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Induction of Phenolic Compounds in Pea (*Pisum sativum* L.) Inoculated by *Rhizobium leguminosarum* and Infected with *Orobanche crenata*

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Abstract

Parasitic plants are among the most important problematic weeds, they are responsible of major losses of many crops. Early growth stages, such as seed germination stimulated by host root exudates and tubercle development, are key phases for these parasites development. Inhibition of these early phases could be a general strategic option for parasitic plants management. In our previous study, we have demonstrated that some *Rhizobium leguminosarum* strains decrease pea infection by *Orobanche crenata* and germinated seeds enhanced browning symptoms. These observations suggested the probability of toxic compounds accumulation such as gallic acid and naringenin used as a defence strategy by inoculated pea plants. In this study, we demonstrate that these two phenolic compounds cause severe physiological disorder of germination broomrape seeds. They inhibited germination of *O. crenata* seeds induced by strigol analogue GR_{24}, and caused a browning reaction in germinated seeds.

Introduction

Broomrape (*Orobanche* spp.), belonging to the family *Orobanchacae*, is an obligate plant parasite, it causes severe damage in many leguminous crops in particular pea (Rubiales et al., 2003). The management of broomrape is often difficult due to its close association with the host for all of its life cycle. Besides, the production of large number of seeds which can lay dormant in the soil for many years and only germinate if stimulated by host root exudates. Once a seed is stimulated, it produces a germ tube that grows in the direction of the host plant. The germ tube develops an appressorium, an organ that functions in attachment, and solute transfer (Stewart and Press, 1990). At the beginning of parasitism process, intrusive cells of *Orobanche* penetrate the host root tissues, eventually connecting the parasite to the vascular system. This phase is a critical step in parasitism (Losner-Ghoshen et al., 1994). Several studies revealed that phenolics can be involved in host resistance as defence compounds against broomrape (Jorrin et al., 1996; Echevarria-Zomeño et al., 2006).

Reduction of pea infection by *Orobanche crenata* with some compatible *Rhizobium leguminosarum* strains has been reported by Mabrouk et al. (2007a,b). Makarova et al. (2003) reported that early stages of symbiotic interactions between legumes and Rhizobium are assumed to involve development of a protective mechanism in plants. And they suggest that this mechanism is similar to hypersensitive response caused by interaction between plant and pathogens, which involves phenolic compounds. It is likely that this mechanism controls symbiotic interactions and thus, nodule formation. The role of phenolic compounds in microsymbiont propagation remains unclear at all stages of symbionts interactions (Hartwig et al., 1991). Accumulation of flavonoids in *Viciae* roots exudates inoculated by *Rhizobium leguminosarum bv viciae* has been reported by Hirsch et al. (2001). Based on the above research, the purpose of the present study was to determine the variation of phenolic compounds in pea roots after inoculation with P.SOM *Rhizobium* strain individually, as well as associated with *O. crenata*.

Materials and Methods

Bacterial strain and growth conditions

Rhizobium leguminosarum strain P.SOM was obtained from INRAT (Mabrouk et al., 2007a,b). This strain was grown at 28°C on a yeast extract mannitol

medium containing 0.08% of yeast extract (w/v) and 1% of mannitol (w/v). The bacterial isolate derived from single colonies. For further root inoculations, the bacterial cultures were prepared in distilled water, with several washes to remove traces of growth medium.

Plant materials

The pea variety Douce de Provence was provided by INRAT. Pea seeds were germinated and grown as previously described for tomato seeds by El-Halmouch et al. (2006). *Orobanche crenata* seeds were collected from flowering spikes in infected pea fields from Ariana (Tunisia) in 2001 and 2003. Once cleaned, the seeds were stored in darkness at room temperature until use. Seed viability was estimated at 70% using the TTC test (Aalders & Pieters, 1985; Mabrouk et al., 2007b).

