Effect of Benzothiadiazole and Salicylic Acid Resistance Inducers on *Orobanche foetida* Infestation in *Vicia faba*

Emna Triki, **Imen Trabelsi**, Institut National de la Recherche Agronomique de Tunisie, Université de Carthage, Rue Hédi Karray, 1004 Tunis-Menzah 1, Tunisia, **Moez Amri**, International Center for Agricultural Research in the Dry Areas, Rabat Morocco, **Fatma Nefzi**, **Mohamed Kharrat**, **and Zouhaier Abbes**, Institut National de la Recherche Agronomique de Tunisie, Université de Carthage, Rue Hédi Karray, 1004 Tunis-Menzah 1, Tunisia

ABSTRACT

Triki, E., Trabelsi, I., Amri, M., Nefzi, F., Kharrat, M., and Abbes, Z. 2018. Effect of benzothiadiazole and salicylic acid resistance inducers on *Orobanche foetida* infestation in *Vicia faba*. Tunisian Journal of Plant Protection 13 (1): 113-125.

The broomrape or orobanche (*Orobanche foetida*) is considered as an important agricultural problem of faba bean (*Vicia faba* var. *minor*) production in Tunisia. The effect of salicylic acid (SA) and benzothiadiazole (BTH) on the induction of faba bean resistance to *O. foetida* was studied. Three application methods (seed soaking, foliar spraying and watering) were used. SA and BTH treatments reduced broomrape infestation under controlled conditions in pot and Petri dish experiments. In pot experiment, SA and BTH treatments reduced broomrape total number. Seed soaking treatments were more effective than foliar spraying and watering. In Petri dish experiment, *O. foetida* seed germination and the number of orobanche tubercles were reduced. The most efficient method was watering for SA and BTH treatments. This reduction was associated to a delay in the tubercle formation. The different application methods of SA and BTH treatment attest that the induced systemic resistance to *O. foetida* can be used in integrated management of broomrapes.

Keywords: Benzothiadiazole, *Orobanche foetida*, resistance inducers, salicylic acid, systemic acquired resistance, *Vicia faba* var. *minor*

Faba bean (*Vicia faba*) is among the most cultivated crop grain legumes in the world. It plays important agronomic and socio-economic roles. However, production, yield and growing areas are variable from year to year (Sillero et al. 2010). In Tunisia, faba bean represents the most important grain legume occupying around 84% of the total grain legume area (DGPA 2016). However, its average yield (about 0.7 t/ha) is below its potential yield in favorable zones of the North, where the average rainfall is higher than 400 mm. The low yield is mainly due to low inputs used, climatic variation, diseases and pests. Broomrapes or orobanches (*Orobanche* spp.) are one of the most important factors reducing faba bean yields in Tunisia (Kharrat and Souissi 2004). These root parasitic plants threaten several crops in many parts of the world. They completely depend on their hosts for their nutritional

Corresponding author: Imen Trabelsi
Email: trabelsiimen11@yahoo.fr

Accepted for publication 18 May 2018

requirements. The most harmful species are *O. crenata*, *O. cumana*, *Phelipanche aegyptiaca*, *P. ramosa* and *O. minor* (Parker 2009). *O. foetida* is considered an important agricultural problem of faba bean production in Tunisia, causing yield losses between 66 to 90% in Beja region (Abbes et al. 2007a). Kharrat and Souissi (2004) indicated that in highly infested areas, farmers generally avoid growing faba bean or other susceptible crops, resulting in substantial reductions of cultivated areas and food legume production. Damages caused in faba bean crops were reported only in Tunisia till now. However, the risk of spread of this parasite to other areas, displaying similar growth conditions, is probable (Abbes et al. 2007a).

Several methods (cultural, chemical, biological, genetic control) have been used for controlling broomrapes but without complete success. In Tunisia, research activities on orobanche were intensified and faba bean resistant cultivars were identified (Abbes et al. 2007a, 2010a, 2011; Kharrat et al. 2010; Trabelsi et al. 2015, 2017). Some chemicals (glyphosate, imazaquin, imazapyr, imazethapyr and sulfosate) were tested and succeeded to induce reduction in *O. foetida* infestation (Kharrat et al. 2002). Late sowing could be an interesting cultural method to reduce significantly the number and the dry weight of emerged *O. foetida* shoots and to significantly increase faba bean seed yield (Abbes et al. 2010b). Bouraoui et al. (2012) indicate that *Rhizobium leguminosarum* reduced *O. foetida* infestation and increased faba bean shoot dry weight. All these methods and strategies resulted to incomplete protection. Thus, alternative or supplementary methods should be considered to prevent or reduce infestation.

Chemically induced resistance enhanced natural defenses of the plant to control pathogens (Hafez et al. 2014; Somaya and El-Sharkawi 2014). Induced resistance of host plants can be used for the control of agronomical important orobanche species (Abbes et al. 2014; Pérez-de-Luque et al. 2004; Sauerborn et al. 2002). It can be activated by exogenous application of salicylic acid (SA) or its synthetic functional analog benzo (1,2,5) thiadiazole - 7 - carbothioic acid S-methyl ester (BTH) (Pérez-de-Luque et al. 2004; Perez et al. 2003).

Systemic Acquired Resistance (SAR) has proven to be effective as a tool for controlling plant pathogens, including fungi, bacteria, viruses, and parasitic weeds. Several studies on different plant species such as clover (Kusumoto et al. 2007), pea (Pérez-de-Luque et al. 2004), tobacco, hemp (Gonsior et al. 2004), sunflower (Buschmann et al. 2005; Muller-Stover et al. 2005; Sauerborn et al. 2002) and oilseed rape (Véronési et al. 2009) indicate that BTH and SA applications also triggers SAR against broomrapes. However, there is a little knowledge about the effects of SAR on faba bean in order to reduce *O. foetida* infestation. Thus, the aim of this study was to evaluate the effect of chemical resistance inducers on *O. foetida* infestation in faba bean.

