Phytopathologia Mediterranea (2016) 55, 1, 54–61
DOI: 10.14601/Phytopathol_Mediterr-16338

RESEARCH PAPERS

Biochemical analysis of induced resistance in chickpea against broomrape (*Orobanche foetida*) by rhizobia inoculation

Yassine MABROUK[1,2,*], Sonia MEJRI[2], Imen HEMISSI[3] and Omrane BELHADJ[2]

[1] Laboratoire de Biotechnologie et Technologies Nucléaires, Centre National des Sciences et Technologies Nucléaires, Technopôle de sidi Thabet, 2020 Tunisie
[2] Université de Tunis El Manar, Faculté des Sciences de Tunis, Laboratoire de Biochimie et de Technobiologie, Campus Universitaire Farhat Hached 2092, Tunis, Tunisie
[3] Institut National de Recherche Agronomique de Tunisie (INRAT), rue hédi karray, 2049 Ariana Tunisie

Summary. This study examined the capacity of *Rhizobium* sp. strain PchAZM to reduce parasitism of chickpea by *Orobanche foetida* under greenhouse conditions, and assessed the relative impact of rhizobia on the expression of chickpea defense response against broomrape. Growth chamber experiments using Petri dishes revealed that rhizobia infection on chickpea roots reduced broomrape seed germination, and restricted the broomrape attachment to host roots while retarding tubercle formation and development by the parasite. In pot experiments, chickpea roots inoculated with rhizobia reduced the total number of broomrape by up to 90%. Broomrape necrosis was observed both before and after parasite attachment to inoculated chickpea roots in Petri dishes and pot experiments. Reduction in infection was accompanied by enhanced levels of the defence-related enzymes phenylalanine ammonia lyase (PAL) and peroxidase (POX). Increased levels of phenolics were recorded in the roots of rhizobia-inoculated plants grown in the presence of broomrape. The results suggest that rhizobia could be used for protection of chickpea against *O. foetida*.

Key words: biocontrol, legume crops, defense related enzymes, phenolic content.

Introduction

Chickpea (*Cicer arietinum* L.) is one of the most utilized legumes in the world because it is considered to be an excellent source of dietary protein (Frias *et al.*, 2000). However, chickpea production in Tunisia falls short of the demand due to biotic and abiotic constraints that reduce crop yields and grain quality. *Orabanche foetida* (broomfape) develops in Tunisia on some cultivated species such as broad beans (Kharrat *et al*., 1992), and can also infect and develop on chickpea, lentil and vetch with variable levels of parasitism (Kharrat, 2002). Recently, attacks by this parasitic plant on Fenugreek (*Trigonella foenum-graecum*) were reported by Amri *et al.* (2009). Faba bean yield losses may reach 50 to 80% at medium to high levels of soil infestation by broomrape (Kharrat and Halila, 1994).

Strategies to control *Orobanche* parasitism are limited because of its extremely broad host range and the long lasting survival of broomrape seeds in fields under various environmental conditions (Rubiales *et al.*, 2003). *Orobanche* spp. are not usually amenable to control by persistent selective herbicides, since herbicides cannot differentiate between crops and these parasitic plants (Rubiales and Fernandez, 2012). The main bio-control components for broomrape management are virulent insects and fungal pathogens, or fungal toxins (Andolfi *et al.*, 2005; El-Kassas *et al.*, 2005; Fernández-Aparicio *et al.*, 2010). For a more integrated *Orobanche* management approach, com-

Corresponding author: Y. Mabrouk
E-mail: yassine.mabrouk@fst.rnu.tn

ISSN (print): 0031-9465
ISSN (online): 1593-2095
www.fupress.com/pm

binations of agronomic practices, chemical and biocontrol approaches would be more suitable.

The ability of rhizobia to limit parasitism by broomrape was demonstrated for different legumes (Mabrouk *et al.*, 2007; Hemissi *et al.*, 2013; Mabrouk and Belhadj, 2014). In this study, experiments were carried out to examine the antagonist effects of *Rhizobium* sp. strain PchAZM to *O. foetida*, and to evaluate the relative impact of rhizobia on the expression of the plant's defence response against the parasite.

Materials and methods

Bacterial strain and growth conditions

Rhizobium sp. strain PchAZM was isolated from nodules on the root systems of chickpea plants harvested from *Orobanche*-free fields. Bacterial cells were stored on yeast extract mannitol agar medium at 4°C (Mabrouk *et al.*, 2007). The *Rhizobium* strain PchAZM used in this study was selected based on its effectiveness as a biocontrol agent against *O. foetida* from among 40 strains after they had been tested in a nodulation test.