Hydroponic co-culture of pea and broomrape

Orobanche crenata seeds were surface-sterilized for 5 min in sodium hypochlorite (3.61%) and rinsed five times with sterile distilled water. They were preconditioned at 25°C for 7 days on glass fibre filter paper moistened with 5 ml sterile distilled water in a Petri dish (9-cm diameter). Pea seeds were surface-sterilized in sodium hypochlorite (3.61%) solution for 5 min and rinsed several times in sterile distilled water, then sown in vials containing glass beads (2-mm diameter) moistened with sterile distilled water. Germination occurred after 7–10 days in adequately watered vials. Seedlings were transferred to Petri dishes (9-cm diameter), 7 days later, as previously described by Labrousse et al. (2001). Roots were covered with a piece of glass fibre filter paper and a 1-cm thick layer of rock wool. The bottom of this system was soaked in a sterile solution of Coïc neutrophile nutrient solution 50% (Coïc and Lesaint, 1975) and the whole was covered with aluminium foil and maintained at 21°C with 100 μmol/m^2/s PAR under a 16 h photoperiod. Preconditioned seeds of *O. crenata* (100 seeds) were placed 1–2 mm from roots of 15-day-old pea plants. The Petri dish system was used for plant material collection from pea roots exposed to four treatments: (i) nutrient solution (control), (ii) *O. crenata*, (iii) *Rhizobium* strain P.SOM (inducer of defence) and (iv) *Rhizobium* P.SOM associated with *O. crenata*. Pea roots from all treatments were sampled 7, 14, 21, 28 and 35 days after transplanting. Roots were frozen in liquid nitrogen and stored at −80°C until phenolic compounds analysis.

Harvesting and effect of root exudates on *O. crenata* seed germination

Two seeds of pea were sown and grown in a pot filled with sand (1 kg), in the presence or not of the P.SOM inoculum (3 ml per pot). Root exudates were collected weekly for 6 weeks of culture at 21°C under 70% relative humidity and a 16 h photoperiod (100 μmol/m^2/s PAR), using the double pot technique as described by Parker et al. (1977). At each root exudate sampling, 40 ml were collected then sterilized by filtration (0.2-mm pore size) until use for bioassay tests.

The effect of root exudates on broomrape germination was assayed according to the method described by El-Halmouch et al. (2006). *Orobanche crenata* seeds, surface-sterilized and preconditioned, were transferred to a small Petri dish (3.5 cm in diameter) containing 0.3 ml of pure root exudate solution and incubated at 21°C for 7 days. Germination rate was determined under a binocular microscope, then estimated as percentage of viable. Seeds were considered to be germinated when the germ tube was at least 0.1-mm long. Control with water instead of GR_{24} was performed to estimate spontaneous germination of the parasite seeds.

Effect of phenolic compounds on *O. crenata* seeds germination

To test the effect of phenolic compounds on *O. crenata* seeds germination, 0.3 ml of either 10 p.p.m. of the germination stimulant GR_{24} (optimal concentration for germination), either alone or in combination with one of the phenolic compounds (gallic, ferulic, cinnamic and *p*-coumaric acids, narengenin or formononetin). These phenolic compounds were tested at different concentrations (0.1, 0.5 and 1 mM). Each phenolic compound was added to Petri dish containing preconditioned seeds and then incubated as indicated above. Germination was evaluated 7 days later using a binocular microscope. Germination was expressed as a percentage of the total seeds. Seeds were considered to be germinated when the germ tube was at least 0.1-mm long.

Phenolic compounds extraction and analysis by high performance liquid chromatography (HPLC)

Pea roots tissues (0.5 g) were powdered finely using a pestle and mortar following by suspension of the ground plant part in 5 ml of % methanol-water (80 : 20; v/v). The homogenate was centrifuged at 10 000 ***g*** for 15 min at 4°C and the supernatants were stored at −20°C for until analysis.

Analysis of the samples was performed with HPLC system. Reverse phase chromatographic analysis was carried out with acetonitrile water gradient with C-18 reverse phase HPLC colomn (150 × 4.6 mm, particle size 5 μm) at 25°C. Running conditions including injection volume (20 μl), mobile phase flow rate 1 ml/min and detection at 280 nm. Samples were filtered through membrane filter (pore size 0.45 μm; Merck) prior to injection in sample loop. tannic, gallic, ferulic, cinnamic, *p*-coumaric, and salicylic acids were used as internal and external standards. Two flavonoids naringenin and formononetin were also used as standards. Phenolic compounds and flavonoids present in the samples were identified by comparing retention time of standards and by co-injection. Concentrations were calculated by comparing peak areas of reference compounds with those in the samples run under the same elution conditions.