MATERIALS AND METHODS

Plant material.

The improved Tunisian variety (cv. Bachaar) of faba bean (*Vicia faba* var. *minor*) known for its high productivity in orobanche-free soils and its high susceptibility to *O. foetida* and *O. crenata* was used (Abbes et al. 2007a; 2007b; JORT 2004). Bachaar seeds were provided by the food legume program, Field Crops Research Laboratory of National Agricultural Research Institute in Tunisia (INRAT). Faba bean seeds were surface-disinfected with 5% calcium hypochlorite for 15 min and then rinsed five times with sterile distilled-water. Disinfected seeds were placed in Petri dishes on a sterile filter paper imbibed with sterile distilled-water and allowed to germinate at 21±2°C in the dark for seven days. *O. foetida* seeds were collected from flowering spikes in infested faba bean fields from Beja (Tunisia) in 2013. Washed seeds were sterilized in 2% calcium hypochlorite for 5 min and rinsed five times with sterile distilled water.

Pot experiment.

Pot experiment was performed under natural conditions at INRAT. Two liters plastic pots were filled up to 2/3 of their height with sterilized soil and then artificially inoculated with 20 mg of *O. foetida* seeds/kg of soil. Plants were watered when necessary.

Chemical inducers salicylic acid (SA, Sigma-Aldrich, purity: ≥ 99.0%) and BTH in the form of acibenzolar acid (Sigma-Aldrich, Pestanal®, analytical standard) were applied to seeds and plants in three different treatments: (i) seed soaking, (ii) foliar spray and (iii) watering. SA and BTH concentrations were chosen because they were frequently reported in the literature as concentrations inducing plant resistance to various pathogens (Abbes et al. 2014; Borges et al. 2003; Daniel and Guest 2006; Elmer 2006; Jackson et al. 2000; Lawton et al. 1996; Pajot et al. 2001; Pérez-de-Luque et al. 2004; Saindrenan et al. 1988; Sauerborn et al. 2002; Véronési et al. 2009).

- Seed soaking: Faba bean seeds were germinated in Petri dishes containing 1 mM water solution of SA or 0.05 g/l of BTH. Control seeds were placed in distilled water for germination.

- Foliar spray: 20-day-old seedlings were sprayed with a hand held sprayer (capacity of 500 ml), with a 1 mM solution of SA or 0.05 g/l of BTH to which Tween 20 was added as wetting agent (3 drops per liter). Each seedling received 10 ml of the solution. Three additional sprays were performed at 35, 50 and 65 days after planting (dap). Control plants received distilled water plus Tween 20.

- Watering: this treatment was used only for SA. Plants (20-day-old) were watered with a 1 mM solution of SA. Each seedling received 100 ml of the solution. Three applications were given at 35, 50 and 65 dap. Control seedlings were watered with distilled water.

Plants were biweekly treated through watering and foliar spraying. The necessity for a repetitive application of low doses of these plant activators was demonstrated by several authors (Pajot et al. 2001; Pérez-de-Luque et al. 2004) in order to increase the efficacy of SAR.

At maturity stage (four months after planting), roots of infected plants were gently removed from the substrate, washed with water, and the orobanche attachments were carefully harvested. Orobanche attachments were sorted according to their developmental stage as reported by Labrousse et al. (2001):

- S1: parasite attachment to the host root,
- S2: small tubercles without root development,
- S3: growing tubercles with crown roots without shoot formation,
- S4: tubercles carrying underground growing shoots,
- S5: emerged parasites.

The first stage (S1) was not observed in the pot experiment. The dry weight of tubercles per plant was recorded. Five replicates per individual treatment were considered.

Petri dish experiment.

The Petri dish experiment was used to evaluate the underground development of root parasitic weeds such as germination and further growth stages since such evaluation is impossible in pot experiments.

Faba bean and orobanche seeds were surface sterilized as described above. Plastic Petri dishes (120 × 120 ×17 mm, Greiner) were filled with autoclaved sand then covered with glass fiber filter paper imbibed with water. Three perforations were made in each Petri dish: the big one was made in the highest board, to allow the shoot out of the dish, and the others were made on the opposite sides to allow root feeding in culture medium (Vincent 1970). Sterilized orobanche seeds (20 mg) were spread between the dish cover and the glass fiber filter paper. Pre-germinated faba bean seeds were placed on the glass fiber filter paper. Petri dishes were closed and vertically stored in a sterile polypropylene tray containing sterile distilled water. The co-culture system was kept in the greenhouse at a temperature above 22±3°C, natural light and in relative humidity above 70%.

As described in pot experiment, the same concentrations and chemical inducers (BTH and SA) were differently applied to seeds and seedlings: seed soaking, foliar spray and watering.

- Seed soaking: Faba bean seeds were germinated in Petri dishes containing the chemical inducers.
- Foliar spray: 20-day-old seedlings were sprayed, with the chemical inducers using a hand held sprayer. Each seedling received 10 ml of the solution. Two additional sprays were performed at 27 and 34 dap.
- Watering: Seedlings were watered with the chemical inducers. Each seedling received 100 ml of the solution. Three applications were given at 27 and 34 dap.

Seed germination was determined by a binocular microscope. Four squares of 1 cm^2 near infested faba bean roots per Petri dish were observed and the number of germinated seeds was counted and expressed as percentage of total seeds. Estimations of percent germination were performed weekly between 21 and 77 dap. In addition, the total number of tubercles was counted weekly between 52 and 101 dap and classified according to their developmental stage (Labrousse et al. 2001).

Statistical analysis.

