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Expression analysis of *Pisum sativum* putative defence genes during *Orobanche crenata* infection

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Abstract. The root holoparasitic angiosperm *Orobanche crenata* is a severe constraint to the cultivation of legumes. Breeding for resistance is a difficult task. Understanding the mechanisms underlying host resistance is a fundamental issue for the genetic improvement of legumes. In this work, the temporal expression patterns of 8 defence-genes known to be involved in different metabolic pathways activated during several plant–pathogen interactions were investigated in *Pisum sativum*. Molecular analyses were carried out using quantitative real-time polymerase chain reaction during the initial stages of the parasitisation process in susceptible (Messire) and incompletely resistant (Ps624) pea genotypes. Transcriptional changes in response to *O. crenata* revealed induction of genes putatively encoding pathogenesis-related proteins, peroxidase activity, and dehydration stress-responsive signalling. This, combined with high constitutive gene expression mediating the phenylpropanoid pathway were observed as part of the defence mechanisms triggered in Ps624 to restrict the growth of the parasite.

Additional keywords: parasitic plants, plant defence, real-time PCR.

Introduction

Crenate broomrape (*Orobanche crenata* Forsk.) is a holoparasitic weed that seriously attacks legume crops such as faba bean, lentils, chickpea, and vetch. This parasitic plant is potentially the major constraint for *Pisum sativum* cultivation in the Mediterranean area and Middle East. The only minor levels of incomplete resistance available in commercial cultivars and the lack of a suitable control method have restricted pea cultivation in infested areas (Pérez-de-Luque *et al*. 2005*a*).

Genetic resistance remains today as one of the most desirable components in an integrated control strategy. Resistance in the strict sense indicates processes which prevent establishment of the parasite. However, resistance to *O. crenata* in legumes is a complex multicomponent event with low heritability, making breeding for resistance a difficult task (Rubiales 2003). A detailed knowledge of the mechanisms underlying such resistance during the host–parasite interaction or the incomplete resistance that reduces the negative effects of the parasite on crop yield is necessary to improve breeding programs. However, despite the enormous economic effect of this disease, little is known about the molecular background of this legume–parasite interaction. Initial screening in pea germplasm led to the identification of valuable sources of resistance (Rubiales *et al*. 2005). Histological studies have revealed lignification of host endodermis and occlusion of host vessels as main mechanisms in preventing parasite intrusion at early infection stages during incompatible reactions (Pérez-de-Luque *et al*. 2005*b*). But so far, studies on changes in gene expression in parasitised plants and the molecular bases of resistance remain at very preliminary stages. Advances in the knowledge of gene expression in infected roots were initiated demonstrating the specific activation of the PR-1 (pathogenesis-related) and HMGR (3-hydroxy-3-methylglutaryl Coenzyme A reductase) gene promoters during the tobacco defence response to *O. aegyptiaca* (Joel and Portnoy 1998; Westwood *et al*. 1998). Recently *in situ* hybridisation techniques have shown the expression of a peroxidase and a β-glucanase involved in resistance (Pérez-de-Luque *et al*. 2006*a*). The use of model plants in transcriptional profiling studies is providing insight into the molecular regulation of plant–parasitic plant interactions (Vieira Dos Santos *et al*. 2003; Die *et al*. 2007). Comparative mapping studies have demonstrated a high degree of synteny between *Medicago truncatula* and pea (Choi *et al*. 2004). But until now, the transfer of knowledge obtained from model plants to crop legumes has been limited. Target gene approaches based on the knowledge gained from these systems allow the identification of orthologous genes involved in pea defence against *Orobanche* being helpful for crop improvement towards resistance.

Based on data obtained for the model legume plant *M. truncatula* (Die *et al*. 2007) and a previous related publication on pea (Pérez-de-Luque *et al*. 2006*a*), we focussed on the gene expression pattern in roots during the initial stages of the parasitisation process from early contact with *Orobanche* radicles to well-developed parasite tubercle formation, leading to

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a detailed temporal expression analysis of 8 putative defence genes in pea. Our data are discussed and compared with those previously obtained through hystological and transcriptional analysis of other plant-parasitic plant systems.