Statistic analysis

The experimental design was completely randomized. Data were tested for homogeneity and analysis of variance and differences of mean values (Duncan's, $P < 0.05$) were performed (SPSS for Windows 12.0, Paris, France). All the data are mean values of five separate replicates per treatment ± standard error.

Results

Kinetics of pea nodulation and infestation under hydroponic conditions

Regardless the presence of *Orobanche*, prenodules was formed at day 21 after inoculation (DAI) and nodules became evident at 28 DAI. *Orobanche* seed germinated rapidly 7 DAI, germination was reduced by a factor 3 in the presence of Rhizobia inoculated plants. A strong decrease in the number of *Orobanche* seedlings that succeeded in attaching pea roots and then in developing tubercles was observed when pea was inoculated with the P.SOM strain. Those data confirmed the observations previously reported by Mabrouk et al. (2007a,b) though co-cultures were performed differently. A major part of germinated *Orobanche* seeds (83%) and high percentage of the established tubercles (58%) on rhizobia inoculated pea became necrotic after 35 DAI (Table 1).

Impact of pea root exudates on *Orobanche* seeds germination.

Pure root exudates obtained from 2- to 6-week-old plantlets triggered germination of broomrape seeds, notably when pea was not inoculated. Germination rate reached the maximal value of 36% of viable seeds in presence of the root exudates collected from 4-week-old healthy pea (Fig. 1). During all the period of exudate harvesting, exudates of the inoculated pea stimulated broomrape germination to a less extent. Indeed, germination rate reached only 11% when elicited with exudates of the 4-week-old pea plants. This could partly explain the reduction of pea infection by *O. crenata* when inoculated by *Rhizobium* strain P.SOM (Mabrouk et al., 2007a,b), suggesting that root exudates of the inoculated pea contains germination inhibitors or low levels of stimulatory substances when compared to healthy pea .To check these hypotheses, the effect of root exudates of both healthy and inoculated plants (4-week old) was determined on parasite seed germination in the presence of the germination stimulant GR_{24} (Fig. 2). The pure exudates of healthy pea displayed no effect on germination levels when stimulated by GR_{24}. In contrast, root exudates collected from the inoculated pea decreased almost by 75%

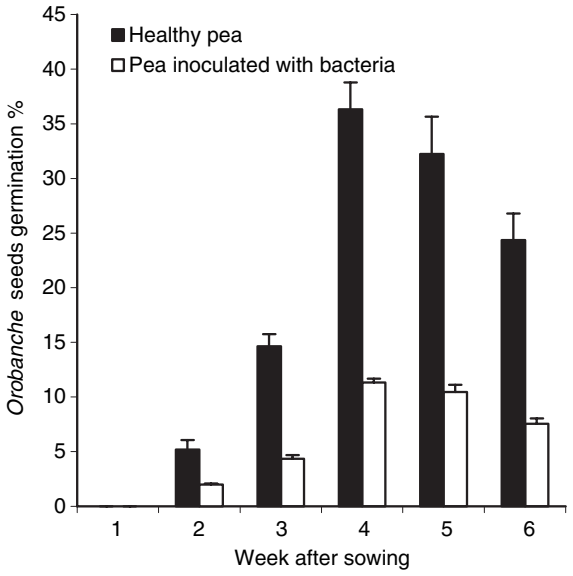

Fig. 1 Influence of pea root exudates on *Orobanche crenata* seed germination *in vitro*. Exudates were collected from 1- to 6-week-old plants. Germination rates are expressed as percentage of viable seeds and results are mean values of five independent replicates ± SD