ANOVA was performed using the SPSS statistical program v.15 (IBM Corporation, Armonk, New York, U.S.A). Tukey's test at P=0.05 was used.

RESULTS

Pot experiment.

SA and BTH foliar spray reduced parasite number by 39 and 32%, respectively (Table 1). Watering with SA reduced *O. foetida* number by 42%. Seed soaking in both SA and BTH were slightly more effective by reducing *O. foetida* number by 45 and 42%, respectively. All treatments reduced significantly the number of underground tubercles but not the number of tubercles reaching the stage S5. Regarding orobanche dry weight, no significant differences were observed between treated and untreated plants (Table 1).

Table 1. Effect of salicylic acid (SA) and benzothiadiazole (BTH) on the total number and dry weight of *Orobanche foetida* in pot experiment

Treatment	Total orobanche number	Orobanche dry weight (g)	Number of underground tubercles (S2+S3+S4)*	Number of emerged orobanche (S5)	Percentage of emerged orobanche (S5)
Control	120.1±14.0b*	6.7±0.5ab	116.5±14.2b	3.5±0.9ab	2.9
Foliar spray SA	73.5±7.0a	8.9±1.2b	68.6±7.3ab	4.8±0.7ab	6.5
Watering SA	69.5±9.0a	7.5±1.1ab	66±9.5a	3.5±1.4ab	5.0
Seeds soaking SA	66.1±11.2a	6.2±1.2ab	62±10.3a	4.1±2.1ab	6.2
Foliar spray BTH	82.2±11.1a	7.4±0.7ab	76±10.8a	6.2±0.8b	7.5
Watering BTH	70±8.1a	4.4±0.8a	68.3±9.8ab	1.6±0.4a	2.2

* S2, S3, S4 and S5 are the stages of orobanche development. Data with the same letter per column are not significantly different (n=5. *P*=0.05, Tukey's test). Rate of S5 is the percentage of emerged orobanche compared to the total number of orobanche.

Petri dish experiment.

SA and BTH treatments strongly reduced the germination percentage of orobanche seeds on faba bean roots (Figs. 1 and 2). The significant reduction was observed with watering method for SA (31% at 42 dap and 38% at 77 dap) and seed soaking method for BTH treatments (51% at 42 dap and 60% at 77 dap).

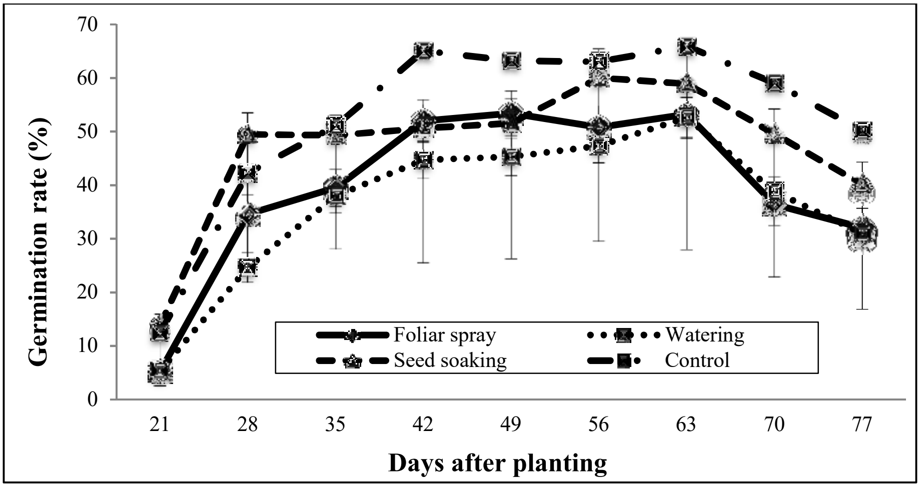

Fig. 1. Effect of salicylic acid (SA) on the germination rate of *Orobanche foetida*. Data are means ± Standard Error (SE).

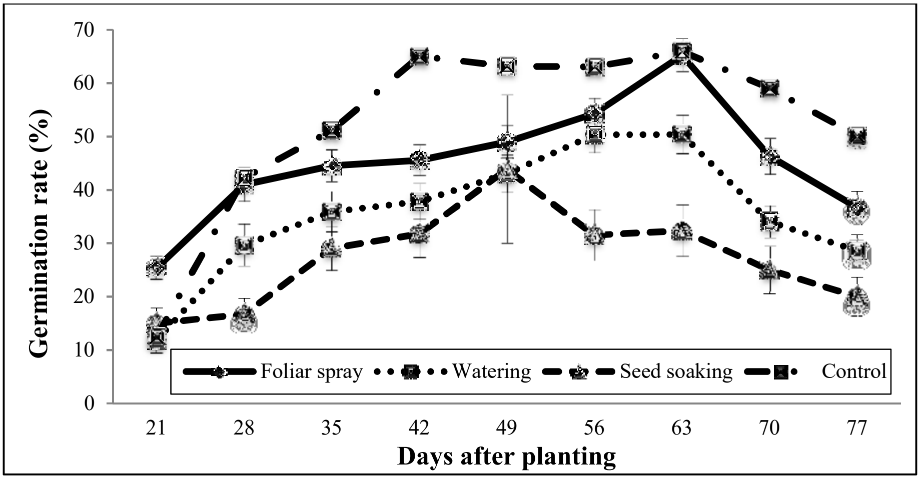

Fig. 2. Effect of benzothiadiazole (BTH) on the germination rate of *Orobanche foetida.* Data are means ± SE.

SA reduced the number of *O. foetida* tubercles on faba bean roots at 80 dap by 52, 55 and 30% with foliar spray, watering and soaking seeds, respectively (Figs. 3 and 4). Watering with SA not only reduced the total number of broomrape attachments but also retarded by one week their formation.