Materials and methods

Plant material and inoculation

The susceptible *P. sativum* cv. Messire, and incompletely resistant accession Ps624, were selected based on previous experiments (Rubiales *et al*. 2005). The following Petri dish assay was carried out according to Pérez-de-Luque *et al*. (2005*a*). Seeds of *P. sativum* were germinated in filter paper and kept in the dark at 20°C for 5 days. Seedlings with roots of 5–7 cm were placed in squared Petri dishes (12 cm by 12 cm) containing a sheet of glass-fibre filter paper (GFFP; Whatman International, Kent, UK), and perlite as substrate. When seedlings showed at least one true leaf, they were inoculated with *O. crenata* seeds at a density of ~50 seeds/cm^2, collected from infested faba bean fields in Córdoba. The synthetic germination stimulant GR24 was applied by adding 3 mL of a 10 ppm solution. *O. crenata* seeds had previously been surface-sterilised (González-Verdejo *et al*. 2005) and stored in the dark at 20°C for 8 days to promote conditioning. Dishes were sealed with Parafilm, covered with aluminium foil, and stored vertically in trays with Hoagland nutrient solution (Hoagland and Arnon 1950). The plants were maintained in a growth chamber at 20°C with 14 h photoperiod and irradiance of 200 µmol/m^2.s. Two serial experiments using 30 plants per experiment and genotype were performed. Fifteen plants were infected and the other 15 used as non-infected controls.

Sample collection and nucleic acids isolation

Observations on host–parasite development were taken every week with using a binocular microscope (Nikon SMZ1000; Nikon Europe BV, The Netherlands). Samples from control and infected *P. sativum* whole roots were harvested at: 15 days post-inoculation (dpi), *O. crenata* radicles in contact with the host roots before attachment; 21 dpi, initial stage of tubercle formation once the vascular systems of the two plants are connected; 35 dpi, before necrosis of most of the developed tubercles in the Ps624 genotype. In order to avoid contamination with parasite tissues, host roots were thoroughly washed with distilled water and blot dried with filter paper. The majority of parasite tubercles from root samples collected at 21 and 35 dpi were carefully removed with a scalpel. Collected samples were frozen in liquid nitrogen. Total RNA samples were isolated from roots (0.1 g) using TRIZOL reagent (Invitrogen, Carlsbad, USA) according to manufacturer's protocols, from different pools of 5 plants in order to minimise variation in gene expression among individual plants in both infected and uninfected control samples. The integrity of total RNA was checked on 2% (w/v) agarose gels and its quantity as well as purity was determined by an optical density at 260 nm and A_{260}/A_{280} absorption ratio using the BioPhotomer (Eppendorf, Germany). Genomic DNA from plants was isolated according to Torres *et al*. (1993) and used for PCR amplification with degenerated primers.

Sequence information and primer design

First, to identify *P. sativum* orthologueous with *M. truncatula* defence-related genes, we checked the pea ESTs database from GenBank. Second, specific peroxidase and glucanase primers were derived from *P. sativum* peroxidase (GenBank accesion no. AF396465) and *P. sativum* glucanase (Chang *et al*. 1992). Third, since no pea cellulose synthase sequence was available in databases, a degenerated primer-based strategy was used. The design of the degenerated primers was based on 5 putative cellulose synthase cDNAs from *M. truncatula*, *Arabidopsis thaliana*, *Eucalypus grandis*, *Vitis vinifera*, and *Gossypium hirsutum*. Polymerase chain reaction was performed with primers CellS1 5′-GNTGAYCCNYTNAARGARCC-3′ and CellS2 5′-TTRCARAANGGANCCCAYTT-3′ in a reaction volume of 25 µL using a template of 1 µL of genomic DNA. Cycling conditions were: 94°C for 35 s, 59°C for 35 s, and 72°C for 1 min for 40 cycles. The amplified 179-bp fragment was cloned into the pGEM-T vector system (Promega, USA), sequenced, and submitted to the GenBank database under accession number EU681279.

Finally, the gene-specific primer sets used for real-time reverse transcription (RT)-PCR were designed with a calculated Tm of 60 ± 0.5°C, GC% between 20% and 80%, and amplification products not larger than 100 bp (Table 1). An orthologous of the *M. truncatula* elongation factor-1α (*ef-1α*, TC106845, The Institute for Genomic Research; TIGR) was used as a constitutively expressed gene for transcript normalisation with primers *efa1* 5′-AAGCTAGGAGGTATTGACAAG-3′ and *efa2* 5′-ACTGTGCAGTAGTACTTGGTG-3′.