Evaluation of *Rhizobium* as resistance inducer against *Orobanche foetida*

Co-culture using Petri dish experiments

Co-culturing was performed as described by Labrousse *et al.* (2001). Chickpea (cv. Beja1) seeds were surface-sterilized with 10% calcium hypochlorite for 30 min and then rinsed three times with sterile water. Seeds were placed in Petri dishes on sterile filter paper imbibed with sterile distilled water and allowed to germinate at 28°C in the dark for 7 d. Chickpea seedlings were transferred to square Petri dishes (120 × 120 × 17 mm, Greiner) lined with fiber filter paper (MN 85/90, 12.5 cm diam., Macherey-Nagel). Roots were spread between the dish cover and filter paper. Two holes were made in the upper rim of each the Petri dish to allow the chickpea shoots to grow through, and two holes were drilled through the lower rim to allow plant roots to feed in culture medium (Figure 1F). A 1 cm thick rock-wool layer (Master from Grodan, Town) was placed under chickpea roots on the other side of the glass fibre filter paper. Petri dishes were closed, covered with aluminium foil and placed vertically in a sterile polypropylene tray contain-

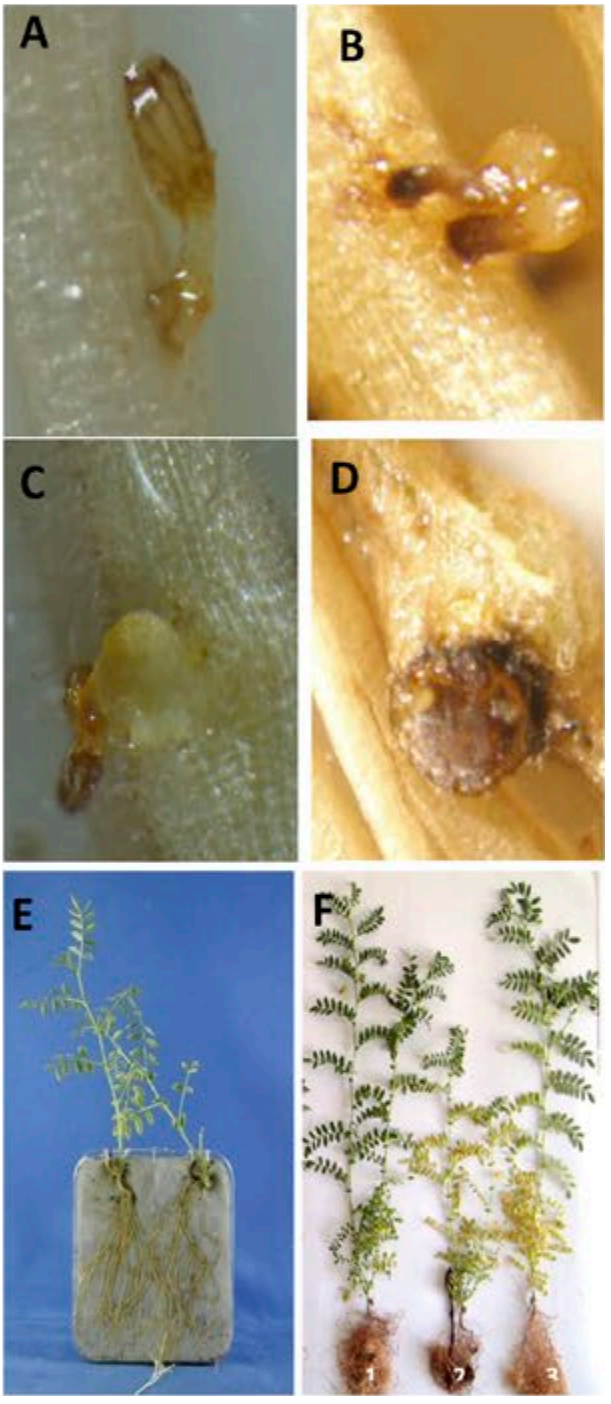

Figure 1. (A) typical germination, (B) seed germination with browning symptoms, (C) healthy tubercle fixed on chickpea roots, (D) tubercle with necrotic symptoms, (E) co-culture system using Petri dishes, and (F) plant growing in pots (1: chickpea plant infested with *O. foetida* and inoculated with Rhizobium strain PchAZM, 2: chickpea infested with *Orobanche* and 3: healthy chickpea plant).

