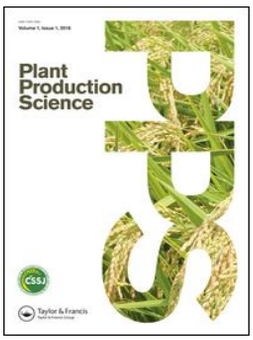

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Study of some resistance mechanisms to *Orobanche* spp. infestation in faba bean (*Vicia faba* L.) breeding lines in Tunisia

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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 Badï. 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 .6 to 3.4 and from 1.4 to 6.4 for the breeding lines against 10.4 and 12.3 for Badï 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 Badï. 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 Badï. 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 Badï. L5 seems to be less resistant even it behaves better than Badï in different culture conditions. The studied breeding lines could be recommended as resistance sources or candidates for varieties registrations.

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CLASSIFICATION
Genetic Resources Evaluation

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

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Table 1. Pedigree, origin, and main characteristics of the studied faba bean entries.

Breeding lines/varieties/Pedigree	Origin/characteristics
L1: XAR-VF00.12–12-3–1-3–1	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	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	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: XBJ92–10-46–1-3–1-2–1-1–1-6-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: XBJ90.04–6-2–1-1–4-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: XBJ90.04–2-3–1-1–1-2A	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*
Badï	Small seeded variety released in 2004 / susceptible to *O. foetida* and *O. crenata*

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 conduced 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. crenata* and *O. foetida* (JORT, 2015; Kharrat et al., 2010). Limited to moderate levels of resistance have been reported recently in several other legume such as pea, lentils, *Lathyrus sativa,* and *Lathyrus cicera* (Fernández-Aparicio et al., 2009a, 2011; Fernández-Aparicio, Sillero et al., 2009; Rubiales et al., 2009). Conversely resistance is prevalent for chickpea and common vetch (Fernández-Martínez et al., 2008; Nefzi et al., 2016; Rubiales, Alcántara et al., 2003; Rubiales, Pérez-De-Luque, Joel et al., 2003). More efforts are still required for the study of resistance inheritance, parasite variability and pathogenicity and resistance mechanism.

The aim of this work was to explore some mechanisms of resistance in seven faba bean breeding lines selected for their resistance to *O. foetida* and *O. crenata* in Tunisia. This was achieved through field trials, pot, and Petri dish experiment. These different culture strategies were used to obtain complementary information about the infection process and the mechanisms involved in faba bean resistance to fetid broomrape. These mechanisms ranged from limited induction of broomrape seed germination and attachment of the germinated seeds to limited growth of established tubercles, a reduction in number of emerged shoots of *Orobanche* per host plant, and thus good seed production in infested conditions (Abang et al., 2007; Abbes et al., 2009, 2010, 2011; Rubiales et al., 2006; Sillero et al., 2005).

1. Material and methods

1.1. Plant material

Ten faba bean entries were used in this study (Table 1); the susceptible control: cv. Badï, 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 north-west 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 m^2) 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 emerged *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. Badï). 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 mm^2) 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 cm^2 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

Table 2. Estimated Severity, Incidence, and Number of emerged *Orobanche* for faba bean entries in infested field by *O. foetida* during two cropping seasons 2012/2013 and 2013/2014.

	Severity			Incidence			Number of Emerged *Orobanche*		
Breeding lines/varieties	2012–2013	2013–2014	Mean	2012–2013	2013–2014	Mean	2012–2013	2013–2014	Mean
L1	4.33a*	5.33abc	4.83ab	88.33ab	100.00b	94.16ab	1.82ab	1.55abc	1.68ab
L2	5.00ab	4.33ab	4.66ab	90.00ab	86.66ab	88.33ab	1.78ab	1.40ab	1.59ab
L3	4.66a	4.00ab	4.33ab	96.66ab	85.00ab	90.83ab	2.06abc	1.23ab	1.65ab
L4	5.00ab	4.33ab	4.66ab	98.33ab	96.66b	97.50ab	2.77bcd	1.55abc	2.16abc
L5	6.33ab	7.00 cd	6.66c	100.00b	100.00b	100.00b	2.92cd	2.92d	2.92c
L6	5.00ab	3.33a	4.16a	96.66ab	73.33a	85.00a	2.18abc	.82a	1.50a
L7	4.66a	5.00ab	4.83ab	80.00a	95.00b	87.50ab	1.37a	1.51ab	1.44a
Baraca	7.00b	4.66ab	5.83bc	98.33ab	96.66b	97.50ab	3.28d	1.67abc	2.48bc
Najeh	4.33a	5.66bc	5.00ab	96.66ab	100.00b	98.33ab	2.43abcd	2.34bcd	2.39abc
Badï	9.00c	8.33d	8.66d	100.00b	100.00b	100.00b	3.11 cd	2.77 cd	2.94c

