. foetida* and *O. crenata*: cv. Najeh (Abbes, Kharrat, & Delavault et al., 2007; Kharrat et al., 2010), the resistant cultivar to *O. crenata*: cv. Baraca (Nadal et al., 2004) and finally seven pure breeding lines developed by the Field Crop Laboratory at the National Institute for Agricultural Research of Tunisia (INRAT). The breeding lines were selected under insect proof cages in naturally infested field by *O. foetida* for more than 10 years (Table 1).

*O. foetida* and *O. crenata* seeds were collected in 2012 from mature spikes in infested faba bean fields, respectively, from Beja and from Ariana (Tunisia).
1.2. Field trial

The tested faba bean entries were sown in Oued Beja Experimental Unit (36°44′ N; 9°13′ E; 150 m a.s.l., in northwest Tunisia in a subhumid climate) in a randomized complete block design with three replicates during two cropping seasons (2012/2013) and (2013/2014). Each breeding line was sown in plot consisted of four rows of 4 m length and .5 m inter-row spacing (8 m²) in the first week of December and the seedling rate was 24 seeds m⁻². At the maturity stage, the severity using a 1–9 scale, the incidence (percentage of faba bean plants presenting Orobanche shoots), (Abbes, Kharrat, & Delavault et al., 2007), the number of emerged Orobanche per plant, and the grain yield in non-infested and naturally infested fields by O. foetida were recorded.

1.3. Pots experiment

Pots experiments were carried out in order to assess the behavior of seven faba bean breeding lines selected for their partial resistance to O. foetida and O. crenata and compare them to the partial resistant cultivars (cvs. Najah and Baraca) and the susceptible one (cv. Badi). Moreover, pot trials are needed to confirm that the breeding lines remaining less infected in the field are truly resistant. Pot methods allow control over the environment, the inoculum density and its origin.

Seeds of different faba bean breeding lines were surface sterilized with calcium hypochlorite (5%) during 15 min then rinsed four times with sterilized distilled water. Artificial inoculation was performed by mixing 20 mg of O. foetida or O. crenata per kg of soil uniformly. Faba bean seeds previously disinfected were planted in 5 L capacity pots (free and infested by fetid or crenate broomrapes). Five pots per breeding line were used for each treatment. Pots were placed under natural conditions at Ariana during 2012/2013 cropping season. Pots were watered with tap water when necessary. Three month later, at the pod setting stage, faba bean plants were uprooted from the soil and washed carefully. The Orobanche attachments were enumerated and classified according to their stage of development based on Sillero et al. (2005) scale (S1–S5). Total and underground attachments per plant (TAN/P, UAN/P) and emerged Orobanche number per plant (ESN/P) were determined. The dry weight of faba bean stems (SDW/P) and roots per plant (RDW/P) and Orobanche attachments dry weight per plant (ADW/P) were recorded after being dried in an oven at 70 °C during 72 h. The contents of the photosynthetic pigments chlorophyll a (chl a), chlorophyll b (chl b) and total chlorophyll (chl t) was determined on leaves of the fifth nodes at pod setting stage for control and infected breeding lines with O. foetida or O. crenata as described by Arnon (1949).

1.4. Hydroponic co-culture experiment

Faba bean breeding lines evaluated in pots were further studied in co-cultures (faba bean breeding line/Orobanche species) experiment carried out in quadratic plastic dishes. The objectives were to test their potential for Orobanche germination induction and dynamic of attachment and Orobanche growth.

O. foetida and O. crenata seeds were surface sterilized for 5 min in sodium hypochlorite (2%) and rinsed five times with sterile distilled water. Twenty (20 mg) of Orobanche seeds were used for each Petri dish. Faba bean seeds were disinfected as described previously for pot experiment before being sown in glass Petri dishes containing water agar and placed in the laboratory in darkness conditions at 22 ± 3 °C for germination. For each breeding line, eleven quadratic plastic Petri dishes (120 × 120 mm²) were filled with sterilized sand and covered with moistened sterilized glass fiber filter paper. Three small holes were made in two opposite sides to allow the stem development outside the Petri dish and nutrient uptake. Orobanche seeds previously disinfected were spread carefully over the glass fiber filter paper surface and faba bean seedlings were transferred to the plastic Petri dishes. Petri dishes were placed in containers and the bottom of this system was soaked in modified nutrient solution (Vincent, 1970) (with reduced amount of nitrogen). The whole was covered with aluminum foil and maintained under natural light at 22 ± 3 °C and in humidity 78% in the green house. The germination rate of O. foetida and O. crenata was determined closely to the faba bean roots weekly from 30 to 58 days after inoculation under a binocular microscope. On the front face of the plastic cover four 1 cm² rectangles were drawn at different levels in order to estimate the percent germination of Orobanche seeds. Broomrape attachments were recorded weekly over a period of 77 days.

1.5. Statistical analysis

ANOVA was performed using the SPSS statistical program v.15 (IBM Corporation, Armonk, New York, U.S.A). Mean comparisons were made using Duncan’s multiple-range test at p = .05. Pearson correlation coefficients were determined on parameters recorded for pot experiment using the same software.