ing sterile solution of Coïc neutrophile nutrient (Coic and Lesaint, 1975), and were maintained at 21°C under a 16 h photoperiod. After 15 d, 3 mL of rhizobial cell suspension were added to the roots. In addition, seeds of *O. foetida* were preconditioned in Petri dishes (35 mm diam.) on fibreglass filter papers moistened with 0.5 mL of distilled water for 7 d at 22°C. About 100 preconditioned *O. foetida* seeds were placed regularly at 1–2 mm from chickpea roots. Broomrape seed germination was evaluated 15, 30 and 45 d after inoculation with rhizobia using a binocular microscope. Four areas per Petri dish were observed and the number of germinated broomrape seeds were counted and expressed as percentage of total seeds. Germination rates were expressed by taking into account the viability of the seed lot used for experiments (82%). By 30 and 45 d after inoculation (DAI), germinated necrotic seeds that did not succeed in attaching to host roots in the selected areas of Petri dishes were counted and expressed as the percentage of total germinated seeds, and numbers of necrotic tubercles per plant were recorded. Non-inoculated chickpea seedlings growing in contact with *Orobanche* served as experimental controls.

Pot experiment

Rhizobium strain PchAZM was tested in a pot experiment with five replicates per treatment. Chickpea seedlings were transferred to plastic pots (0.5 L capacity) containing a sterilised mixture of local field soil and sand (1:1, v/v) and then either left as such or inoculated with *O. foetida* seeds (5 mg per pot). Four sets of pot cultures were managed simultaneously; (i) chickpea grown in *Orobanche*-free soil, (ii) chickpea grown in infested soil, (iii) chickpea grown in *Orobanche*-free soil inoculated with *Rhizobium* strain PchAZM, and (iv) chickpea grown in infested soil inoculated with *Rhizobium* strain PchAZM. Each chickpea plant was treated with 3 ml of rhizobial cell suspension. Plants were irrigated weekly with N-free nutrient solution. The impact of rhizobia on chickpea infection by *O. foetida* was estimated for 70-d-old plants, at which time broomrape seeds started to emerge. Chickpea roots were gently harvested, washed with water, the total number of tubercles per plant, the necrotic tubercle as the percentage of total fixed tubercles, and the dry matter of *Orobanche* were recorded, and chickpea shoot lengths and shoot dry weights were measured.

Analysis of some biochemical parameters

Peroxidase (POX) and Phenylalanine ammonia lyase (PAL) assays

Procedures were carried out as described by Anderson *et al.* (1995) and Lin and Kao (1999) for the peroxidase assay and by Westcott and Henshaw (1976) for the PAL assay. After grinding chickpea roots in liquid nitrogen, extraction buffer was added to the frozen powder in the ratio (1:3). The extraction buffer contained 100 mM KH_2PO_4/K_2HPO_4 (pH 7), 1% (V/V) Triton X-100 and 2% (wt/V) insoluble polyvinylpyrrolidone. The mixture was homogenized and centrifuged for 20 min at 10,000 g (4°C). The supernatant was used immediately for total protein quantification (Bradford, 1976) and both enzyme assays.

Soluble peroxidase activity was read in a spectrophotometer using gaiacol as substrate and hydrogen peroxide to initiate the reaction. Peroxidase activity was measured at 470 nm at 25°C in a reaction medium containing 9 mM gaiacol, 1 mM hydrogen peroxide and crude enzyme extract and expressed as U mg^{-1} protein (U: μmole tetragaïacol produced per min).

Phenylalanine ammonia lyase activity was determined spectrophotometrically at 290 nm by measuring the amount of cinnamic acid formed after incubation of the crude enzyme with L-phenylalanine for 2 h. The reaction mixture contained 1.4 mL of borate buffer (100 mM, pH 8.8), 0.6 mL of L-phenylalanine (100 mM) and 0.3 mL of the crude enzyme. Following 2 h of incubation at 40°C, the reaction was stopped by the addition of 0.05 mL of 5 M HCl. Activity was expressed as amount of cinnamic acid produced (μg h^{-1} mg^{-1} protein).

Estimation of total soluble phenolics in chickpea roots

Frozen chickpea roots from different treatments were extracted three times in 80% aq. MeOH at 4°C under continuous stirring. Total soluble phenolic content was measured spectrophotometrically at 760 nm, using the Folin–Ciocalteu reagent method (Waterman and Mole, 1994). The reaction mixture contained 790 μL of distilled water, 10 μL of sample and 50 μL of Folin–Ciocalteu reagent. After 1 min, 150 μL of aqueous sodium carbonate (20%) was added and the mixture was vortexed and allowed to stand at room temperature in the absence of light for 30 min. The total phenol concentration (expressed as μg catechin g^{-1} root fresh weight) was calculated from a calibration curve, using catechin as standard.