Two-step real-time RT-PCR

Total RNA (1 µg) was reverse-transcribed using the QuantiTec Reverse Transcription Kit (Quiagen, Germany), according to the manufacturer's instructions. Genomic DNA was eliminated during this procedure by RNase-free DNase I treatment. In order to ensure equal starting cDNA amounts, real-time PCR amplification of *ef-1α* was run for all the different templates and, depending on the C_T (threshold cycle), 3-fold to 10-fold serial dilutions of cDNA were prepared to obtain similar C_T values for products of equal starting amounts of cDNA, before initiating real-time PCR experiments. Polymerase chain reactions were performed in a 96-well plate with a M×3000P Real-Time PCR System (Stratagene, USA), using SYBR Green to monitor dsDNA synthesis. Reactions contained 0.5 µL 50× SYBR Green Solution, 12.5 µL 2× SensiMix (dT) (Quantace, London), 2.5 µL of cDNA, and 200 nM of each gene-specific primer in a final volume of 25 µL. The following standard thermal profile was used for all PCR reactions: polymerase activation (95°C for 10 min), amplification and quantification cycles repeated 40 times (95°C for 1 min, 60°C for 1 min). Each measurement was performed in triplicate and the C_T was determined.

Verification of amplified products

Specificity of the primer amplicons was checked by melting-curve analysis performed by the PCR machine after 40 amplification cycles (60−95°C with one fluorescence reading every 0.6°C). All investigated RT-PCR products that showed

Table 1. Primer sequences used in RT-PCR for amplifying defence-related genes in *Pisum sativum*
(1) Die *et al*. (2007); (2) Pérez-de-Luque *et al*. (2006*a*)

Target gene	Left Primer 5′–3′	Right Primer 5′–3′	PCR product size (bp)	PCR product melting temp. (°C)	Reference, accession
Cellulose synthase	CCGCGATGTTGACATTTGAA	GGGAGCCCATTTTCTTGCA	70	78.68 ± 0.29	EU681279
Hypersensitive reaction 203J	CACTACCACCAACGACAACTTCA	GGCGTTTTCTCCGGTAGGTAT	70	79.10 ± 0.57	(1), ABO26296[A]
Peroxidase	TGTTTGAATCAGATGCTGCATTG	CATTTGATTGAAGATGTTGTGCAA	75	75.26 ± 0.03	(2), AF396465[A]
Glutathione S-transferase	GTTCGTCCTCCTCCGCTAACT	GTTCGTCCTCCTCCGCTAACT	70	79.87 ± 0.43	(1), AB087837[A]
Chalcone synthase	TGATGTACCAACAAGGGTGCTT	CCAAATCCTTAGCCAAACGAA	70	79.93 ± 0.25	(1)
Dehydrin-like protein	CCAAACACGTAAGACCGATGAA	GCCACTAATAGGGTTGCCATATTG	73	76.24 ± 0.38	(1)
β-glucanase	TGACATGACCCTTATTGGAAACTC	GGTATGACCTAACATCACTTCTGAAAGA	71	76.27 ± 0.74	(2)
Ripening-related protein	TGATACGGTTAAACACTGGACATATG	TCAACTTCTTCAATACTCTCGTGACA	75	75.37 ± 0.38	(1)

[A]Unique sequence for primer design.

only single peaks and no primer-dimer peaks or artefacts were considered for further analysis.

In order to confirm the plant origin of the transcripts, amplification products were checked on 2% (w/v) agarose gel using cDNA from Messire infected roots (21 dpi) and cDNA from *Orobanche* nodules (21 dpi) developed in Messire plants. A primer pair was used as the *Orobanche* expressed control gene *ocr1* 5′-GTCTGCAGTAGTATGTTGCAT-3′ and *ocr2* 5′-GACAAATTCCTCAAAATCTTC-3′.