*Data with the same letter per column are not significantly different according to Duncan's test ($p = 0.05$).

Table 3. Grain yield (g plant^{-1}) of faba bean entries in non-infested and infested field by *O. foetida* during cropping seasons 2012/2013 and 2013/2014.

	Grain yield (g plant^{-1})						
	Non-infested field			Infested field			
Breeding lines/varieties	2012–2013	2013–2014	Mean	2012–2013	2013–2014	Mean	Mean reduction (%)
L1	11.61a*	13.23c	12.42c	4.89b	3.83bc	4.36b	64.89
L2	11.84a	10.81bc	11.32bc	4.80b	4.07c	4.83b	57.33
L3	12.66a	13.59c	13.12c	5.14b	6.47c	5.80b	55.79
L4	12.30a	13.03c	12.66c	3.16ab	4.32bc	3.74b	70.45
L5	10.43a	11.69c	11.06bc	1.97ab	1.73ab	1.85a	83.27
L6	9.72a	10.49bc	10.10bc	3.28b	5.23c	4.26b	57.82
L7	9.79a	11.53b	10.66bc	4.06b	4.10bc	4.08b	61.72
Baraca	8.87a	13.04bc	10.96bc	2.57ab	5.08c	3.83b	65.05
Najeh	9.26a	8.13b	8.70ab	4.00a	4.12bc	4.06b	53.33
Badï	8.62a	3.84a	6.23a	.25b	.70a	.16a	97.43

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

two cropping seasons, results revealed that the susceptible check cv. Badï 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 Badï for these two breeding lines. In non-infested fields, the grain yield of the seven breeding lines was significantly higher ($p = .000$) than Badï (Table 3). It varied from 10.1 g plant^{-1} to 13.1 g plant^{-1} against 6.2 g plant^{-1} for Badï. Nevertheless, in infested field, the grain yield decreased for the studied breeding lines and the grain yield reductions reached the maximum for Badï (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 Badï. 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 Badï (5 g/plant). The breeding line L7 showed low average number (.8) and weight (.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 Badï (Table 4). For the partial resistant check of Baraca infested by *O. crenata*,

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.

	Total Attachments (S1-S5) Number/Plant (TAN/P)		Attachments (S1-S5) Dry Weight/Plant (g) (ADW/P)		Underground Attachments (S1-S4) Number/Plant (UAN/P)		Emerged Spikes (S5) Number/Plant (ESN/P)	
Breeding lines/varieties	*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	2.46 abc	2.62 a	.20 a	4.60 b	1.80 a	1.80 a
L3	2.00 a	5.00 ab	3.57 bc	2.86 a	.40 a	2.00 ab	1.60 a	3.00 a
L4	3.40 a	1.40 a	1.56 ab	1.59 a	1.80 a	.20 a	1.60 a	1.20 a
L5	2.80 a	4.00 ab	2.80 abc	3.00 a	1.80 a	1.20 ab	1.00 a	2.80 a
L6	2.00 a	5.00 ab	1.27 ab	3.27 a	1.40 a	1.40 ab	.60 a	3.60 a
L7	.80 a	3.80 ab	.17 a	1.58 a	.80 a	3.20 ab	.00 a	.60 a
Baraca	5.20 a	2.00 ab	3.04 abc	3.20 a	2.60 a	.00 a	2.60 a	2.00 a
Najeh	1.40 a	2.75 ab	1.39 ab	2.85 a	.80 a	.25 a	.60 a	2.50 a
Badï	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.