Statistical analyses

In all the experiments, ten plants were grown per treatment. Consequently, the data are means ± confidence limits (n =10, $\alpha = 0.05$ Student's t-test). Data were analyzed by multifactorial analysis of variance (ANOVA, SPSS 13.0 for Windows) and significant differences among treatments were considered at the $P < 0.05$ level.

Results

Effects of *Rhizobium* inoculation on broomrape parasitism in chickpea

Effects of treatment on yield parameters

Infection of chickpea plants with broomrape led to a significant reduction in plant growth as shown in the measured shoot lengths and shoot dry weights (Table 1). In contrast, *Rhizobium* sp. strain PchAZM established an effective symbiotic association with chickpea, as demonstrated by the large numbers of root nodules and significant increases in chickpea shoot lengths and shoot dry weights in comparison with infected plants. We observed that plants not inoculated with rhizobia showed no nodulation (Table 1). Chickpea roots inoculated with *Rhizobium* strain PchAZM had decreased numbers of *O. foetida* tubercles (Table 1). Total *Orobanche* dry matter per plant was reduced by 95% in chickpea roots inoculated with rhizobia. When plants grew in *Orobanche*-infested soil in pot experiments, chickpea roots inoculated with the *Rhizobia* carried few broomrape tubercles by 70 d after seeding in comparison with non-inoculated chickpeas (Table 1, Figure 1F).

Effects of rhizobia on underground stages of Orabanche foetida

Inoculation with the *Rhizobium* strain PchAZM, resulted in a significant decrease in germination of *O. foetida* seeds (up to 50%) and in tubercles formed on chickpea roots 45 DAI (Table 1). Changes in *O. foetida* seed germination started to be visible at 25 DAI. The chickpea radicles turned from colourless and translucid to brownish, seedling elongation ceased, and *Orobanche* development stopped (Figure 1 A and B). These results confirm those obtained previously in the case of pea inoculated with some *Rhizobium leguminosarum* strains (Mabrouk *et al.*, 2007). Some disease symptoms during early developmental stages of broomrape were observed in the case of inoculated chickpea with rhizobia, and the proportion of necrotic tubercles increased with time. Forty-five d after transplanting, the greatest percentage of necrotic tubercles was observed on chickpea inoculated with rhizobia. Healthy tubercles developed on the host roots within 18 d after attachment (Figure 1C). In contrast, when *O. foetida* attacked the chickpea roots inoculated with *Rhizobium* it died at an early stage of tubercle development (Figure 1D).

Effects of inoculation on defense enzyme activities and accumulation of phenolic compounds in chickpea roots.

Enzyme activities were investigated at 35 DAI in both infected and inoculated chickpeas (Figures 2 and 3), and compared with activity levels of the control plants including healthy and infected non-inoculated chickpeas. In chickpea plants inoculated

Table 1. Impact of root inoculation by rhizobia on *Orobanche* tubercle formation and dry matter in pot experiments

Treatment	*O. foetida* tubercle number / plant	*O. foetida* necrotic tubercles (% of total tubercles)	*O. foetida* DW (g)	Chickpea Shoot dry weight (g)	Chickpea shoot length (cm)	Nodule number/ plant
Chickpea	0^a	0^a	0^a	0.9 ± 0.1^a	41 ± 1.2^a	0^a
O. foetida infected chickpea	15.23 ± 3.25^c	0^a	2.5 ± 0.21^b	0.4 ± 0.1^b	22.1 ± 1.7^b	0^a
PchAZM inoculated chickpea	0^a	0^a	0^a	2.02 ± 0.1^c	58.4 ± 2.3^c	64.1 ± 2.8^b
O. foetida infected chickpea and inoculated by PchAZM	1.3 ± 0.3^b	50.4 ± 2.3^b	0.1 ± 0.05^a	1.8 ± 0.1^c	55.4 ± 3.2^c	63.2 ± 3.2^b