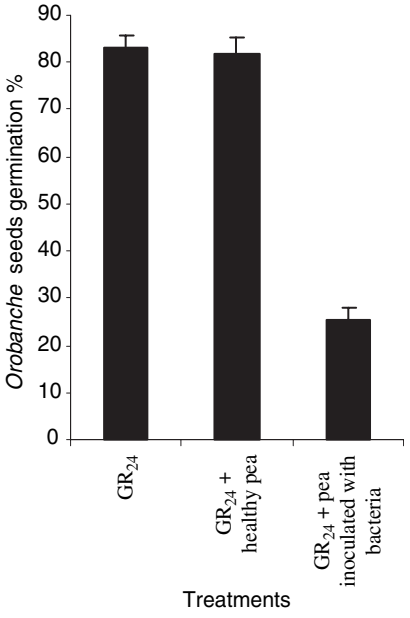

Fig. 2 Effect of pea root exudates on *Orobanche crenata* seeds germination *in vitro*. Exudates were harvested from 4-week-old plants. Germination rates were expressed as percentage of viable seeds (in presence of GR_{24} 10 p.p.m.) and results presented are mean values of five independent replicates ± SD

Table 1
Behaviour of *Orobanche crenata* seeds and tubercles on roots of pea plants growing in Petri dish assays and inoculated or not with *Rhizobium* strain P.SOM

	Necrotic germinated seeds (% of total germinated seeds)	Total tubercle number/plant	Necrotic tubercles (% of total tubercle number)
Infected pea	5.51 ± 2.37	14.5 ± 1.32	0
Infected pea + P.SOM	83.72 ± 5.71	3.00 ± 0.40	58.33 ± 9.42

parasite seed germination rates in the presence of GR_{24}.

Phenolic compounds induction in response to *Rhizobium* inoculation and *O. crenata* infection

High performance liquid chromatography profiles of pea root extracts displayed eight major peaks. Six of them, identified as ferulic, gallic, cinnamic, *p*-coumaric acids, formononetin and naringenin, showed significant changes in amount according the treatment (Fig. 3). Healthy pea roots accumulated mainly gallic acid (around 20 $\mu g/g$ FW), other phenolic acids and flavonoids were detected only at trace. Infection by *O. crenata* did not change these profiles in the non-inoculated pea. In contrast, from 7 DAI bacteria inoculation induced a strong accumulation of these phenolic compounds and flavonoids in pea roots. Levels of accumulation peaked for both gallic and ferrulic acids following 21 DAI (Fig 3a,e) while the other compounds displayed unchanged high levels during 35 DAI. Similar patterns of accumulation were observed in the inoculated pea when parasitized by *O. crenata*.

Impact of phenolic compounds and flavonoids on *O. crenata* seeds germination *in vitro*

In the controls containing only GR_{24} at 10 p.p.m., the rate of *O. crenata* seed germination reached the high value around 80%. From 0.1 mM, both naringenin and gallic acid inhibited strongly GR_{24}-induced germination (Fig. 4) and optimal inhibitory effect was observed at 0.5 mM. Naringenin was a more potent inhibitor than gallic acid at all the concentrations tested. When added to GR_{24}-germinated *O. crenata* seeds, naringenin and gallic acid stopped radicle elongation and caused browning of radicles (Fig. 5), in a similar way to symptoms induced by the exudates of pea roots inoculated with the P.SOM strain of *R. leguminosarum* (Figs 1 and 2). In contrast, the other phenolics did not affect significantly the parasite seed germination when tested at 0.1–0.5 mM. The highest level (1 mM) decreased only slightly seed germination (Fig. 4).

Discussion

The aim of this work was to understand the mechanisms involved in decreasing broomrape seed germination near to pea roots inoculated by some *R. leguminosarum* strains. Indeed the *Orobanche* seeds incubated with root exudates collected from healthy pea and pea inoculated with the P.SOM strain of *R. leguminosarum* differ strongly in their ability to germinate, even in the presence of the germination stimulant GR_{24}. Thus root exudates of the P-SOM inoculated pea trigger low level of seed germination. This may result from a low stimulatory substance concentration or the presence of germination inhibitors. Germination of parasite seeds is effectively known to be dependent on chemical exsuded from roots of host plants. Besides, Whitney and Carsten (1981) showed that host root exsudates affect germination of broomrape seeds, containing inhibitory complements that influence the size and direction of growth of the resulting radicle. Moreover, El-Halmouch et al. (2006) showed the presence of non-identified inhibitory compounds in the root exudates of a resistant genotype of tomato upon infection by the species *Orobanche aegyptiaca*. As yet, natural substances from root exudates inhibiting broomrape germination have not been isolated.