2. Results

2.1. Field trial

During the two cropping seasons 2012/2013 and 2013/2014, the severity, the incidence and the number of emerged Orobanche spikes per plant were recorded at the maturity stage in infested field by O. foetida in Beja. By meaning the
two cropping seasons, results revealed that the susceptible check cv. Badi followed by L5 presented significantly the highest value for the three previously mentioned parameters (Table 2). The remaining breeding lines carried a high level of resistance to O. foetida. L6 followed by L7 were the less affected by Orobanche parasitism according to the severity, the incidence and the number of emerged Orobanche. Thus, the number of Orobanche spikes was two-fold lower than the susceptible check Badi for these two breeding lines. In non-infested fields, the grain yield of the seven breeding lines was significantly higher (p = 0.000) than Badi (Table 3). It varied from 10.1 g plant\(^{-1}\) to 13.1 g plant\(^{-1}\) against 6.2 g plant\(^{-1}\) for Badi. Nevertheless, in infested field, the grain yield decreased for the studied breeding lines and the grain yield reductions reached the maximum for Badi (97.4%). For lines L1–L4, L6, and L7 the grain yield reductions did not exceed 70.4%. However, the reduction was higher for L5 than the remaining lines (83.2%). Despite the important yield losses due to Orobanche parasitism, these breeding lines maintained good seed production in infested field compared to the susceptible check (Table 3).

### 2.2. Pots experiments

The results showed significant differences (p = .001) between the studied breeding lines for the average number of attachments per plant which varied from .6 to 10.4 in O. foetida-inoculated pots and from 1.4 to 12.3 in O. crenata-inoculated pots (Table 4). For both Orobanche species, the number of attachment was significantly lower for the tested breeding lines and both resistant checks (Najeh and Baraca) compared to the susceptible cultivar Badi. No significant differences were observed between tested breeding lines for O. crenata dry matter weight per plant (p = .92) meanwhile, significant differences were observed between the breeding lines inoculated with O. foetida (p = .05). Breeding lines L1, L4, L6, L7, and Najeh had significant lower dry weight of O. foetida per plant than the susceptible check Badi (5 g/plant). The breeding line L7 showed low average number (.8) and weight (1.1 g) of O. foetida attachments per plant without emerged spikes. Nevertheless, it presented higher number of O. crenata attachments per plant (3.8) which is maintained significantly lower than the susceptible check Badi (Table 4). For the partial resistant check of Baraca infested by O. crenata,
all attachments evolved in emerged spikes, nevertheless the susceptible cultivar Badi showed limited development of attached tubercle to stage 5 (emerged spikes). In fact, 21.1% of *O. foetida* and 27% of *O. crenata* attachments evolved toward emerged spikes. High significant differences (*p < .001*) were observed between breeding lines for the number of subterranean attachments (S1–S4) for both *Orobanche* species meanwhile there were no significant differences in emerged spikes (S5). No necrosis of attached tubercles was observed in this experiment.

Stem and root dry weight and pod number per plant were recorded in the pot experiment (infested and non-infested) and average data are presented in Table 5. ANOVA analysis showed high significant differences between the breeding lines in pod number per plant in non-infested pots (*p = .002*) and in infested pots with *O. foetida* (*p = .003*), however in infested pots with *O. crenata* there were no significant differences (*p = .308*). Almost all the breeding lines, except L7 and cv. Najeh infected by *O. foetida*, were seriously affected by *Orobanche* parasitism especially *O. crenata*. The pod number per plant in infested pots by *O. crenata* was nil for the resistant cultivar Baraca and the susceptible check Badi. Regarding the stems dry weight per plant, the differences were significant between the breeding lines in infested pots by *O. crenata* (*p = .038*) and *O. foetida* (*p < .001*) and the non-infested pots (*p = .031*). *O. crenata* affected more the stems dry weight of /plant than *O. foetida*. For the roots dry weight per plant, the differences between the breeding lines were significant only in pots infested with *O. foetida* (*p = .032*) and non-infested pots (*p = .01*). Nevertheless, the decrease of the root dry weight by comparing the non-infested and infested pots by *O. crenata* is remarkable. The cv. Badi (susceptible check) was the most affected by both *Orobanche* species as it can be observed for the traits reported in Tables 4 and 5. It presented the lowest stem and root dry weight in the presence of both *Orobanche* species. Moreover, no pod development was observed for Badi infected with *O. crenata* and only .6 pod/plant with *O. foetida* (Table 5).

In general, based on the three studied traits (the pod production and the development of the host plants by weighting the dried stems and roots), it appears that *crena*te broomrape is more aggressive than fetid one (Table 5).

The contents of photosynthetically active pigments (chl a, chl b) estimated in leaves of *V. faba* plants in pots experiments were shown in Figure 1. Chlorophyll a content is predominant for all entries. ANOVA showed significant differences between entries for the Chl a (*p = .001*),

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**Table 4.** The attachments number (TAN/P, UAN/P and ESN/P) and dry weight (ADW/P) of *O. foetida* and *O. crenata* in pots.