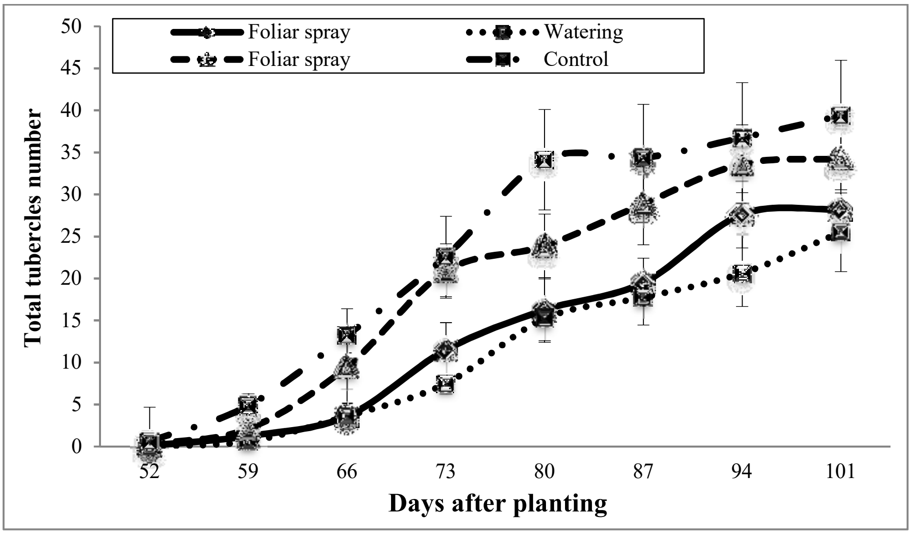

Fig. 3. Effect of salicylic acid (SA) on the total number of *Orobanche foetida* tubercles. Data are means ± SE.

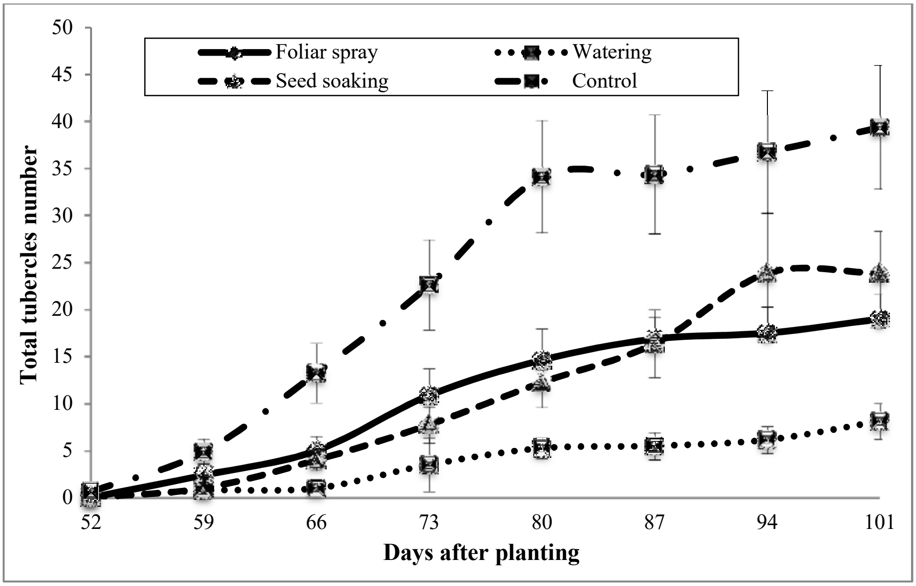

Fig. 4. Effect of benzothiadiazole (BTH) on the total number of *Orobanche foetida* tubercles. Data are means ± SE.

BTH treatments strongly reduced the number of broomrape attachments on faba bean roots. A significant reduction in the number of attachments was observed in BTH-watered plants with less than six attachments per plant. Significant decrease in broomrape infestation was also observed after BTH seed soaking or BTH foliar spray (Figs. 3 and 4). Watering with BTH was the most efficient treatment. It has reduced broomrape infestation by 84% at 80 dap. This reduction was also associated to a delay in the tubercle formation with all BTH treatments (delay of one week). As observed in the pot experiments, no tubercle necrosis was observed following SA and BTH treatments.

DISCUSSION

In pot experiment, the reduction of orobanche number is in agreement with previous studies (Abbes et al. 2014; Buschmann et al. 2005; Fan et al. 2007; Pérez-de-Luque et al. 2004; Véronési et al. 2009). This reduction was characterized by a reduced number of underground tubercles without significant changes in orobanche dry weight or number of tubercles reaching Stage 5. In the present work, the untreated control presented the highest number of orobanche but not necessarily the highest dry weight of orobanche. This can be explained by the high competition level between tubercles for water and nutrients, thus they remain small (Aalders and Pieters 1987; Rubiales et al. 2006; Ter Borg et al. 1994; Trabelsi et al. 2016; Zeid et al. 2013). According to Ter Borg et al. (1994), the major indicator of resistance to broomrape is the tubercle number per plant. Sillero et al. (1996) reported also that indices based only on size and weight of broomrapes can be misleading.

Several authors (Abbes et al. 2014; Bigirimana and Hofte 2002; Lopez and Lucas 2002; Perez et al. 2003) mentioned that, in addition to the reduction of orobanche number, SA and BTH treatments induced observable growth reduction in hosts plants. Heil et al. (2000) explain the biomass reduction as allocation cost of plants treated with resistance-inducing agents which is a result of a metabolic competition between biomass production and defense. These plants use energy and assimilates for defense reactions and not for biomass production. In order to reduce the negative effects of BTH on plant biomass and to increase the efficacy of orobanche control, the timing of the first treatment and the number of treatments should be studied as suggested by Buschmann et al. (2005).

