

OROBANCHE FOETIDA CONTROL IN FABA BEAN BY FOLIAR APPLICATION OF BENZOTHIADIAZOLE (BTH) AND SALICYLIC ACID

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Abstract

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Orobanche foetida is considered as an important agricultural problem of faba bean production in Tunisia. The effect of salicylic acid SA and 1, 2, 3-Benzothiadiazole-7-carbothioic acid, S-methyl ester (BTH) as foliar application on the induction of faba bean resistance to *O. foetida* was studied. Foliar application of 1 mM SA or 0.05 g/l BTH reduced broomrape infection under controlled conditions in pot and Petri dish experiments. In pot experiment, SA and BTH applications reduced the total number of faba bean broomrape by 46.45% and 77.06%, respectively, and the dry weight of broomrape by 47.03% and 70.3%, respectively. In Petri dish experiment, *O. foetida* seed germination was reduced by 33.8% and 48.3%, respectively. Foliar application of SA and BTH demonstrate that the induced resistance to *O. foetida* is systemic. These results demonstrate that SAR is capable of being an important method to control broomrapes and to form an integrated control strategy leading to reduce soil infestation by *Orobanche*.

Key words: *O. foetida*, faba bean, Salicylic acid, BTH, Control, Systemic acquired resistance SAR

Introduction

Broomrapes (*Orobanche* spp.) are root parasitic plants that threaten agricultural production in many parts of the world. They completely depend on their host for their nutritional requirements. *O. foetida* is considered as an important agricultural problem of faba bean production in Tunisia, causing yield losses between 66 to 90% in the 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 to both the extent of cultivated areas and to food legume production.

Several strategies (cultural, chemical and biological) have been used for controlling broomrapes but without complete success. In Tunisia, research activities on *Orobanche* were intensified and some methods result to incomplete protection

against broomrapes in faba bean (Abbes et al., 2007a, 2010; Kharrat et al., 2010; Bouraoui et al., 2012). Thus alternative or supplementary methods should be considered to prevent infection.

Salicylic acid (SA) is a chemical defence-inducer that enhanced resistance to various pathogens and induces disease resistance and SAR gene expression (Ward et al., 1991). BTH (1,2,3-Benzothiadiazole-7-carbothioic acid, S-methyl ester) is a synthetic functional analogue of SA (Gorlach et al., 1996; Bokshi et al., 2003) and its application to plants has been proposed to be an applicable strategy to control *Orobanche* parasitism. For example, BTH soil drenching can reduce *O. ramosa* attachments in hemp, tobacco and oilseed rape (Gonsior et al., 2004; Véronési et al., 2009) and *O. cumana* in sunflower (Muller-Stover et al., 2005; Fan et al., 2007). Soaking seeds in BTH or foliar spray of BTH were also reported to reduce *O. cumana* at-

tachments in sunflower (Sauerborn et al., 2002), *O. crenata* attachments in pea (Perez-de-Luque et al., 2004) and *O. ramosa* in oilseed rape (Véronési et al., 2009). In clover, root application of SA and BTH significantly reduced the number of established *O. minor* parasites by more than 75% (Kusumoto et al., 2007).

The aim of this study was to evaluate for the first time the potential of chemically induced resistance in the pathosystem faba bean - *O. foetida*.

Materials and Methods

Plant materials

The faba bean variety (Badi) used in this work was known for its high productivity in non *Orobanche* infested fields (JORT, 2004). This variety is known also for its susceptibility to *O. foetida* and *O. crenata* (Abbes et al., 2007a, 2007b, 2011). *O. foetida* seeds were collected from shoots harvested from an infested faba bean field in 2012 (Beja, Tunisia) and kept at 20°C before use. Faba bean seeds were surface-sterilized with 5% calcium hypochlorite for 15 min and then rinsed five times with sterile distilled-water and allowed to germinate at 21 ± 2°C in the dark for seven days. *O. foetida* seeds were sterilized in 2% calcium hypochlorite for 5 min and rinsed five times with sterile distilled-water. Seeds were provided by legume program, Field Crops Laboratory of National Agronomic Research Institute in Tunisia (INRAT).