| Breeding lines/varieties | Total Attachments (S1-55) Number/Plant (TAN/P) | Attachments (S1-55) Dry Weight/Plant (g) (ADW/P) | Underground Attachments (S1-54) Number/Plant (UAN/P) | Emerged Spikes (SS) Number/Plant (ESN/P) |
|--------------------------|-----------------------------------------------|--------------------------------------------------|-----------------------------------------------------|----------------------------------------|
|                          | *O. foetida* | *O. crenata* | *O. foetida* | *O. crenata* | *O. foetida* | *O. crenata* | *O. foetida* | *O. crenata* | *O. foetida* | *O. crenata* |
| L1                       | .60 a*       | 3.80 ab     | 1.96 ab     | 3.22 a      | .20 a       | 1.40 ab     | .40 a       | 2.40 a      |
| L2                       | 2.00 a       | 6.40 b      | .50 ab      | 2.62 a      | .20 a       | 4.60 b      | 1.80 a      | 1.80 a      |
| L3                       | 3.40 a       | 4.00 ab     | 1.27 ab     | 3.27 a      | 1.80 a      | 1.20 ab     | 1.00 a      | 2.80 a      |
| L4                       | 5.20 a       | 3.80 ab     | .17 a       | 1.58 a      | 1.20 ab     | .00 a       | 2.60 a      | 2.00 a      |
| Baraca                   | 1.40 a       | 7.25 ab     | 1.39 ab     | 2.85 a      | .80 a       | .25 a       | .60 a       | 2.50 a      |
| Badi                     | 10.40 b      | 12.33 c     | 5.08 c      | 4.32 a      | 8.20 b      | 8.66 c      | 2.20 a      | 3.33 a      |

*Data with the same letter per column are not significantly different according to Duncan’s test (*p = .05).*

**Table 5.** The stem (SDW) and root dry weight (RDW) and pod number/plant (PN/P) of different entries infested or not by *O. foetida* and *O. crenata* in pots.

| Breeding lines/ varieties | Stem Dry Weight/Plant SDW/P (g) | Root Dry Weight/Plant RDW/P (g) | Pod Number/plant (PN/P) |
|---------------------------|--------------------------------|---------------------------------|------------------------|
|                           | Non Infested | *O. foetida* | *O. crenata* | Non Infested | *O. foetida* | *O. crenata* | Non Infested | *O. foetida* | *O. crenata* | Non Infested | *O. foetida* | *O. crenata* |
| L1                        | 9.47 ab*     | 6.03 bc     | 3.16 abc    | 1.22 a      | 2.11 abc    | 1.16 a      | 2.80 ab      | 2.00 ab      | .40 a       |
| L2                        | 13.38 c      | 5.49 b      | 4.41 bc     | 2.74 bc     | 1.91 ab     | 1.39 a      | 5.40 c       | 2.20 ab      | 1.00 a      |
| L3                        | 11.42 abc    | 7.20 bcd    | 3.52 abc    | 1.20 a      | 1.68 ab     | 1.36 a      | 3.60 ab      | 3.20 ab      | .60 a       |
| L4                        | 11.94 bc     | 10.80 e     | 5.57 c      | 3.59 c      | 3.20 ab     | 1.48 a      | 4.40 bc      | 3.40 bc      | 1.00 a      |
| L5                        | 9.92 ab      | 7.33 bcd    | 3.97 abc    | 1.82 ab     | 1.66 ab     | 1.23 a      | 5.80 c       | 2.80 bc      | 1.40 a      |
| L6                        | 9.66 abc     | 9.16 cde    | 5.65 c      | 1.82 ab     | 3.80 c      | 1.96 a      | 4.40 bc      | 3.40 bc      | 1.00 a      |
| L7                        | 11.87 bc     | 8.71 bcd    | 4.29 abc    | 2.70 abc    | 2.73 bc     | .90 a       | 3.40 ab      | 3.80 bc      | 1.00 a      |
| Baraca                    | 8.48 ab      | 9.85 de     | 2.24 abc    | 1.82 ab     | 2.83 bc     | .59 a       | 3.20 ab      | 2.40 b       | .00 a       |
| Najeh                     | 8.75 ab      | 8.21 bcd    | 3.40 abc    | 3.07 bc     | 1.88 ab     | .60 a       | 2.40 a       | 4.20 c       | 1.25 a      |
| Badi                      | 7.70 a       | 2.27 a      | 1.60 a      | 2.42 abc    | .70 a       | .46 a       | 2.80 ab      | .60 a        | .00 a       |

*Data with the same letter per column are not significantly different according to Duncan’s test (*p = .05).*
Chl b ($p = .002$), and Chl t ($p = .001$) in *O. crenata*-infested pots. However, no significant differences were recorded in infested pots by *O. foetida*. For the non-infested pots, the differences were significant only for Chl a ($p = .001$) and Chl t ($p = .007$). Parasitism by *Orobanche* leads to decrease chlorophyll content for all entries except Baraca (Figure 1). Generally, in non-infested pots, chlorophyll content was higher than in infested pots due to the *Orobanche* effect and it decreased in pots infested by *O. crenata* more than in pots infested by *O. foetida*.

Pearson correlation coefficients between different recorded parameters are presented in Table 6. It appears that chlorophyll content was negatively correlated to *Orobanche* infestation traits (TAn, ESN, ADW, and UAn) and positively correlated with plant growth traits (SDW, RDW). The stem dry weight (SDW) was negatively correlated with TAN, UAn, ESN, and ADW, whereas the root dry weight (RDW) was negatively correlated only with the three previous traits.