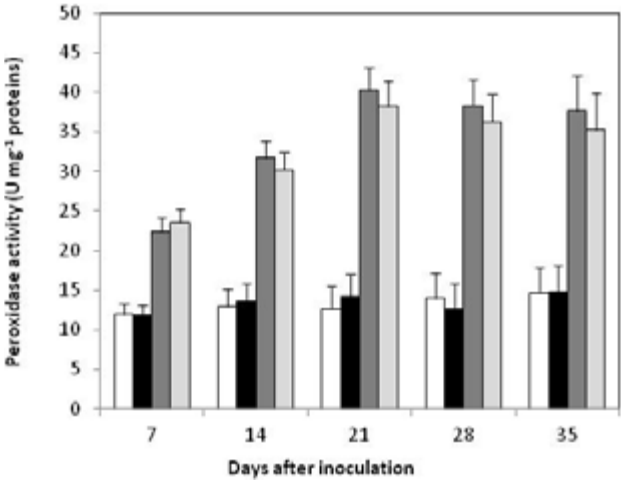

Figure 2. Peroxidase activities in chickpea roots inoculated with *Rhizobium* strain Pch AZM and infested by *Orabanche foetida*. Activities were measured at 7, 14, 21, 28 and 35 d after inoculation with rhizobia and infection with *Orobanche* (light grey). Experimental controls were with healthy chickpeas (□) singly inoculated (dark gray) or singly infected with *O. foetida* (■)

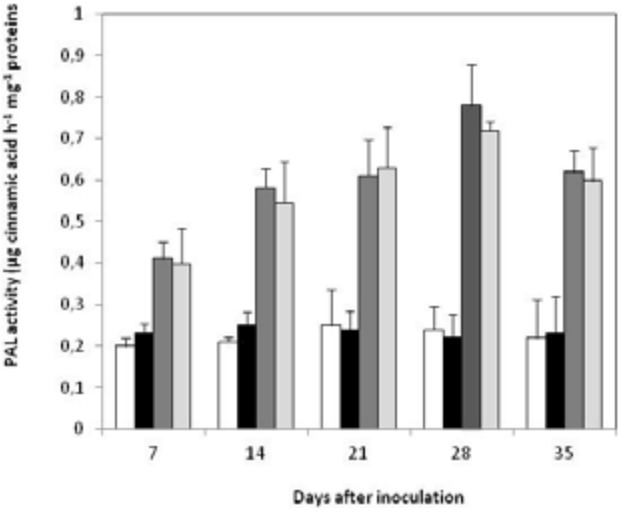

Figure 3. Levels of phenylalanine ammonia-lyase (PAL) in chickpea roots inoculated with *Rhizobium* strain PchAZM and infested by *Orabanche foetida*. Activities were measured at 7, 14, 21, 28 and 35 d after inoculation with rhizobia and infection with *O. foetida* (light grey). Experimental controls were performed with healthy chickpeas (□) singly inoculated (dark gray) or singly infected with *O. foetida* (■).

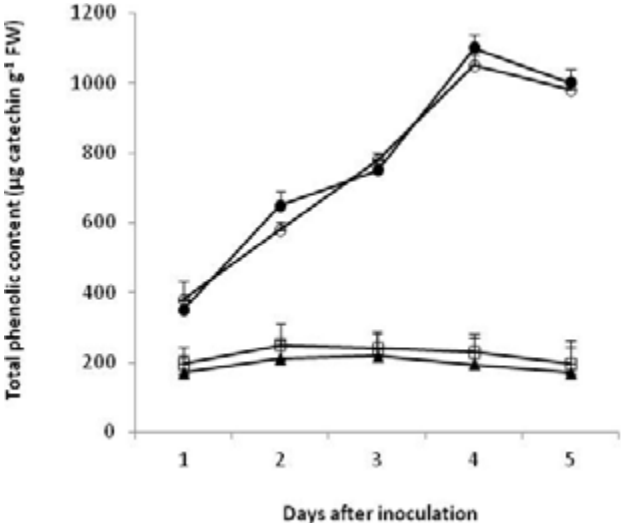

Figure 4. Phenolic content in chickpea root inoculated with *Rhizobium* strain PchAZM and infested by *O. foetida*. Contents were measured on 7, 14, 21, 28 and 35 days after inoculation with rhizobia and infection with *Orobanche* (●). Control was performed with healthy chickpeas (□) singly inoculated (○) or singly infected with *O. foetida* (▲).

with rhizobia, the expression of induced resistance was associated with enhanced enzyme activities.

Peroxidase activity increased gradually from 7 to 35 DAI when chickpeas were inoculated singly by rhizobia (Figure 2). A similar pattern was observed in roots inoculated with rhizobia and concomitantly infected by the parasitic plants. On the other hand, low and relatively constant activity occurred in healthy and *O. foetida*-infected chickpeas when not inoculated by *Rhizobium*.