	Stem Dry Weight/Plant SDW/P (g)			Root Dry Weight/Plant RDW/P (g)			Pod Number/plant (PN/P)		
Breeding lines/ varieties	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	3.40 ab	2.40 b	.60 a
L4	11.94 bc	10.80 e	5.57 c	3.59 c	3.20 bc	1.48 a	3.60 ab	3.00 bc	1.80 a
L5	9.92 abc	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 bcde	4.29 abc	2.70 abc	2.73 bc	.90 a	3.40ab	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 bcde	3.40 abc	3.07 bc	1.88 ab	.60 a	2.40 a	4.20 c	1.25 a
Badï	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 ($p = .05$).

all attachments evolved in emerged spikes, nevertheless the susceptible cultivar Badï 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 Badï. 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. Badï (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 Badï 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 crenate 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 ($p = .001$),

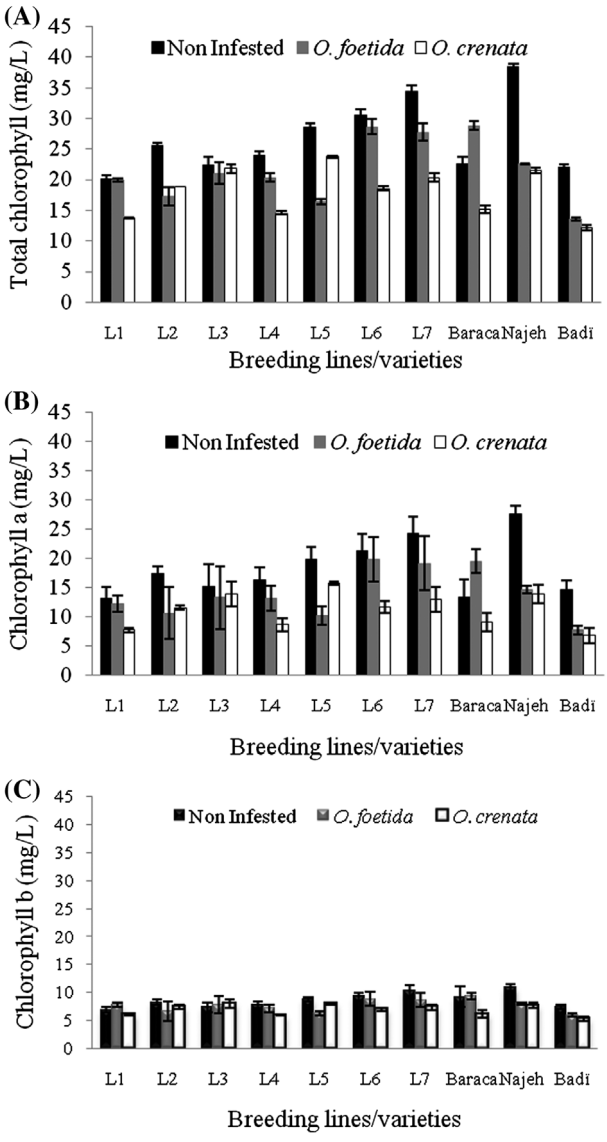

Figure 1. Effect of *O. foetida* and *O. crenata* on the total chlorophyll (A), chlorophyll a (B), and chlorophyll b (C) contents in different faba bean entries.
Note. Data are means ± SE.

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 Badï, 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 Badï showed the highest infestation level after 77 days with 22.30 and 24.60 attachments, respectively, for *O. crenata* and *O.*

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).