### 2.3. Co-cultures experiment

Percentage of germination for both *Orobanche* species, reached the maximum after 44 days for most of the faba bean entries (Figures 2 and 3). High significant differences ($p < .001$) between the breeding lines were observed for germination induction for both *Orobanche* species. The percentage of *Orobanche* germination varied from 6.3 to 69.9% for *O. crenata* and from 4.8 to 59.5% for *O. foetida*. The highest broomrapes germination level was recorded for the susceptible check Badî, inoculated with *O. foetida*. For the remaining breeding lines, the germination rate did not exceed 44.9% recorded for L5 with almost similar behavior toward both *Orobanche* species.

For the different studied breeding lines, high significant differences ($p < .001$) were observed for the tubercles number per plant. The susceptible check Badî showed the highest infestation level after 77 days with 22.30 and 24.60 attachments, respectively, for *O. crenata* and *O. foetida*.

### Table 6. Pearson phenotypic correlation between different traits: Stem Dry Weight (g) (SDW); Root Dry Weight (g) (RDW); Total Attachments Number/Plant (TAN); Underground Attachments Number/Plant (UAN); Emerged Spikes Number/Plant (ESN); Attachments Dry Weight (g)/Plant (ADW); Chlorophyll a (mg/L) (Chl a); Chlorophyll b (mg/L) (Chl b); Total Chlorophyll (mg/L) (Chl t).

|                  | SDW(g) | RDW(g) | TAN     | UAN     | ESN     | ADW(g) | Chl a (mg/L) | Chl b (mg/L) | Chl t (mg/L) |
|------------------|--------|--------|---------|---------|---------|--------|--------------|--------------|--------------|
| SDW(g)           | 0.625*** | -0.419* | -0.266** | -0.486** | -0.473** | 0.501** | 0.386**      | 0.484**      |              |
| RDW(g)           | -0.297ns | -0.191ns | -0.34ns  | -0.269* | 0.451*  | -0.320ns | 0.438*       |              |              |
| TAN              | 0.902*** | 0.744*** | 0.382ns  | 0.490** | -0.274ns | -0.320ns | 0.278ns      |              |              |
| UAN              |         |        |         |         |         |        |              |              |              |
| ESN              |         |        |         |         |         |        |              |              |              |
| ADW(g)           |         |        |         |         |         |        |              |              |              |
| Chl a (mg/L)     |         |        |         |         |         |        |              |              |              |
| Chl b (mg/L)     |         |        |         |         |         |        |              |              |              |
| Chl t (mg/L)     |         |        |         |         |         |        |              |              |              |

nsNon Significant; ***$p \leq .001$; **$0.001 < p \leq .01$; *$0.01 < p \leq .05$
Orobanche tubercle reached stage 4 on all the tested lines inoculated by O. crenata. Compared to the susceptible check Badi for which tubercle stage 4 was observed earlier (56 days after inoculation) with a high percentage of tubercles reaching stage 4 (39.8%), tubercle growth on other tested lines was slower and took 70–77 days after inoculation to reach stage 4. The percentage of tubercles reaching stage 4 ranged from 12.5 to 35.7% for these lines. No necrosis of attached tubercles was observed even in Petri dish experiment.

3. Discussion

In the present study, the level of resistance of some faba bean breeding lines to Orobanche spp. was performed (i) under open-field conditions in infested and non-infested field by O. foetida and (ii) under controlled conditions in pots and Petri dishes co-cultures experiments. These experiments allowed a better understanding of the resistance mechanisms involved in the tested breeding lines to O. foetida and O. crenata.

In many previous studies, the resistance level to Orobanche species in many cultivated crops was evaluated by authors using different approaches and parameters based mainly on the number of Orobanche tubercles/shoots per host plant and the impact of the parasite on grain yield and host plant development (Abbes & Kharrat et al., 2007; Fernández-Aparicio et al., 2007; Kharrat et al., 2010; Rubiales et al., 2006; Zeid et al., 2013). In this study, the field evaluation performed in infested plot by O. foetida was based mainly on the severity, incidence, number of Orobanche spikes, and the grain yield. Results showed that the seven tested breeding lines, except L5, showed good resistance level to O. foetida that was expressed by a low parasitism severity and number of emerged Orobanche spikes. The breeding line L5 appeared to behave differently compared to previous results showed by Trabelsi et al. (2015). This can be explained by the influence of cultural and climatic conditions as indicated by several authors (Maalouf et al., 2011; Rubiales, Alcántara et al., 2003). The other breeding lines, especially L6 and L7, were significantly less affected by O. foetida parasitism compared to the susceptible check Badi. Similar results were found by Trabelsi et al. (2015) in infested fields by O. foetida.

The use of the parameter number of emerged Orobanche shoots per host plant as the best index of the resistance that gives the most reliable estimation of the total level of infestation (Rubiales et al., 2006) is not always obvious especially in highly infested fields where the number of emerged spikes is not a good criterion for screening. Thus, an important number of tubercles per one host plant can be in concurrence for nutrients resulting in a limited Orobanche development and emergence. In such
maximum recorded for L3 against almost total yield loss recorded for the susceptible check.

In order to confirm results obtained in field conditions, artificial infestation experiments were carried out in pots and Petri dishes using two *Orobanche* species. In pots experiment, for both *Orobanche* species, the most important infestation level was observed for the susceptible check Badï, which presented the highest number of tubercles against an important resistance level observed for the seven breeding lines. In this experiment, the seven breeding lines were significantly less affected compared to cv. Badï which is the most affected by *Orobanche* parasitism conditions, grain production and the development capacity of the host plant are the main parameters for screening for resistance to *Orobanche*.