The exchange of signals between the host plant and Rhizobia is involved in the secretion of phenolic compounds, flavonoids and/or isoflavonoids (phytoalexins) by host plants (Peters and Verma, 1990), thus activating the *nod* gene expression of Rhizobia (Peters et al., 1986). As a consequence, phytoalexins or phenolic compounds associated with plant growth promoting rhizobacteria and biocontrol, offer a practical way of immunizing plants against pathogen ingress (Weller and Cook, 1986). A close relationship between high phenolic concentration in inoculated pea roots and low rate of broomrape germination is in conformity with some earlier reports which argue in favour of the important role of phenolic compounds in sunflower defence against the species *Orobanche cumana* (Labrousse et al., 2001; Serghini et al., 2001; Sauerborn et al., 2002; Echevarria-Zomeño et al., 2006), modifying root cell wall properties (lignification, suberization) and producing a physical barrier to root invasion by the intrusive cells of the parasite. In this context, further studies are useful to determine changes in pea roots structure following *Rhizobium* inoculation.

On the other hand, the present investigation brings some new information about chemical toxicity of phenolic acids against *Orobanche*, showing that changes in the composition of host defence molecules of the P.SOM inoculated pea, including some phenolic acids and flavonoids, leads to a strong reduction in the stimulatory activity of the root exudates. There are some other evidences, notably in pea, that *Rhizobacteria* trigger accumulation of soluble phenolics (mainly gallic, chlorogenic and cinnamic acids), leading to a better plant performance upon infection by pathogens (Singh et al., 2002, 2003; Mishra et al., 2006). As secondary metabolites, these compounds function as antioxidants or phytoalexins in the host plant response to parasites (Nicholson and Hammerschmidt, 1992; Grace and Logan, 2000). Furthermore, enhanced levels of cinnamic acid in pea inoculated by the P.SOM strain of *R. leguminosarum* are in conformity with enhanced phenylalanine ammonia lyase activity (Mabrouk et al., 2007b). Nevertheless, cinnamic acid does not inhibit broomrape germination, in contrast to gallic acid and narigenin which also accumulated in pea roots when inoculated. The data presented here indicate clearly that the induced synthesis, accumulation and excretion of toxic compounds constitute an important defensive mechanism for preventing parasitic plant infection in pea following inoculation with the P.SOM strain of *R. leguminosarum*. In addition to reduce strongly the parasite germination, gallic acid and narigenin have a noteworthy impact on the germinated seeds, in terms