Phenylalanine ammonia lyase activity remained unchanged by 35 DAI at a low value in healthy chickpea roots (Figure 3). Furthermore, infection by *O. foetida* did not significantly affect PAL activity in roots. In contrast, chickpea inoculation with rhizobia induced a threefold increase in PAL activity with a maximum at 21 DAI coinciding with no parasite attachment to chickpea roots.

Total soluble phenolic compounds accumulated later, from 28 to 35 DAI in chickpea inoculated by rhizobia (Figure 4). A similar pattern was observed when chickpea was inoculated with *Rhizobium* and then infected by *O. foetida*. On the other hand, healthy chickpea and plants only infected by *O. foetida* exhibited low relative amounts of total soluble phenolics.

Discussion

Our results show that inoculation with rhizobia significantly reduced parasitism of chickpea plants by *O. foetida*. These findings are in agreement with those of Bouraoui *et al.* (2012) who found that pre-inoculation with *Rhizobium* led to a significant reduction in disease severity caused by *O. foetida* in faba bean. These rhizobia also increased nitrogen content and dry weight of nodules, roots and shoots. Several other workers have noticed the beneficial effects of rhizobia on plant growth and reduction in parasite germination and tubercle formation (Hemissi *et al.*, 2013; Mabrouk and Blhadj, 2014). Indeed, the mechanism of action includes reducing parasite seed germination and radical growth, blocking host tissue penetration and connection to the vascular system. Our results show that inoculation with *Rhizobium* strain PchAZM significantly reduced the percentage of broomrape seed germination and tubercle formation on chickpea roots. *In vitro* co-culturing provided evidence that a much greater proportion of the successfully germinated *Orobanche* seedlings close to *Rhizobium*-inoculated roots turned brown before or during root penetration and finally failed to attach to host roots (Figures 1B and D; Table 2). This explains the significant reduction of tubercle formation on inoculated chickpea roots (Tables 1 and 2), and confirms potential mechanisms previously discussed by several authors. Among these are the typical plant mechanisms of defense against pathogenic microorganisms, such as the induction of pathogenesis-related (PR) proteins, peroxidases and phytoalexin biosynthesis enzymes (Joel and Portnoy, 1998; Castillejo *et al.*, 2004; Pérez de Luque *et al.*, 2006).

Biochemical and histological studies in several plant-parasite interactions revealed that production and secretion of these toxic compounds are responsible for arresting parasite development (Serghini *et al.*, 2001; Echevarría-Zomeño *et al.*, 2006; Pérez-de-Luque *et al.*, 2009). Several gene expression studies have revealed the induction of genes encoding enzymes involved in the phenylpropanoid pathway in response to *Orobanche* infection (Griffitts *et al.*, 2004; Dita *et al.*, 2009). In order to obtain a more comprehensive analysis of biochemical mechanisms of induced resistance in chickpea against *O. foetida* by inoculation with rhizobia, we analyzed the defense-related enzymes and changes in toxic compounds in roots.

The obvious increase in the total phenolics levels in the broomrape infected chickpea roots compared to healthy roots of plants previously inoculated with rhizobia, indicated the effectiveness of the rhizobia for inducing host resistance. In this regards, soluble phenolics have been shown to accumulate in plant roots, playing important roles in the parasitic infection processes and host resistance (Mabrouk *et al.*, 2007).

The activities of two defense enzymes in chickpea roots were significantly affected by inoculation with *Rhizobium* strain PchAZM. Phenylalanine ammonialyase (PAL) is the first enzyme of the phenylpropanoid pathway, whose induction and accumulation is a typical defense response associated with resistance in several plant systems and against diverse pathogenic organisms, especially broomrape (Daayf *et al.*, 1997; Mabrouk *et al.*, 2010). Peroxidases can polymerise polysaccharides and polyphenols to produce stable vascular occluding gels (Crews *et al.*, 2003).

In this study, rhizobia inoculated chickpeas displayed enhanced peroxidase activity, in addition to constantly high PAL activity in comparison with non-inoculated chickpeas. This was observed from 14 DAI when broomrape attachment had not yet oc-

Table 2. *Orobanche foetida* germination and tubercle formation in co-culture experiments on chickpea roots in relation to rhizobia inoculation.