Sillero et al. (1996) suggested that a screening based only on the number of emerged stems was misleading, and that the health of the host plant must also be considered. In our study, the parameters’ severity and grain yield are sufficient to distinguish the resistance of the breeding lines (except for L5) from the susceptible check Badï. All the breeding lines showed lower severity parasitism than cv. Badï. The low infection level recorded for these breeding lines resulted in relatively important grain yield with a

**Figure 4.** Evolution of the total tubercle number (*O. foetida* and *O. crenata*) of 10 faba bean entries.

*Note.* Data are means ± SE.
as indicated by the significant reduction of shoot and root DW and pod number per plant.

This could be related to a source–sink competition for nutrients between the plant development (pod setting) and *Orobanche* tubercles development (Grenz et al., 2005). Ter Borg et al. (1994) signaled that the tubercle number per plant is the major indicator of resistance to broomrape and that higher is the number of *Orobanche* on the host plant, lower is the average weight of the host plant biomass. A significant decrease was also observed in chlorophyll content under *Orobanche* infection compared to non-infested plants for all breeding lines. This was observed for other pathosystems as chickpea/*O. foetida* and tomato/*Phelipanche ramosa* (Mauromica et al., 2008; Nefzi et al., 2016). These parasites caused significant reduction in their hosts’ development and in the chlorophyll content of their leaves resulting in an altered photosynthetic capacity in the host plant.

In addition, the proportion of tubercles reaching the stage 5 was recorded on all entries. Results showed that for L7, none of *O. foetida* attached tubercles has reached the stage 5 and only 15.7% of *O. crenata* tubercles succeeded to evolve into stage 5. For the resistant check of Baraca inoculated by *O. crenata*, all attachments (2) emerged above ground against limited development of tubercles observed on the susceptible check Badi (21.1 and 27% for, respectively, *O. foetida* and *O. crenata*). This can be explained by the high competition level between tubercles for water and nutrients (Rubiales et al., 2006; Ter Borg et al., 1994; Zeid et al., 2013).

In Petri dishes experiment, the germination of *Orobanche* seed and the number and the growth of the attached tubercles for both *Orobanche* species were recorded for the different faba bean entries. For both *Orobanche* species, the faba bean selected lines showed low germination levels compared to the resistant cv. Najeh and the susceptible check Badi. Except L5, for which 44.9% of *Orobanche* seed germinated after 44 days, the *Orobanche* seed germination rate on the other breeding lines did not exceed 17.2% with similar behavior toward both *Orobanche* species. These results indicated that *Orobanche* seeds germination rate can be taken as an indicator of resistance of the majority of breeding lines to both *Orobanche* species. Most studies on legume resistance to broomrape concluded that resistance is correlated with low stimulatory activity by root exudates of the host plant (Abbes, Kharrat, & Simier et al., 2007, 2010; Rubiales, Pérez-De-Luque, Cubero et al., 2003; Rubiales et al., 2004; Rubiales et al., 2006). According to Cubero and Hernández (1991), the percentage of *Orobanche* seed germination is considered as the best criteria for distinguishing between susceptible and resistant lines. In several previous studies, the low germination stimulant production reported in some host plant species was also advanced as one of mechanisms of resistance in some breeding genotypes (Fernández-Aparicio et al., 2009b; Pérez-de Luque et al., 2010). Yoneyama et al. (2010) reported that any low germination probably derives from a low production of stimulants. The resistance expressed by the breeding lines L1–L4, L6, L7, and cv. Najeh can be explained by a low secretion level of germination stimulant by the host plant root system (Trabelsi et al., unpublished data). In a recent study, Fernández-Aparicio et al. (2014) confirmed resistance to parasitic weeds based on low strigolactone exudation within faba bean germplasm. On the other hand, the studied resistant breeding lines may produce also inhibitors in the root exudates as it was reported by Serghini et al. (2001) for sunflower and Evidente et al. (2007) for fenugreek. Conversely, previous studies indicated that resistant lines produced similar or even higher parasite germination compared to susceptible lines (Ter Borg et al., 1994). This could explain the partial resistance reaction of L5 (in pots and Petri dishes experiments) which presented almost high germination rate as compared by the remaining breeding lines suggesting that other additional resistance/tolerance mechanisms were involved. For example, the resistance of Giza 402 to *O. crenata* was explained by the delay of release of *Orobanche* germination stimulants (Al-Menouf, 1991). The low infection of the Tunisian cultivar Najeh was related to a delay of the parasite attachment and a deeper root system allowed to the cultivar to escape *Orobanche* seeds (Abbes, Kharrat, & Delavault et al., 2007; Abbes, Kharrat, Simier et al., 2007).