Plant culture and treatments

For pot experiments, broomrape seeds (20 mg kg⁻¹ of soil) were mixed with a sterilized soil in a 2 L pot. Two faba bean seeds were then sown directly into each pot. Two weeks after faba bean emergence, seedlings were thinned to one per pot. Pots containing broomrape-free soil were used as controls. Plants were grown under greenhouse conditions at 25 ± 5°C with humidity of 70% and a 16 h photoperiod and watered as needed. Two chemical resistance inducers are used: BTH in the form of “1,2,3-Benzothiadiazole-7-carbothioic acid, S-methyl ester” and Salicylic Acid SA. Treatment procedure was made as foliar application. Treatment was started on 4-week-old faba bean plants by spraying BTH (0.05 g/l) or Salicylic Acid (1 mM) solutions on leaves until saturated. Excess of stimulator was collected at the soil surface on a filter paper. Treatments were repeated on all the leaves at 5, 6 and 7 weeks after sowing. Tween 20 (3 drops per liter) was added as a surfactant for leaf application of BTH and SA. Controls were performed by spraying water plus Tween 20.

Twelve weeks after sowing, faba bean plants were uprooted. The total number of broomrape attachments per host

plant were measured and classified according to their developmental stage (1: attachment of haustorium to host root; 2: small tubercles without root development; 3: tubercles with crown roots without shoot formation; 4: underground tubercles with shoot formation; 5: emergence of spikes) (Labrousse et al., 2001). In addition, the dry weight of broomrape attachments was determined following incubation of fresh tubercles in an oven at 80°C for 72 h.

For Plastic Petri dish experiment, faba bean and *Orobanche* seeds were surface sterilized as described above. Petri dishes (120 x120 x17 mm, Greiner) were filled with sterilized sand and prepared according to Abbes et al. (2010) with some modifications. Filter paper was placed between the sand and the plastic cover. Twenty milligrams of *O. foetida* seeds were spread uniformly on the filter paper. Germinated faba bean seeds were transferred into each Petri dish and put in contact with the parasite seeds. Petri dishes were then transferred to greenhouse (25 ± 5°C, 12 h photoperiod, natural light) and placed side by side into a plastic box in order to shield the roots from light without disturbing shoot development.

Treatment by the two chemical inducers (0.05 g/l BTH or 1 mM Salicylic Acid) on plant leaves was made as foliar application on 2-week-old faba bean until saturated. Two other treatments are repeated at 28 and 36 days after transplantation (dat).

O. foetida seed germination was evaluated 43 days after transplantation (dat) by using a binocular microscope. Four squares of 1 cm² near infested faba bean roots per Petri dish were observed and the number of germinated seeds counted and expressed as percentage of total seeds. The total number of tubercles per plant was determined from the whole root system of the host plant at 57 dat. Tubercles were classified according to their developmental stage (Labrousse et al., 2001).

Statistical analysis

Results were analyzed using the SPSS 11.5 software (Windows edition). Mean comparisons were made using Duncan's multiple-range classification test at P = 0.05.

Results

In pot experiments, the number and dry weight of attachments formed and their development stage were measured twelve weeks after planting. Non-treated plants showed up to 17 broomrape attachments per faba bean plant (Table 1).

Foliar application of SA and BTH reduced the total number of faba bean broomrape by 46.45% and 77.06%, respectively. Most attachments were at subterranean stages, and the

number of those reaching stages S5 was significantly reduced (Table 1).

Spraying SA and BTH did not affect only the number of total *Orobanche* but also the *Orobanche* dry weight. SA reduced the dry weight of broomrape by 47.03%, however this reduction was 70.3% under BTH treatment.

In Petri dish experiments, SA and BTH applications reduce *O. foetida* seed germination by 33.8% and 48.3% respectively (Table 2). The number of broomrape attachments per faba bean plant was significantly reduced in BTH treated plants (69.42%). SA treated plants show non-significant reduction (46.94%). No tubercle necrosis was observed by SA and BTH treatments.