The germination of broomrape seeds in Petri dish experiment was significantly affected by SA and BTH treatments in comparison with the untreated control. The most effective reduction was observed with the watering method for SA and seed soaking method for BTH. Chemicals seemed to induce low production of stimulant substances or increase the release of inhibitory substances by the host. In order to determine whether BTH or SA effects on parasitism were mediated through host responses or via direct impacts on the parasite, several authors treated orobanche seeds with activator chemicals. These authors did not show a significant reduction of germination of treated orobanche seeds, demonstrating that there are no toxic effects of chemicals on orobanche seeds (Kusumoto et al. 2007; Pérez-de-Luque et al. 2004; Sauerborn et al. 2002; Véronési et al. 2009). Buschmann et al. (2005) showed that BTH applied as a root chamber drench did not interfere with *O. cumana* seed germination. Our study demonstrated that BTH or SA foliar applications (no direct contact with orobanche seeds) reduced significantly orobanche germination percentage. Based on these data, we can strongly suggest that BTH and SA do not act via an herbicidal activity but via induction in faba bean roots of the SAR pathway. Foliar applications demonstrate that the induced resistance to orobanche is systemic.

In addition, the three methods of applications of SA and BTH resulted in a reduction of *O. foetida* infestation, but the most efficient was watering for both SA and BTH treatments. This reduction was associated to a delay in the tubercle formation with these two treatments (delay of one week). No necrosis of developing parasite tubercles was observed. Similar data were reported in tobacco and hemp infected by *O. ramosa* (Gonsior et al. 2004), in pea infested with *O. crenata* (Pérez-de-Luque et al. 2004), in sunflower attacked by *O. cumana* (Buschmann et al. 2005; Fan et al. 2007; Muller-Stover et al. 2005), in oilseed rape infected with *O. ramosa* (Véronési et al. 2009) and in faba bean infected by *O. foetida* (Abbes et al. 2014). The reduction in the number of established parasites by SA and BTH can be related to the reduction in orobanche germination. Kusumoto et al. (2007) show that the reduction of *O. minor* tubercles in red clover caused by SA and BTH was due to the inhibited elongation of *O. minor* radicles and the activation of defense responses in the host root including lignification of the endodermis. Sauerborn et al. (2002) indicated that the total number of *O. cumana* shoots was reduced with BTH treatment, and this was due to synthesis of scopoletin (coumarin phytoalexin) and of hydrogen peroxide in the BTH-treated sunflower roots, but with no increase in lignification. Other studies showed that lignification has been reported as a defense reaction against *Orobanche* spp. penetration, connection to the vascular system and/or tubercle development (Barandiaran et al. 1999;

Goldwasser et al. 1999; Stermer et al. 1987).

Other mechanisms such as an increased synthesis of the scopoletin and ayapin in sunflower (Prats et al. 2002; Sauerborn et al. 2002; Serghini et al. 2001), or the accumulation of reactive oxygen species and hydroxyl coumarin phytoalexins (Buschmann et al. 2002) can be induced by BTH or SA application. Those mechanisms of action of SA and BTH against orobanche infestation were also reported in the control of some plant diseases. *Fusarium oxysorum* f.sp. *ciceris* incidence on chickpea reduction was found to be associated with phytoalexin, pathogenesis-related proteins and chitinase and β-1,3-glucanase induction (Kuc 2006; Sarwar *et al.* 2005). Same results were reported for infections of potato by *Rhizoctonia solani* (Hadi and Balali 2010), faba bean by chocolate spot (Hassan et al. 2006; Mbazia et al. 2016), cucumber and bean by *Botrytis cinerea*, pepper by Fusarium wilt/ root rot causal agent (Abdel-Monaim et al. 2010) and tobacco by TMV (Achuo et al. 2004).

These studies suggested that these chemical defense-inducers could prevent orobanche infestation by activating the SA dependent pathway in host plants. The present study suggests that SAR could be an important method to control broomrapes. This control method should be confirmed under field conditions and could be a useful tool for an integrated control program leading to reduced soil infestation by orobanche. More researches and studies by varying rates, treatment methods and application times are necessary in order to reduce the danger of biomass reduction in faba bean and to increase the efficacy of *O. foetida* control.

ACKNOWLEDGMENTS

The authors which to thank the Ministry of Higher Education and Scientific Research and the Ministry of Agriculture, Hydraulic Resources and Fisheries of Tunisia for the financial support to this study.

RESUME

Triki E., Trabelsi I., Amri M., Nefzi F., Kharrat M. et Abbes Z. 2018. Effet de l'acide salicylique et du benzothiadiazole comme inducteurs de la résistance, sur l'infestation de *Vicia faba* par *Orobanche foetida.* Tunisian Journal of Plant Protection 13 (1): 113-125.

L'orobanche (*Orobanche foetida*) est considérée comme étant un problème majeur entravant la production de féverole (*Vicia faba* var. *minor*) en Tunisie. L'effet de l'acide salicylique (SA) et du benzothiadiazole (BTH) sur l'induction de la résistance à *O. foetida* chez la féverole a été étudié. Trois méthodes d'application (trempage des graines, pulvérisation foliaire et arrosage) ont été testées. Les traitements ont réduit l'infestation par l'orobanche en pots et en boîtes de Pétri. En pots, SA et BTH ont réduit le nombre total d'orobanches. Le trempage des graines était généralement plus efficace que l'arrosage et la pulvérisation foliaire. En boîtes de Pétri, la germination des graines d'*O. foetida* et le nombre de tubercules d'orobanche ont été réduits. La méthode la plus efficace était l'apport de SA et BTH par arrosage. Cette réduction est associée à un retard de la formation des tubercules. Les différentes méthodes d'application de SA et BTH montrent que la résistance induite à *O. foetida* est systémique. Ces résultats démontrent que la résistance systémique acquise est une méthode à utiliser dans les programmes de lutte intégrée contre l'orobanche.