In this experiment, the tubercles number was low for the seven breeding lines and did not exceed 5.7 and 9.8, respectively, for *O. foetida* and *O. crenata* against high infection level for the susceptible cv. Badi with 22.3 and 24.6 attachments, respectively, for *O. crenata* and *O. foetida*. Some breeding lines were characterized by a delay in *Orobanche* attachments which can reach two weeks in comparison to those attached on cv. Badi. The reduced number of tubercles fixed on the roots of the different breeding lines was also associated to a slow parasite development once attached to their roots expressed by the very low percentage of tubercles reaching the stage 4. In contrast, the high number of observed attachments on cv. Badi was associated to higher parasite growth rate. This late tubercle growth was observed in many other pathosystems: sunflower – *O. cumana*; pea – *O. crenata*; faba bean – *O. crenata*; chickpea – *O. foetida*, and faba bean – *O. foetida* (Abbes et al., 2010; Labrousse et al., 2001; Nefzi et al., 2016; Pérez-de-Luque & Rubiales et al., 2005; Pérez-de-Luque et al., 2007). The delay in the attachments of *Orobanche* tubercles and the slow in their growth once fixed on host roots can be explained by various resistance mechanisms.
For example, this can be related to the production of physical barriers that reduce water and nutrient fluxes between host and parasite as a result of changes in host cell walls at the infection site, such as lignification, calse apposition in host phloem cells, or accumulation of secretions or gels leading to xylem occlusion (Abbes et al., 2010; Labrousse et al., 2001; Pérez-de-Luque & Jorrin et al., 2005; Pérez-de-Luque et al., 2007). For cv. Najeh, the slow in the tubercles growth after their formation is due to low soluble invertase activity, low osmotic potential of the infected roots and the organic nitrogen deficiency of the host phloem sap (Abbes et al., 2009a, 2009b). Slow growth tubercles were also observed on faba bean roots following inoculation by some rhizobium strains (Bouraoui et al., 2016).

Finally, in this study, no necrosis of attached tubercles was observed in the different experiments. In general, based on the different experiments, it appears that crenate broomrape is more aggressive than fetid one.

4. Conclusion

In the present work, the studied breeding lines showed high level of resistance to Orobanche spp. infestation in field, pots, and co-culture in Petri dishes, except L5 which appeared to behave differently. Under infested field conditions, the tested lines developed few number of Orobanche and remain productive in infested field by O. foetida. In pots, the resistant breeding lines developed few tubercles. Furthermore, Orobanche tubercle development (but not seed germination) was delayed for some studied breeding lines in Petri dishes experiments. Different explanations could be proposed for this. It could be assigned to the low percentage of O. foetida and O. crenata germination or other resistance mechanisms acting after seeds germination such as the development of mechanical and physiological barriers as it was reported by some authors (Khan et al., 2009; Perez-de-Luque & Jorrin et al., 2005).

So far more investigations on the capacity of the tested breeding lines to induce broomrape seed germination and their ability to limit the parasite attachments and development are required. The presence of inhibitors must be confirmed and their role must be more investigated.

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References

Abbes, Z., Kharrat, M., Delavault, P., Simier, P., & Chaibi, W. (2007). Field evaluation of the resistance of some faba bean (Vicia faba L.) genotypes to the parasitic weed Orobanche foetida Poir. Crop Protection, 26, 1777–1784.

Abbes, Z., Kharrat, M., Simier, P., & Chaibi, W. (2007). Characterization of resistance to crenate broomrape (Orobanche crenata) in a new small-seeded line of Tunisian faba beans. Phytoprotection, 88, 83–92.

Abbes, Z., Kharrat, M., Delavault, P., Chaibi, W., & Simier, P. (2009a). Nitrogen and carbon relationships between the parasitic weed Orobanche foetida and susceptible and tolerant faba bean lines. Plant Physiology and Biochemistry, 47, 153–159.

Abbes, Z., Kharrat, M., Delavault, P., Chaibi, W., & Simier, P. (2009b). Osmoregulation and nutritional relationships between Orobanche foetida and faba bean. Plant Signaling and Behavior, 4, 336–338.

Abbes, Z., Kharrat, M., Shaaban, K., & Bayaa, B. (2010). Comportement de différentes accessions améliorées de féverole (Vicia faba L.) vis-à-vis d’Orobanche crenata Forsk. et Orobanche foetida Poir. Cahiers Agriculture, 19, 194–199.

Abbes, Z., Sellami, F., Amri, M., & Kharrat, M. (2011). Variation in the resistance of some faba bean genotypes to Orobanche crenata. Pakistan Journal of Botany, 43, 2017–2021.

Abbes, Z., Mkadmi, M., Trabelsi, I., Amri, M., & Kharrat, M. (2014). Orobanche foetida control in faba bean by foliar application of benzothiadiazole (BTH) and salicylic acid. Bulgarian Journal of Agricultural Science, 20, 1450–1454.

Abang, M. M., Bayaa, B., Abu-Irmaileh, B., & Yahyaoui, A. (2007). A participatory farming system approach for sustainable weed management in the near East (Vicia faba L.) vis-à-vis d’Orobanche crenata Forsk. et Orobanche foetida Forsk. Obst. et Hortic. Mediterranéennes, 1, 336–338.

Al-Menoufi, O. A. (1991). Breeding Faba bean (Vicia faba L.) genotypes to the parasitic weed Orobanche foetida Poiret. Proceedings of the International Workshop on Orobanche Research (pp. 241–247). Obermarchtal, Germany.

Arnon, D. I. (1949). Copper enzymes in isolated chloroplasts. Polyphenol-oxidase in Beta vulgaris. Plant Physiology, 24, 1–15.

Bouraoui, M., Abbes, Z., Abdí, N., Hmissi, I., & Sifi, B. (2012). Evaluation of efficient Rhizobium isolates as biological control agents of Orobanche foetida Poir. Parasitizing Vicia faba L. minor in Tunisia. Bulgarian Journal of Agricultural Science, 18, 557–564.