Discussion

The effect of 1 mM of salicylic acid or BTH (0.05 g/l) on the biological cycle of the parasite was evaluated in pot and Petri dish experiments. These SA and BTH concentrations were chosen because they were frequently reported in the literature as concentrations inducing plant resistance to various pathogens (Saindrenan et al., 1988; Lawton et al., 1996; Jackson et al., 2000; Sauerborn et al., 2002; Borges et al., 2003; Perez de luque et al., 2004; Daniel and Guest, 2006; Elmer, 2006; Véronési et al., 2009).

In pot experiment, the three applications of SA or BTH weekly resulted in a high reduction of *O. foetida* infestation essentially with BTH treatment. This reduction was characterized by a reduced number and dry weight of *O. foetida* parasites and also low tubercle reaching stage 5.

In Petri dish experiments, the germination of *O. foetida* seeds was significantly affected by treatments with SA or BTH in comparison with the controls. Knowing that in our study SA and BTH were used as foliar application and thus there is no direct contact between these molecules and *O. foetida* seeds, we can postulate that these molecules induce low production of stimulant substances or increase in the

release of inhibitory substances by the host. Several studies show no significant reduction of germination of BTH-treated *Orobanche* seeds, demonstrating that there are no toxic effects of BTH on *Orobanche* seeds (Sauerborn et al., 2002; Pérez-de-Luque et al., 2004; Kusumoto et al., 2007; Véronési et al., 2009). Buschmann et al (2005a) showed that BTH applied as a root chamber drench did not interfere with *O. cumana* seed germination. 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. These results of foliar applications demonstrate that the induced resistance to *Orobanche* (a root pathogen) is systemic.

Also, BTH and SA applications reduced the total *O. foetida* number and the number of tubercles reaching stage 5, but did not induce necrosis of developing parasite tubercles. Similar data were reported in tobacco and hemp infected by *O. ramosa* (Gonsior et al., 2004), in pea infested with *O. crenata*, in sunflower attacked by *O. cumana* (Buschmann et al., 2005b; Muller-Stover et al., 2005; Fan et al., 2007) and in oilseed rape infected with *O. ramosa* (Véronési et al., 2009). 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.*

Table 2
Effect of Salicylic acid (1 mM) and BTH (0.05 g/l) treatment (leaf spray) on the infestation of faba bean with *Orobanche foetida* in petri dish experiment

	Germination percentage, 43 dat	Total Orobanche number, 57 dat
Control	29.20 a	6.54 a
SA	19.33 b	3.47 ab
BTH	15.12 b	2.00 b

Plants were weekly treated. Data with the same letter are not significantly different (Duncan, P = 0.05).

Table 1
Effect of Salicylic acid (1 mM) and BTH (0.05 g/l) treatment (leaf spray) on the infestation of faba bean with *Orobanche foetida* in pot experiment

	Total Orobanche	S2	S3	S4	Subterranean Orobanche	Emerged Orobanche	Total Orobanche
	Number, 84 dat				number, S2-S4	number, S5	dry weight, g
Control	17.48 a	3.44 a	2.08 a	9.56 a	15.08 a	2.40 a	2.02 a
SA	9.36 b	1.25 ab	1.20 a	6.27 a	8.72 b	0.64 b	1.07 b
BTH	4.01 b	0.80 b	0.81 a	2.20 b	3.81 b	0.20 b	0.60 c

Plants were weekly treated. Data with the same letter are not significantly different (Duncan, P = 0.05).

minor tubercles in red clover caused by SA and BTH was due to the inhibited elongation of *O. minor* radicles and the activation of defence 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 phytoalexin scopoletin 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 (Goldwasser et al., 1999; Stermer et al., 1987).

Other mechanisms like the synthesis of the coumarin phytoalexins scopoletin and ayapin in sunflower and its increased synthesis can be induced by BTH or SA application (Prats et al., 2002; Sauerborn et al., 2002; Serghini et al., 2001). These studies suggested that these chemical defence-inducers could prevent *Orobanche* infection by activating the SA dependent pathway in the host plants.

Conclusion

These results demonstrate that SAR is capable of being an important method to control broomrapes. This control method should be confirmed in field conditions and could be a useful tool for an integrated control strategy leading to reduce soil infestation by *Orobanche*. More researches and studies by varying doses, treatment methods and application times are necessary for an efficient control of the fetid broomrape.

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