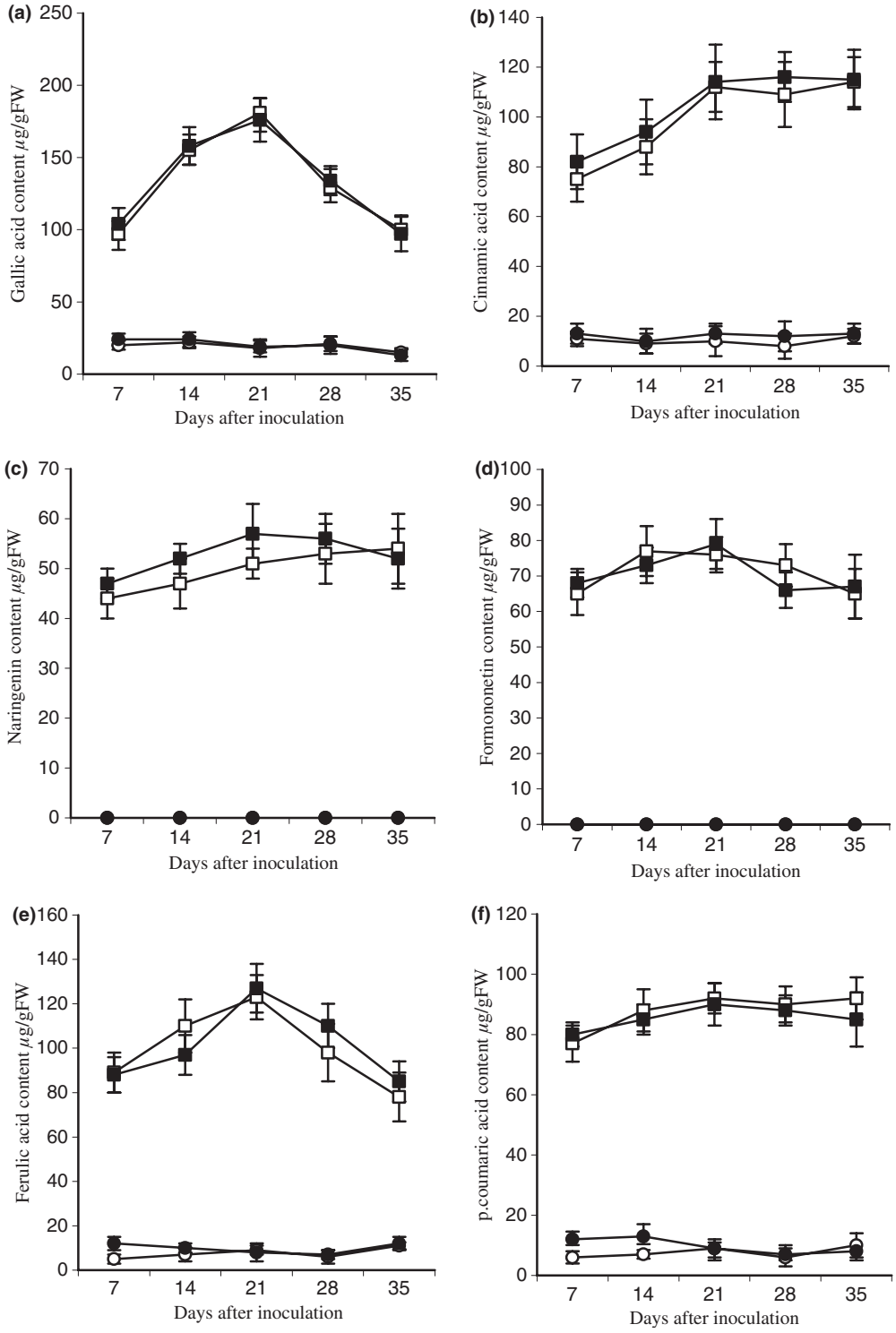

Fig. 3 Effect of *Rhizobium leguminosarum* pea inoculation on phenolic compounds and flavonoids accumulation in pea roots. Levels of gallic acid (a), cinnamic acid (b), naringenin (c), formononetin (d), ferulic acid (e) and *p*-coumaric acid (f). Phenolic contents were measured at 7, 14, 21, 28 and 35 DAI in pea inoculated with *R. Leguminosarum* and concomitantly infected by *Orobanche crenata* (■). Controls were performed with healthy pea (○), pea singly inoculated with *R. Leguminosarum* (□) singly or infected by *O. crenata* (●). Results presented are mean values of five independent replicates ± SD

of both reduced size and shape of the germ tube, then limiting capability of reaching the host root. The flavonoid derivative narigenin may be part of the pea defence strategy by acting as allelochemicals (preventing *O. crenata* seed germination or killing germinated seeds) or phytoalexin (preventing root penetration and connection to the vascular system). The hypothesis that phytoalexin may act as defensive compounds against parasitic weeds was effectively proposed in chickpea and sunflower (Wegmann et al., 1991;

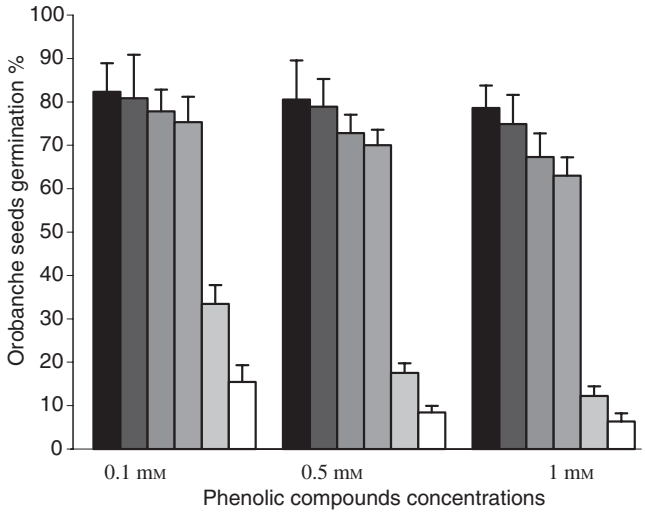

Fig. 4 Inhibition of the GR_{24}-induced *Orobanche crenata* seed germination by formononetin ■, cinnamic acid ■, coumaric acid ■, ferulic acid ■, gallic acid ■ and naringenin □. After conditioning, seeds were incubated for 7 days in darkness at 25°C in the presence of GR_{24} alone or with phenolic compounds at different concentrations. Germination with GR_{24} reached 83%. Results presented are mean values of five independent replicates ± SD