Bouraoui, M., Abbes, Z., Rouissi, M., Abdí, N., Hmissi, I., Kouki, S., & Sifi, B. (2016). Effect of rhizobia inoculation, N and P supply on Orobanche foetida parasitizing faba bean (Vicia faba minor) under field conditions. Biocontrol Science and Technology, 26, 776–791.

Cubero, J. I., & Hernández, L. (1991). Breeding Faba bean (Vicia faba L.) for resistance to Orobanche crenata Forsk. Options Méditerranéennes, 10, 51–57.

Evidente, A., Fernández-Aparicio, M., Andolfi, A., Rubiales, D., & Motta, A. (2007). Trigoxazonane, a monosubstituted trioxazonane from Trigonella foenum-graecum root exudate, inhibits Orobanche crenata seed germination. Phytochemistry, 68, 2487–2492.
Fernández-Martínez, J. M., Domínguez, J., Pérez-Vich, B., & Velasco, L. (2008). Update on breeding for resistance to sunflower broomrape. *Helia*, 31, 73–84.

Fernández-Aparicio, M., Flores, F., & Rubiales, D. (2009a). Field response of *Lathyrus ciceria* germplasm to crenate broomrape (*Orobanche crenata*). *Field Crops Research*, 113, 321–327.

Fernández-Aparicio, M., Flores, F., & Rubiales, D. (2009b). Recognition of root exudates by seeds of broomrape (*Orobanche* and *Phelipanche*) species. *Annals of Botany*, 103, 423–431.

Fernández-Aparicio, M., Kisugi, T., Xie, X., Rubiales, D., & Yoneyama, K. (2014). Low Strigolactone root exudation: A novel mechanism of broomrape (*Orobanche* and *Phelipanche spp.*) resistance available for faba bean breeding. *Journal of Agricultural and Food Chemistry*, 62, 7063–7071.

Fernández-Aparicio, M., Pérez-de-Luque, A., Lozano, M. D., & Rubiales, D. (2007). Inoculation and growth with root parasitic weeds. In U. Mathésius, E. P. Journet, & L. W. Sumner (Eds.), *Medicago truncatula handbook*. Ardmore, OK: Samuel Roberts Noble Foundation.

Fernández-Aparicio, M., Sillero, J. C., & Rubiales, D. (2009). Resistance to broomrape in wild lentils (*Lens spp.*). *Plant Breeding*, 128, 266–270.

Fernández-Aparicio, M., Westwood, J. H., & Rubiales, D. (2011). Agronomic breeding and biotechnological approaches to parasitic plant management through manipulation of germination stimulant levels in agricultural soils. *Botany*, 89, 813–826.

Grenz, J. H., Manschadi, A. M., Uygur, F. N., & Sauerborn, J. (2005). Effects of environment and sowing date on the competition between faba bean (*Vicia faba*) and the weed parasite *Orobanche crenata*. *Field Crops Research*, 93, 300–313.

JORT. (2015). *Official Journal of Tunisian Republic*, 2015. *Ministry of Agriculture, Hydraulic Resources and Fishery*, 42, 1049–1051.

Khan, M. A., Sharif, T., Ahmad, M., Zafar, M., & Tareen, R. B. (2009). Anatomical characterization of parasitic plants of Pakistan. *Pakistan Journal of Botany*, 41, 2661–2669.

Kharrat, M., Abbès, Z., & Amri, M. (2010). A new faba bean small seeded variety Najeh tolerant to *Orobanche* registered in the Tunisian Catalogue. *Tunisian Journal of Plant Protection*, 5, 125–130.

Kharrat, M., Halila, M. H., Linke, K. H., & Haddar, T. (1992). First report of *Orobanche foetida* Poiret on faba bean in Tunisia. *Fabis Newsletter*, 30, 46–47.

Labrousse, P., Arnaud, M. C., Seriès, H., Bervillé, A., & Thalouarn, P. (2001). Several mechanisms are involved in resistance of *Helianthus* to *Orobanche cumana* Wallr. *Annals of Botany*, 88, 859–868.

Maaloulf, F., Khalil, S., Ahmed, S., Akintunde, A. N., Kharrat, M., & Shama’a, K. E., … Hajjar, S. (2011). Yield stability of faba bean lines under diverse broomrape prone production environments. *Field Crops Research*, 124, 288–294.

Mauromicale, G., Lo Monaco, A., & Longo, M. G. A. (2008). Effect of branched broomrape (*Orobanche ramosa*) infection on the growth and photosynthesis of tomato. *Weed Science*, 56, 574–581.

Nadal, S., Moreno, M. T., & Cubero, J. I. (2004). Registration of *Baraca* faba bean. *Crop Science*, 44, 1864–1865.

Nassib, A. M., Hussein, A. H. A., & El Rayes, F. M. (1984). Effect of variety chemical control sowing date and tillage on *Orobanche* spp. infestation and faba bean yield. *FABIS Newsletter*, 10, 11–15.

Nefzi, F., Trabelsi, I., Amri, M., Kharrat, M., & Abbès, Z. (2016). Response of some chickpea (*Cicer arietinum*) genotypes to *Orobanche foetida* Poiret. parasitism. *Chilean Journal of Agricultural Research*, 76, 170–178.