Mots clés: Acide salicylique, benzothiadiazole, inducteurs de résistance, *Orobanche foetida*, résistance systémique acquise, *Vicia faba* var. *minor*

ملخص

تريكي، آمنة وإيمان□طرابلسي ومعز□عامري وفاطمة□نفزي ومحمد□خراط وزهير عباس. 2018. تأثير محفزات □مقاومة، حامض □سا□يسيليك ومستحضر □بانزوثياديازول) على الإصابة با□ها□وك (*Orobanche foetida*) على □فول□مصري (*Vicia faba*). Tunisian Journal of Plant Protection13 (1): 113-125.

يعتبر الطفيل الهالوك (*Orobanche foetida*) مشكلة رئيسية□حد من إنتاج الفول المصري (*Vicia faba* var. *minor*) في□ونس.□مت□راسة□أثير □امض الساليسيليك ومستحضر البانزوثيا□يازول لتحفيز المقاومة □د الهالوك لدى الفول المصري □م استعمال ثلاثة طرق□طبيق: غمس البذور، رش الأوراق والري. وقد خفض استعمال □امض الساليسيليك ومستحضر البانزوثيا□يازول من الإصابة بالهالوك في الأصص وفي أطباق بتري. فيما يخص□جربة الأصص، خفضت□ميع طرق استعمال□امض الساليسيليك ومستحضر البانزوثيا□يازول العد□ الإ□مالي لدرنات الهالوك، ولكن غمس البذور عموما كان أكثر فاعلية للحد من عد□رنات الهالوك. أما مخبريا، فإن إنبات□ب الهالوك وعد□ر□ا□ه انخفض أيضا. كانت الطريقة الأكثر فعالية هي الري. ير□بط هذا الانخفاض بتأخير في□كون الدرنات. □ميع الطرق المستعملة مع □امض الساليسيليك ومستحضر البانزوثيا□يازول□بين أن مقاومة الطفيل هي مقاومة □هازية مكتسبة. □ظهر هذه النتائج أن المقاومة الجهازية المكتسبة يمكن أن□كون عنصر من عناصر المكافحة المتكاملة □د الهالوك.

كلما مفتا□ية: □امض الساليسيليك، محفزات المقاومة، مستحضر البانزوثيا□يازول، مقاومة □هازية مكتسبة، *Orobanche foetida*، *Vicia faba var. minor*

LITERATURE CITED

Aalders, A.J.G., and Pieters, R. 1987. Resistance in *Vicia faba* to *Orobanche crenata*: true resistance versus hidden susceptibility. Euphytica 36: 227-236.

Abbes, Z., Kharrat, M., Delavault, P., Simier, P., and Chaïbi, W. 2007a. Field evaluation of the resistance of some faba bean (*Vicia faba* L.) genotypes to the parasitic weed *Orobanche foetida* Poiret. Crop Protection 26: 1777-1784.

Abbes, Z., Kharrat, M., Simier, P., and Chaïbi, W. 2007b. 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., Shaaban, K., and Bayaa, B. 2010a. Comportement de différentes accessions améliorées de féverole(*Vicia faba* L.)vis-à-vis d'*Orobanche crenata* Forsk. et *Orobanche foetida* Poiret. Cahiers Agricultures 19: 194-199.

Abbes, Z., Sellami, F., Amri, M., and Kharrat, M. 2010b. Effect of sowing date on *Orobanche foetida*infection and seed yield of resistant and susceptible faba bean cultivars. Acta Phytopathologica et Entomologica Hungarica 45: 267-275.

Abbes, Z., Sellami, F., Amri, M., and 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., and Kharrat, M. 2014. *Orobanche foetida* control in faba bean by foliar application of Benzothiadiazole (BTH) and salicylic acid. Bulgarian Journal of Agricultural Science 20: 1439-1443.

Achuo, E., Audenaert A., Meziane, K., and Höfte, M. 2004. The salicylic acid dependent defense pathway is effective against different pathogens in tomato and tobacco. Plant Pathology 53:65-72

Abdel-Monaim, M.F., and Ismail, M.E. 2010. The use of antioxidants to control root rot and wilt diseases of pepper. Notulae Scientia Biologicae 2: 46-55

Azami-Sardooei, Z., SorenSeifi, H., De Vleesschauwer, D., and Höfte, M. 2013. Benzothiadiazole (BTH)-induced resistance against *Botrytis cinerea* is inversely correlated with vegetative and generative growth in bean and cucumber, but not in tomato**.**Australasian Plant Pathology 42: 485-490.

Anonyme. 2005 Statistiques de la Direction Générale de la Production Agricole (DGPA), Ministère de l'Agriculture et des Ressources Hydrauliques,

Barandiaran, X., Moral, R., Gil, J., and Moreno, M.T. 1999. Efecto del glutation reducido sobre la germinacion y el crecimiento de *Orobanche crenata.* Pages 145-149. In: Actas del Congreso 1999 de la Sociedad Espanola de Malherbologa Comunidad Autonoma de La Rioja Suso, ML and A Pardo, (eds), Spain.