Treatment	*O. foetida* seed germination (%)	Necrotic germinated seeds (%)	Number of tubercles/ plant	Necrotic tubercles (% of total tubercle number)
O. foetida infected chickpea	59.6±6.6[a]	2.9 ±0.4[a]	48.15± 4.9[a]	0[a]
O. foetida infected chickpea and inoculated by PchAZM	28.3±4.6[b]	52.9±4.7[b]	0.98±0.1[b]	50[b]

curred. Consequently, we hypothesize that these enzymes could be involved in chickpea resistance to *O. foetida* induced by *Rhizobium* inoculation during early and later stages of infection. These two enzymes could be implicated in broomrape avoidance of inoculated chickpea by preventing parasite penetration of the host roots, or by lowering nutrient fluxes towards the parasite when connection succeeds.

High levels of the defense enzymes POX and PAL in response to *Rhizobium* inoculation were correlated with reduced broomrape seed germination, and by prevention of parasite attachment and growth of installed tubercles. We conclude that the plants were primed for defense. Nevertheless, further studies are needed to characterize the mechanisms involved in *Rhizobium*-induced chickpea resistance to *O. foetida*, to improve *Rhizobium* strains used as bio-fertilizers and biocontrol agents in chickpea fields.

Acknowledgments

This work was supported by funds from the project "PHC Utique 13G0925" managed by the Joint University Committee of Cooperation (Tunisie-France). The authors thank Prof. Martina Rickauer (INP-ENSAT Toulouse, France) for critical review of the manuscript of this paper.

Literature cited

Amri M., F. Sellami and M. Kharrat, 2009. First Report of the Parasitic Plant *Orobanche foetida* on Fenugreek (*Trigonella foenum-graecum*) in Tunisia. *Tunisian Journal of Plant Protection* 4(2), 235–238.

Anderson M.D., T.K. Prasad and C.R. Stewart, 1995. Changes in isozyme profiles of catalase, peroxidase and glutathione reductase during acclimation to chilling in mesocotyls of maize seedlings. *Plant Physiology* 109, 1247–1257.

Andolfi A., A. Boari, A. Evidente and M. Vurro, 2005. Metabolites inhibiting germination of *Orobanche ramosa* seeds produced by *Myrothecium verrucaria* and *Fusarium compactum*. *Journal of Agriculture and Food Chemistry* 53, 1598–1603.

Bouraoui M., Z. Abbes, N. Abdi, I. Hemissi and B. Sifi, 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 (4), 557–564.

Bradford M., 1976. A rapid and sensitive method for the quantification of microgram quantities of proteins utilizing the principle of protein dye binding. *Analytical Biochemistry* 72, 248–254.

Castillejo M.A., N. Amiour, E. Dumas-Gaudot, D. Rubiales and J.V. Jorrín, 2004. A proteome approach to studying plant response to crenate broomrape **(***Orobanche crenata***)** in pea (*Pisum sativum*). *Phytochemistry* 65, 1817–1828.

Coic Y. and C. Lesaint, 1975. La nutrition mine´rale et en eau des plantes en horticulture avancée. *La Documentation Technique de la SCPA* 23, 1–22.

Crews L.J., M.E. McCully and M.J. Canny, 2003. Mucilage production by wounded xylem tissue of maize roots – time course and stimulus. *Functional Plant Biology* 30, 755–766.

Daayf F., R. Bel-Rhlad and R.R. Belanger, 1997. Methyl ester of *p-coumaric* acid- a phytoalexin like compound from long English cucumber leaves. *Journal of Chemical Ecology* 23, 1517–1526.

Dita M.A., J.V. Die, B. Román, F. Krajinski, H. Küster, M.T. Moreno, J.I. Cubero and D. Rubiales, 2009. Gene expression profiling of *Medicago truncatula* roots in response to the parasitic plant *Orobanche crenata. Weed Research* 49, 66–80.

Echevarría-Zomeño S., A. Pérez-de-Luque, J. Jorrín and A.M. Maldonado, 2006. Pre-haustorial resistance to broomrape (*Orobanche cumana*) in sunflower (*Helianthus annuus*): cytochemical studies. *Journal of Experimental Botany* 2006, 57, 4189–4200.

El-Kassas M., Z.Karem El-Din, M.H. Beale, J.L. Ward and R.N. Strange, 2005. Bioassay-led isolation of *Myrothecum verrucaria* and verrucarin A as germination inhibitors of *Orobanche crenata*. *Weed Research* 45, 212–219.

Fernández-Aparicio M., J.M. García-Garrido, J.A. Ocampo and D. Rubiales, 2010. Colonisation of field pea roots by arbuscular mycorrhizal fungi reduces *Orobanche* and *Phelipanche* species seed germination. *Weed Research* 50, 262–268.