	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^{ns}	−0,486**	−0,473**	0,501**	0,386^{ns}	0,484**
RDW(g)		−0,297^{ns}	−0,191^{ns}	−0,34^{ns}	−0,269*	0,451*	0,361^{ns}	0,438*
TAN			0,902***	0,744***	0,672***	−0,336^{ns}	−0,320^{ns}	−0,338^{ns}
UAN				0,382^{ns}	0,490**	−0,274^{ns}	−0,272^{ns}	−0,278^{ns}
ESN					0,679***	−0,295^{ns}	−0,264^{ns}	−0,293^{ns}
ADW(g)						−0,374^{ns}	−0,324^{ns}	−0,369^{ns}
Chl a (mg/L)							0,905**	0,995***
Chl b (mg/L)								0,942***

^{ns}Non Significant; ***$p \leq .001$; **$.001 < p \leq .01$; *$.01 < p \leq .05$

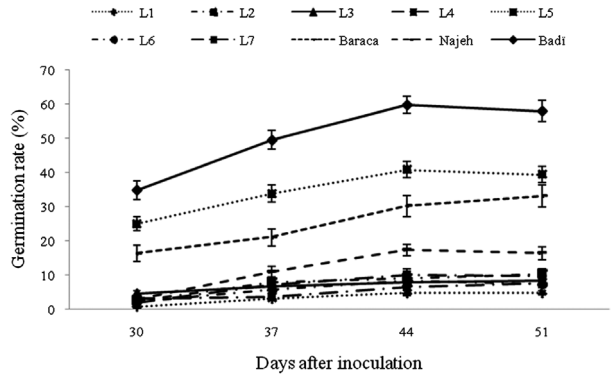

Figure 2. Germination rate of *O. foetida* seeds for 10 faba bean entries.
Note. Data are means ± SE.

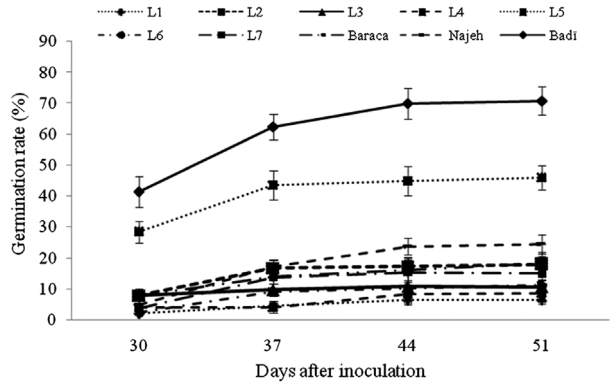

Figure 3. Germination rate of *O. crenata* seeds for 10 faba bean entries.
Note. Data are means ± SE.

foetida (Figure 4). Nevertheless, the tubercles number was relatively low for the other breeding lines and remained constant. They did not exceed 5.70 and 9.80, respectively, for *O. foetida* and *O. crenata*. Compared to the susceptible check Badï, attachments on the root system of the breeding lines L1, L2, L4, L6, and Najeh inoculated by fetid broomrape and L1–L4, and Najeh inoculated by crenate broomrape occurred one week later (55 days after inoculation). Conversely, for L7 inoculated by *O. crenata,* the attachments were observed two weeks later than Badï (63 days after inoculation).

Tubercles reaching stage 4 in the check and tested breeding lines were examined in co-cultures. Starting from 48 to 55 days after inoculation by *O. foetida*, the percentage of *Orobanche* tubercles reaching stage 4 increased significantly for the cultivars Badï and Baraca to reach, respectively, 40.6 and 36.2% 77 days after inoculation. However, for the breeding line L4, the percentage of tubercles reaching stage 4 was nil. Tubercle Stage 4 appeared 7 days later for lines L2, L3, L5, and cv. Najeh and 15 days later for L1, L6, and L7. The percentage of tubercles reaching stage 4 ranged from 0 to 21.1% for the seven breeding lines.