Bigirimana, J., and Höfte, M. 2002. Induction of systemic resistance to *Colletotrichum lindemuthianum* in bean by a benzothiadiazole

derivative and rhizobacteria. Phytoparasitica 30: 159-168.
Bokshi, A.I., Morris, S.C., and Deverall, B.J. 2003. Effects of benzothiadiazole and acetylsalicylic acid on β-1,3-glucanase activity and disease resistance in potato. Plant Pathology 52: 22-27.
Borges, A.A., Cools, H.J., and Lucas, J.A. 2003. Menadione sodium bisulphite: a novel plant defense activator which enhances local and systemic resistance to infection by *Leptosphaeria maculans* in oilseed rape. Plant Pathology 52: 429-436.
Bouraoui, M., Abbes, Z., Abdi, N., Hmissi, I., and 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.
Buschmann, H., Ghiasi, K.G., and Sauerborn, J. 2002. Induced resistance in sunflower against *Orobanche cumana.* IOBC Bulletin 25: 145-148.
Buschmann, H., Fan, Z.W., and Sauerborn, J. 2005. Effect of resistance-inducing agents on sunflower (*Helianthus annuus* L.) and its infestation with the parasitic weed *Orobanche cumana* Wallr. Journal of Plant Disease and Protection 112: 7386-7397.
Daniel, R., and Guest, D. 2006. Defense induced by potassium phosphonate in *Phytophthora palmivora*-challenged *Arabidopsis thaliana.* Physiological and Molecular Plant Pathology 67: 194-201.
DGPA. Direction Générale de la Production Agricole en Tunisie, 2011
Elmer, W.H. 2006. Effect of acibenzolar-S-methyl on the suppression of Fusarium wilt of cyclamen. Crop Protection 25: 671-676.
Fan, Z.W., Buschmann, H., Muller-Stover, D., and Sauerborn, J. 2007. Main effect and interactions among acibenzolar-S-methyl, a biocontrol fungus and sunflower cultivar on control of *Orobanche cumana* Wallr. Journal of Plant Diseases and Protection 114: 76-81.
Goldwasser, Y., Hershenhorn, J., Plakhine, D., Kleifeld, Y., and Rubin, B. 1999. Biochemical factors involved in vetch resistance to *Orobanche aegyptiaca.*Physiological and Molecular Plant Pathology 54:87-96.
Gonsior, G., Buschmann, H., Szinicz, G., Otmar, S., and Sauerborn, J. 2004. Induced resistance - an innovative approach to manage branched broomrape (*Orobanche ramosa*) in hemp and tobacco. Weed Science 52: 1050-1053.
Gorlach, J., Volrath, S., Knauf-Beiter, G., Hengy, G., Beckhove, U., Kogel, K.H., Oostendorp, M., Staub, T., Ward, E., Kessmann, H., and Ryals, J. 1996. Benzothiadiazole, a novel class of inducers of systemic acquired resistance, activates gene expression and disease resistance in wheat. Plant Cell 8: 629-643.
Hadi, M. R., and Balali, G.R. 2010. The effectof salicylic acid on the reduction of *Rhizoctonia solani* damage in the tubers ofmarfona potato cultivar. American-Eurasian Journal of Agricultural and Environmental Sciences 7: 492-496.
Hafez, Y.M., Soliman, N.K., Saber, M.M., Imbabi, I.A., and Abd-Elaziz, A.S. 2014. Induced Resistance against *Puccinia triticina*, the Causal Agent of Wheat Leaf Rustby Chemical Inducers. Egyptian Journal of Biological Pest Control 24: 173-181.
Hassan, M.E.M., Abd El-Rahman, S.S., El-Abbasi,I.H., and Mikhail, M.S. 2006. Inducing resistance against faba bean chocolate spot disease. Egyptian Journal of Phytopathology 34: 69-79.
Heil, M., Hilpert, A., Kaiser, W., and Linsenmair, K.E. 2000. Reduced growth and seed set following chemical induction of pathogen defense: does systemic acquired resistance (SAR) incur allocation costs? Journal of Ecology 88: 645 -654.
Jackson, T.J., Burgess, T., Colquhoun, I., and Hardy, G.E.St.J. 2000. Action of fungicide phosphite on *Eucalyptus marginata* inoculated with *Phytophthora cinnamomi.*Plant Pathology 49: 147-154.
JORT. 2004. Journal Officiel de la République Tunisienne 63: 2246-2248.
Kharrat, M., Zouaoui, M., Saffour, K., and Souissi, T. 2002. Lutte chimique contre l*'Orobanche foetida* sur la fève. Page 90. In: Devenir des légumineuses alimentaires dans le Maghreb Kharrat, M., Abbad Andaloussi, F., Maatoughi, M.E.H., Sadiki, M., and Bertenbreiter, W., (eds), GTZ, Tunis, Tunisia.
Kharrat, M., and Souissi, T. 2004. Research on *Orobanche foetida* and *O. crenata* in Tunisia. Pages 106-110. In: Integrated Management of Orobanche in Food Legumes in the Near East and North Africa, Proceedings of the Expert Consultation on IPM for Orobanche in Food Legume Systems in the Near East and North Africa Dahan, R. and El-Mourid, (eds), ICARDA, Aleppo.
Kharrat, M., Abbes, Z., and Amri, M. 2010. A new faba bean small seeded variety 'Najeh' tolerant to Orobanche registered in the Tunisian catalogue Tunis. Tunisian Journal of Plant Protection 5: 125-130.
Kuc, J. 2006. What's old and what's new in concepts of induced systemic resistance in plants, and its applications. Pages 9-20. In: Tuzun, S. and Bent, E. (eds.), Multigenic and Induced Resistance in Plants. Springer NY, USA.

Kusumoto, D., Goldwasser, Y., Xie, X., Yoneyama, K., Takeuchi, Y., and Yoneyama, K. 2007. Resistance of red clover (*Trifolium pratense*) to the root parasitic plant *Orobanche minor* is activated by salicylate but not jasmonate. Annals of Botany 100: 537-544.

Labrousse, P., Arnaud, M.C., Serieys, H., Berville, A., and Thalouarn, P. 2001. Several mechanisms are involved in resistance of *Helianthus* to *Orobanchecumana* Wallr. Annals of Botany 88: 859-868.