Pérez de Luque, A., Eizenberg, H. G., Grenz, J. H., Sillero, J. C., Ávila, C., Sauerborn, J., & Rubiales, D. (2010). Broomrape management in faba bean. *Field Crops Research*, 115, 319–328.

Pérez-de-Luque, A., Jorrin, J., Cubero, J. I., & Rubiales, D. (2005). *Orobanche crenata* resistance and avoidance in pea (*Pisum spp.*) operate at different developmental stages of the parasite. *Weed Research*, 45, 379–387.

Pérez-de-Luque, A., Lozano, M. D., Moreno, M. T., Testillano, P. S., & Rubiales, D. (2007). Resistance to broomrape (*Orobanche crenata*) in faba bean (*Vicia faba*): Cell wall changes associated with prehaustorial defensive mechanisms. *Annals of Applied Biology*, 151, 89–98.

Rubiales, D., Alcántara, C., Pérez-de-Luque, A., Gil, J., & Sillero, J. C. (2003). Infection of chickpea (*Cicer arietinum*) by crenate broomrape (*Orobanche crenata*) as influenced by sowing date and weather conditions. *Agronomie*, 23, 359–362.

Rubiales, D., Alcántara, C., & Sillero, J. C. (2004). Variation in resistance to crenate broomrape (*Orobanche crenata*) in species of *Cicer*. *Weed Research*, 44, 27–32.

Rubiales, D., Fernández-Aparicio, M., Pérez-De-Luque, A., Prats, E., Castillejo, M. A., Sillero, J. C., … Fondevilla, S. (2009). Breeding approaches for crenate broomrape (*Orobanche crenata Forsk.*) management in pea (*Pisum sativum* L.). *Pest Management Science*, 65, 553–559.

Rubiales, D., Flores, F., Emeran, A. A., Kharrat, M., Amri, M., Rojas-Molina, M. M., & Sillero, J. C. (2014). Identification and multi-environment validation of resistance against broomrapes (*Orobanche crenata* and *Orobanche foetida*) in faba bean (*Vicia faba*). *Field Crops Research*, 166, 58–65.

Rubiales, D., Pérez-De-Luque, A., Cubero, J. I., & Sillero, J. C. (2003). Crenate broomrape (*Orobanche crenata*) infection in field pea cultivars. *Crop Protection*, 22, 865–872.

Rubiales, D., Pérez-De-Luque, A., Joel, D. M., Alcántara, C., & Sillero, J. C. (2003). Characterization of resistance in chickpea to broomrape (*Orobanche crenata*). *Weed Science*, 51, 702–707.

Rubiales, D., Pérez-de-Luque, A., Fernández-Aparicio, M., Sillero, J. C., Román, B., Kharrat, M., … Riches, C. (2006). Screening techniques and sources of resistance against parasitic weeds in grain legumes. *Euphytica*, 147, 187–199.

Sato, D., Awad, A. A., Chae, S. H., Yokota, T., Sugimoto, Y., Takeuchi, Y., & Yoneyama, K. (2003). Analysis of strigolactones, germination stimulants for *Striga* and *Orobanche* by high-performance liquid chromatography/tandem mass spectrometry. *Journal of Agricultural and Food Chemistry*, 51, 1162–1168.
Serghini, K., De Luque, A. P., Castejón-Muñoz, M., García-Torres, L., & Jorrin, J. V. (2001). Sunflower (*Helianthus annuus* L.) response to broomrape (*Orobanche cernua* Loefl.) parasitism: Induced synthesis and excretion of 7-hydroxylated simple coumarins. *Journal of Experimental Botany, 52*, 2227–2234.

Sillero, J. C., Moreno, M. T. & Rubiales, D. (1996). Preliminary Screening for Broomrape (*Orobanche crenata*) resistance in *Vicia* species. *Proceedings of 6th International Parasitic Weed Symposium Advances in Parasitic Plant Research* (p. 929). Cordoba, Spain.

Sillero, J. C., Moreno, M. T., & Rubiales, D. (2005). Sources of resistance to crenate broomrape among species of *Vicia*. *Plant Disease, 89*, 23–27.

Ter Borg, S. J., Willemsen, A., Khalil, S. A., Saber, H. A., Verkleij, J. A. C., & Pieterse, A. H. (1994). Field study of the interaction between *Orobanche crenata* Forsk. And some new lines of *Vicia faba* L. in Egypt. *Crop Protection, 13*, 611–616.

Trabelsi, I., Abbès, Z., Amri, M., & Kharrat, M. (2015). Performance of faba bean genotypes with *Orobanche foetida* and *Orobanche crenata* infestation in Tunisia. *Chilean journal of agricultural research, 75*, 27–34.

Vincent, J. M. (1970). *A manual for the practical study of root nodule bacteria*. Oxford: Blackwell Scientific.

Yoneyama, K., Awad, A. A., Xie, X., Yoneyama, K., & Takeuchi, Y. (2010). Strigolactones as germination stimulants for root parasitic plants. *Plant and Cell Physiology, 51*, 1095–1103.

Zeid, M., Nawar, A., El-Bebany, A., & Link, W. 2013. Development and evaluation of faba bean breeding materials suitable for mapping resistance/tolerance to *Orobanche crenata* using molecular markers. *Proceedings of Control of Orobanche crenata in legumes* (p. 21), Rabat, Morocco.