Frias J., C. Vidal-Valverde, S. Sotomayor, C. Diaz-Pollan and G. Urban, 2000. Influence of processing on available carbohydrate content and antinutritional factors of chickpeas. *European Food Research and Technology* 5, 340–345.

Griffitts A., C. Cramer and J. Westwood, 2004. Host gene expression in response to Egyptian broomrape (*Orobanche aegyptiaca*). *Weed Science* 52, 697–703.

Hemissi I., Y. Mabrouk , N. Abdi, M. Bouraoui, M. Saidi and B. Sifi, 2013. Growth promotion and protection against *Orobanche foetida* of chickpea (*Cicer aerietinum*) by two *Rhizobium* strains under greenhouse conditions. *African Journal of Biotechnology* 12(12), 1371–1377.

Joel D.M. and V. Portnoy, 1998. The angiospermous root parasite *Orobanche* L. (*Orobanchaceae*) induces expression of a pathogenesis related (PR) gene in susceptible tobacco roots. *Annals of Botany* 81, 779–781.

Kharrat M. and M.H. Halila, 1994. *Orobanche* species on faba bean (*Vicia faba* L.) in Tunisia: Problem and Management. In: *Biology and Management of Orobanche* (A.H. Pieterse J.A.C. Verkleij and S.J. ter Borg, eds.) Proceedings of the 3rd International Workshop on *Orobanche* and Related *Striga* Research, November, 8-12, 1993. Amsterdam, The Netherlands, 639–643.

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

Kharrat M., M. Zouaoui, K. Saffour and T. Souissi, 2002. Lutte chimique contre *l'Orobanche foetida* sur la fève. In: M. Kharrat, F. A. Andaloussi, M. E. H. Maatoughi, M. Sadiki and

W. Bertenbreiter (ed.), *Proceedings sur le devenir des légumineuses alimentaires dans le Maghreb*, GTZ, Tunisie, pp. 90.

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

Lin C.C. and C.H. Kao, 1999. NaCl-induced changes in ionically bounds peroxidase activity in root of rice seedlings. *Plant and Soil* 216, 147–153.

Mabrouk Y. and O. Belhadj, 2014. Effect of the inoculation of chickpea by rhizobia on growth promotion and protection against *Orobanche crenata*. *Global Journal of Biology, Agriculture and Health Sciences* 3(3), 55–59.

Mabrouk Y., L. Zourgui, B. Sifi, P. Delavault, P.Simier and O. Belhadj, 2007. Some compatible *Rhizobium leguminosarum* strains in peas decrease infections when parasitised by *Orobanche crenata*. *Weed Research* 47, 44–53.

Mabrouk Y., S. Mejri, I. Hemissi, P. Simier, P. Delavault, M. Saidi O. Belhadj, 2010. Bioprotection mechanisms of pea plant by *Rhizobium leguminosarum* against *Orobanche crenata*. *African Journal of Microbiology Research* 4, 2570–2575.

Pérez-de-Luque A., C. González-Verdejo, M. Lozano, M. Dita, J. Cubero P. González-Melendi, M. Risueño and D. Rubiales, 2006. Protein cross-linking, peroxidase and b-1,3-endoglucanase involved in resistance of pea against *Orobanche crenata*. *Journal of Experimental Botany* 57, 1461–1469.

Pérez-de-Luque A., S. Fondevilla, B. Pérez-Vich, R. Aly, S. Thoiron, P. Simier, M.A. Castillejo, J.M. Fernández-Martínez, J. Jorrín, D. Rubiales and P. Delavault, 2009. Understanding *Orobanche* and *Phelipanche*– host plant interaction and developing resistance. *Weed Research* 49, 8–22.

Rubiales D. and M. Fernández-Aparicio, 2012. Innovations in parasitic weeds management in legume crops. A review. *Agronomy for Sustainable Development* 32 (2), 433–449.

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

Serghini K., A. Perez de Luque, M. Castejon-Munoz, L. Garcia-Torres and J.V. Jorrin, 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.

Waterman P.G. and S. Mole, 1994. Analysis of phenolics plant metabolites. Blackwell Scientific Publications, Oxford, UK.

Westcott R.J. and G.G. Henshaw, 1976. Phenolic synthesis and phenylalanine ammonia-lyase activity in suspension cultures of Acer pseudoplatanus L. *Planta* 131, 67–73.

Accepted for publication: October 26, 2015
Published online: May 14, 2016