Lawton, K.Y., Friedrich, L., Hunt, M., Weymann, K., Delaney, T., Kessmann, H., Staub, T., and Rayals, J. 1996. Benzothiazole induces disease resistance in *Arabidopsis* by activation of the systemic acquired resistance signal transduction pathway. Plant Journal 10: 71-82.

Lopez, A.M.Q., and Lucas, J.A. 2002. Effects of plant defense activators on anthracnose disease of Cashew. European Journal of Plant Pathology 108: 409-420.

Mbazia, A., Omri Ben Youssef, N., and Kharrat, M. 2016. Effect of some chemical inducers on chocolate spot disease of faba bean in Tunisia. Journal of Crop Protection 5: 541-552.

Muller-Stover, D., Buschmann, H., and Sauerborn, J. 2005. Increasing control reliability of *Orobanche cumana* through integration of a biocontrol agent with a resistance- inducing chemical. European Journal of Plant Pathology 111: 193-202.

Pajot, E., Le Corre, D., and Silue, D. 2001. Phytogard (R) and DL-beta-amino butyric acid (BABA) induce resistance to downy mildew (*Bremia lactucae*) in lettuce (*Lactuca sativa* L.). European Journal of Plant Pathology 107: 861-869.

Parker, C. 2009. Observations on the current status of *Orobanche* and *Striga* problems worldwide. Pest Management Science 65: 453-459.

Perez, L., Rodriguez, M.E., Rodriguez, F., and Roson, C. 2003. Efficacy of acibenzolar-S-methyl, an inducer of systemic acquired resistance against tobacco blue mould caused by *Peronospora hyoscyami* f sp *tabacina*. Crop Protection 22: 405-413.

Pérez-de-Luque, A., Jorrin, J.V., and Rubiales, D. 2004. Crenate broomrape control in pea by foliar application of benzothiazole (BTH). Phytoparasitica 32: 21-29.

Prats, E., Rubiales, D., and Jorrin, J. 2002. Acibenzolar-S-methyl-induced resistance to sunflower rust (*Puccinia helianthii*) is associated with an enhancement of coumarins of foliar surface. Physiological and Molecular Plant Pathology 60:155-162.

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

Saindrenan, P., Barchietto, T., and Bompeix, G. 1988. Modification of the phosphite induced resistance response in leaves of cowpea infected with *Phytophthora cryptogea* by a-aminooxyacetate. Plant Science 58: 245-252.

Sarwar, N., Hayet, M.Z.C.H., Haq, I., and Jamil, F.F. 2005. Induction of systemic resistance in chickpea against Fusarium wilt by seed treatment with salicylic acid and Bion. Pakistan Journal of Botany 37:989-995.

Sauerborn, J., Buschmann, H., Ghiasvan-Ghiasi, K., and Kogel, K.H. 2002. Benzothiazole activates resistance in sunflower (*Helianthus annuus*) to the root-parasitic weed *Orobanche cumana.*Phytopathology 92: 59-64.

Serghini, K., Pérez-De-Luque, A., Castejon-Munoz, M., Garcia-Torres, L., and 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., Rubiales, D., and Cubero J.I. 1996. Risks of *Orobanche* resistance screening based only on number of emerged shoots per plant. Pages 651-657. In Moreno, M.T., Cubero, J.I. Berner, D. Joël, D. Musselman, L.J. and Parker C. (eds.), Advances in Parasitic Plant Research Cordoba, Spain.

Sillero, J.C., Villegas-Fernandez, A.M., Thomas, J., Rojas-Molina, M.M., Emeran, A.A., Fernandez-Aparicio, M., and Rubiales, D. 2010. Faba bean breeding for disease resistance.Field Crops Research 115: 297-307.

Somaya, T., and El-Sharkawy, H.H.A. 2014. Efficacy of some resistance inducers for controlling wheat rust disease (*Puccinia triticina* fsp tritici). Egyptian Journal of Biological Pest Control 24: 203-209.

Stermer, B.A., and Hammerschmidt, R. 1987. Association of heat shock induced resistance to disease with increased accumulation of insoluble extensin and ethylene synthesis. Physiological and Molecular Plant Pathology 31:453-461.

Ter Borg, S.J., Willemsen, A., Khalil, S.A., Saber, H.A., Verkleij, J.A.C., and 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., Abbes, Z., Amri, M., and Kharrat, M. 2015. Performance of faba bean genotypes with *Orobanche foetida* Poir. and *Orobanche crenata* Forsk. infestation in Tunisia. Chilean Journal of Agricultural Research 75: 27-34.

Trabelsi, I., Abbes, Z., Amri, M., and Kharrat, M. 2016. Response of some selected faba bean (*Vicia faba* L.) genotypes to *Orobanche* spp. in Tunisia. Plant Production Science 19: 562-573.

Trabelsi, I., Yoneyama, K., Abbes, Z., Xie, X., Amri, M., Kharrat, M., and Yoneyama, K. 2017. Characterization of strigolactones produced by *Orobanche foetida* and *Orobanche crenata* resistant and susceptible faba bean genotypes and effect of phosphorous, nitrogen, and potassium deficiencies on strigolactone production. South African Journal of Botany 108: 15-22.

Véronési, C., Delavault, P., and Simier, P. 2009. Acibenzolar-S-methyl induces resistance in oilseed rape (*Brassica napus* L) against branched broomrape (*Orobanche ramosa* L). Crop Protection 28: 104-108.

Vincent, J.M. 1970. A manual for the practical study of root nodule bacteria. Oxford: Blackwell Scientific, UK, 164 pp.

Ward, E.R., Uknes, S.J., William, S.C., Dincher, S.S., Wiederhold, D.L., Alexander, D.C., Ahl-Goy, P., Métraux, J.P., and Ryals, J. 1991. Coordinate gene activation in response to agents that induce systemic acquired resistance. Plant Cell 3: 1085-109.

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