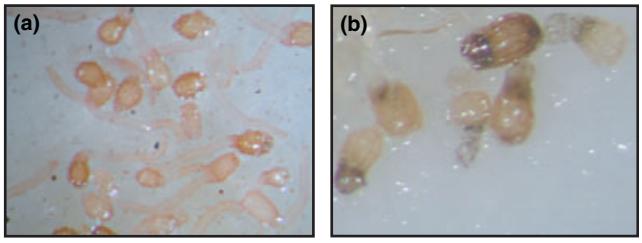

Fig. 5 Effect of gallic acid or naringenin (0.5 mM) on germ tube form and colourless of *Orobanche crenata* seed. (a) Germinated seeds of *O. crenata* only in the presence of GR_{24} (10 p.p.m.). (b) Abnormal germinated seeds of *O. crenata* in the presence of GR_{24} (10 p.p.m.) + naringenin or gallic acid

Serghini et al., 2001; Echevarria-Zomeño et al., 2006). Finally, pisatin is the major phytoalexin produced by pea plants (Sweigard et al., 1986). Complementary studies are in progress aiming to characterize its contribution to the induced resistance against *O. crenata* in pea following inoculation with some effective *R. leguminosarum strains*.

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References

Aalders AJG, Pieters R. (1985) *In vitro* testing with 2,3,5 triphenyl tetrazolium chloride (TTC) of Orobanche crenata seed metabolism. *FABIS Newsletter* **13**:35–37.

Coïc Y, Lesaint C. (1975) La nutrition minérale et en eau des plantes en horticulture avancée. *Doc Tech SCPA* **23**:1–22.

Echevarria-Zomeño S, Perez-de-Luque A, Jorrin J, Maldonado AM. (2006) Pre-haustorial resistance to broomrape (*Orobanche cumana*) in sunflower (*Helianthus annuus*): cytological studies. *J Exp Bot* **57**:4189–4200.

El-Halmouch Y, Benharrat H, Thalouarn P. (2006) Effect of root exudates from different tomato genotypes on broomrape (*O. aegyptiaca*) seed germination and tubercle development. *Crop Prot* **25**:501–507.

Grace SC, Logan BA. (2000) Energy dissipation and radical scavenging by the plant phenylpropanoid pathway. *Philos Trans R Soc Lond B Biol Sci* **355**:1499–1510.

Hartwig UA, Josef CM, Phillips DA. (1991) Flavonoids released naturally from alfalfa seeds enhance growth rate of *Rhizobium meliloti*. *Plant Physiol* **95**:797–803.

Hirsch AM, Lum Michelle R, Allan Downie J. (2001) What makes the rhizobia-legume symbiosis so special? *Plant Physiol* **127**:1484–1492.

Jorrin J, De Ruck E, Serghini K et al. Biochemical aspects of the parasitism of sunflower by *Orobanche*. In: Moreno MT, Cubero JI, Berner D, Joel D, Musselman LJ, Paker C (eds), *Proceedings of the 6th International Parasitic Weed Symposium*, Cordoba, Spain, Advances in Parasitic Plant Research, Junta de Andalucia Dirección General de Investigación Agraria, 1996, pp. 551–558.

Labrousse P, Arnaud MC, Serieys H, Bervillé A, Thalouarn P. (2001) Several mechanisms are involved in resistance of helianthus to Orobanche cumana Wallr. *Ann Bot* **88**:859–868.

Losner-Ghoshen D, Mayer AM, Ben Hold G, Joel DM. Host-parasite interaction: an immunocytochemical approach using antibodies for protein and pectic enzymes. In: Pieterse AH, Verkleij JAC, Ter-Borg SJ (eds), *Proceedings of the 3rd International Workshop on Orobanche and related Striga research*, Amsterdam, the Netherlands, Royal Tropical Institute, 1994, pp. 248–254.