Orobanche tubercle reached stage 4 on all the tested lines inoculated by *O. crenata*. Compared to the susceptible check Badï 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 Badï. 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

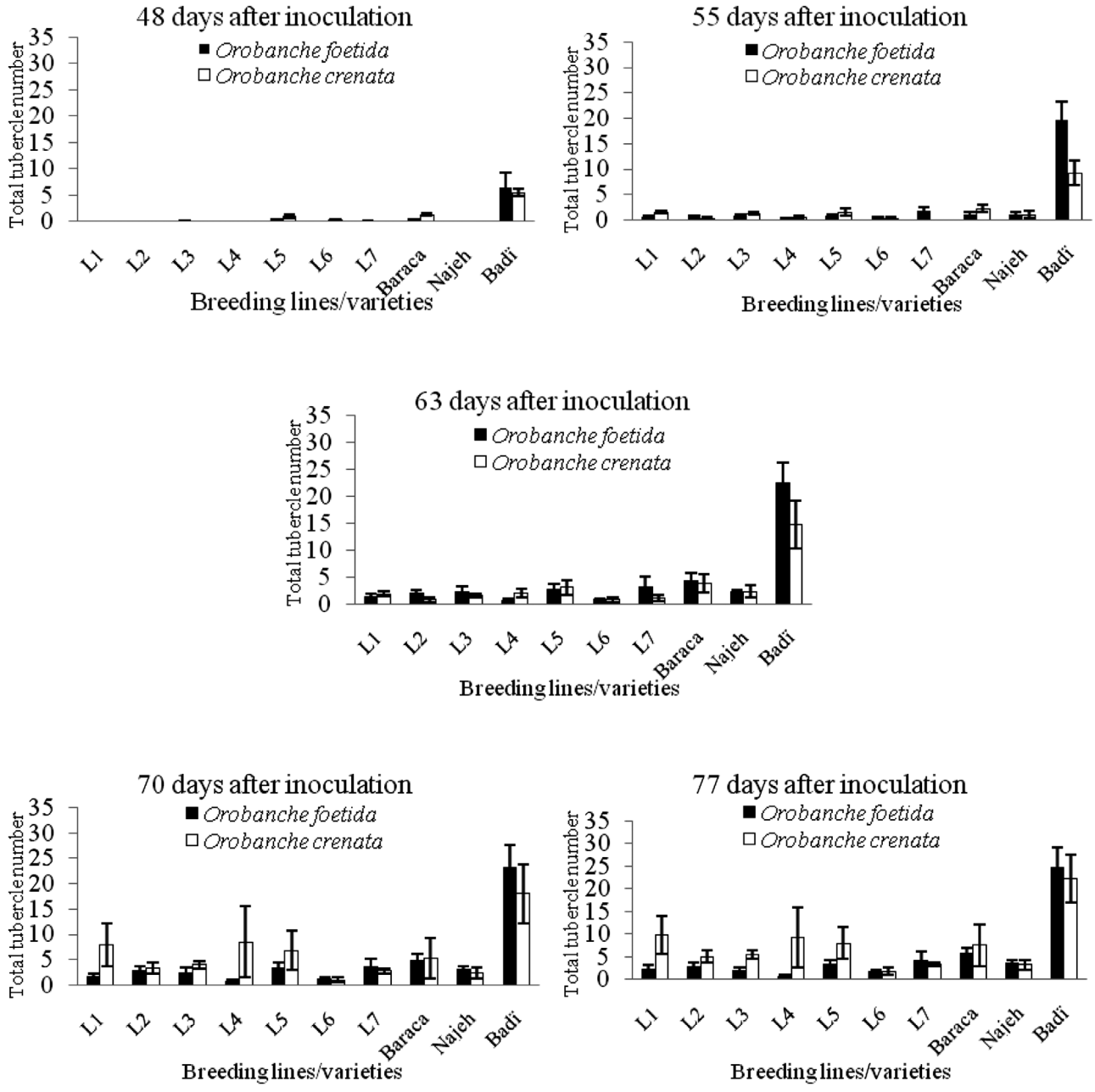

Figure 4. Evolution of the total tubercle number (*O. foetida* and *O. crenata*) of 10 faba bean entries.
Note. Data are means ± SE.

conditions, grain production and the development capacity of the host plant are the mains 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 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

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* (Mauromicale 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 Badï (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 Badï. 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-Menoufi, 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. Badï 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. Badï. 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. Badï 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, callose 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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Disclosure statement

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