Mabrouk Y, Zourgui L, Sifi B, Belhadj O. (2007a) The potential of *Rhizobium* strains for biological control of *Orobanche crenata*. *Biologia* **62**:1–5.

Mabrouk Y, Zourgui L, Sifi B, Delavault P, Simier P, Belhadj O. (2007b) Some compatible *Rhizobium leguminosarum* strains in peas decrease infections when parasitized by *Orobanche crenata*. *Weed Res* **47**:44–53.

Makarova LE, Latysheva SE, Ekimova EG. (2003) The involvement of endogenous phenolic compounds in the response of pea seedling roots to inoculation with *Rhizobium leguminosarum* at various temperatures. *Russ J Plant Physiol* **50**:261–264.

Mishra RP, Singh RK, Jaiswal HK, Kumar V, Maurya S. (2006) *Rhizobium*-mediated induction of phenolics and plant growth promotion in rice (*Oryza sativa* L.). *Curr Microbiol* **52**:383–389.

Nicholson RL, Hammerschmidt R. (1992) Phenolic compounds and their role in disease resistance. *Annu Rev Phytopathol* **30**:369–389.

Parker C, Hitchcock AM, Ramaiah KV. The germination of Striga species by crop root exudates: techniques for selecting resistant cultivars. In: *Proceedings Sixth Asian–Pacific Weed Science Society Conference July 11–17*, Jakarta, Indonesia, 1977, 67 pp.

Peters NK, Verma DPS. (1990) Phenolic compound as regulators of gene expression in plant-microbe interactions. *Mol Plant Microbe Interact* **3**:4–8.

Peters NK, Frost JW, Long SR. (1986) A plant flavone, luteolin induces expression of *Rhizobium meliloti* nodulation genes. *Science* **233**:977–980.

Rubiales D, Perez de Luque A, Cubero JI, Sillero JC. (2003) Crenata broomrape (*Orobanche crenata*) infection in field pea cultivars. *Crop Prot* **22**:865–872.

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

Serghini K, Perez de Luque A, Mercedes CM, Luis GT, Jesus VJ. (2001) Sunflower (*Helianthus annuus* L.) response to broomrape (*Orobanche cernua* Loefl.) parasitism: induced synthesis and excretion of 7-hydroxylated simple coumarins. *J Exp Bot* **52**:2227–2234.

Singh UP, Sarma BK, Singh DP, Bahadur A. (2002) Plant growth-promoting rhizobacteria-mediated induction of phenolics in pea (*Pisum sativum*) after infection with *Erysiphe pisi*. *Curr Microbiol* **44**:396–400.

Singh UP, Sarma BK, Singh DP. (2003) Effect of plant growth-promoting Rhizobacteria and culture filtrate of *Sclerotium rolfsii* on phenolic and salicylic acid contents in chickpea (*Cicer arietinum*). *Curr Microbiol* **46**:131–140.

Stewart GR, Press MC. (1990) The physiology and biochemistry of parasitic angiosperms. *Annu Rev Plant Physiol Plant Mol Biol* **41**:127–151.

Sweigard JA, Matthews DE, Vanetten HD. (1986) Synthesis of the phytoalexin pisatin by a methyltransferase from pea. *Plant Physiol* **80**:277–279.

Wegmann K, Von Elert E, Harloff HJ, Stadler M. Tolerance and resistance to Orobanche. In: Wegmann K, Musselman L. (eds), *Progress in Orobanche Research. Proceedings of the International Workshop on Orobanche Research*, Tuebingen, Germany, Eberhard-Karls-University, 1991, pp. 318–321.

Weller DM, Cook RJ. Suppression of root disease of wheat by fluorescent pseudomonads and mechanism of action. In: Sinsburne TR (ed.), *Iron, Siderophore, and Plant Diseases*, New York, NY, USA, Plenum, 1986, pp. 99–107.

Whitney PJ, Carsten C. (1981) Chemotropic response of broomrape radicals to host root exudates. *Ann Bot* **48**:919–921.