Data analysis

Data were analysed using the M×3000P analysis software version 3.00 (Stratagene, USA). All amplification plots were analysed with an R_n threshold of 0.035 to obtain C_T values for each gene-cDNA combination. The PCR efficiency (E) of each primer pair in each individual reaction was estimated from the data obtained from the exponential phase of each individual amplification plot and the Eqn $(1+E)=10^{slope}$ (Ramakers *et al*. 2003). Primer efficiency values with an R^2 value less than 0.997 were ignored. The expression levels of the gene of interest (GOI) relative to the *ef-1α* were calculated for each cDNA sample using the equation: relative ratio $_{GOI/ef\text{-}1\alpha} = (E_{GOI}{}^{-CTGOI})/(E_{ef\text{-}1\alpha}{}^{-CTef\text{-}1\alpha})$. The values of 6 infected and 6 control samples (from the 2 independent experiments) were used in a Student's *t* test to calculate probabilities of distinct induction or repression, and the average ratio of these values was used to determine the fold change in transcript level in infected samples compared with uninfected control plants as described by McGrath *et al*. (2005).

Results

The susceptible *P. sativum* cv. Messire and the incompletely resistant accession Ps624 were selected and used to monitor the transcript accumulation of genes encoding several defence-related proteins assayed by real-time reverse transcription (RT)-PCR. Real-time PCR reactions resulted in a single product with the specific temperature shown in Table 1. Amplification products were obtained using cDNA from pea root tissues but no products were detected using cDNA from *O. crenata* nodules, proving that the gene expression observed was transcribed in roots of *P. sativum* (Fig. 1). The different mechanisms of resistance to *O. crenata* in Ps624 were reflected by a low number of established tubercles per plant, which demonstrated a delay in tubercle development in accordance with previously characterised differences in resistance to broomrape (Ángeles Castillejo *et al*. 2004; Pérez-de-Luque *et al*. 2005*a*). Moreover, most of the tubercles formed became necrotic (Table 2) and died 35 days after inoculation (Fig. 2).

Gene expression patterns in susceptible pea genotype

The penetration of *O. crenata* radicles, parasite attachment, and further development of tubercles into host roots led to a transient induction of selected genes at the three time-points addressed (Table 3). The induction of a gene encoding a dehydrin-like protein (*dhl*) exhibited a 1302.92-fold difference in expression level in infected Messire roots at 35 dpi compared with the corresponding controls, which was the highest difference expression level measured in this study. A remarkable higher level of glutathione S-tranferase gene (*gst*) expression was detected during the initial contacts with *Orobanche* radicles (15 dpi) and the developed tubercles stage (35 dpi) in infected Messire plants when compared with infected Ps624 plants (Fig. 3).

Gene expression patterns in the incompletely resistant pea genotype

Significant induction in Ps624 ranged from at least 2-fold and up to 22-fold difference in expression level between infected and control plants. Genes identified with more than a 2-fold change in relative expression in the resistant compared with susceptible genotype were tentatively classified as associated with the molecular resistance response. A distribution of the ratios [(Ps624 infected/*ef-1α*)/(Messire infected/*ef-1α*)] using the 2-fold cutoff is shown in Fig. 3. Expression levels were higher in Ps624 for all transcripts analysed at least in one of the time points studied, except for the glutathione S-tranferase gene (*gst*), which showed a remarkable higher level in Messire plants at 15 and 35 dpi and the hypersensitive reaction 203J gene (*hsr203J*) which showed a 4.73-fold difference at 35 dpi in infected Messire compared with infected Ps624 plants.

Interestingly the highest comparative expression level detected in the Messire genotype for the *dhl* gene (1302.92-fold difference between infected and uninfected plants) did not reach the relative level observed in Ps624, which showed a 22.39-fold up-regulation change in infected plants. Thus, in spite of this high up-regulation in both genotypes, the *dhl* gene was finally induced to similar levels in both infected genotypes (Fig. 3).

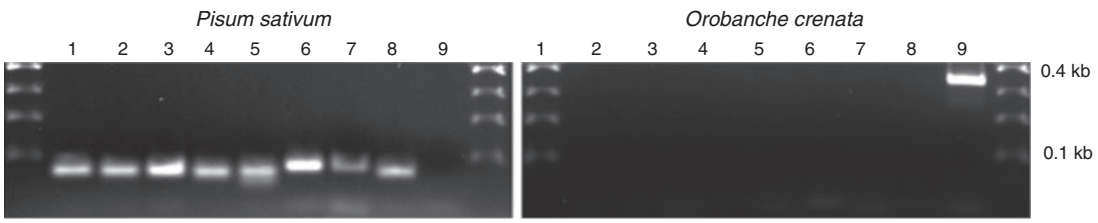

Fig. 1. Transcript accumulation of (*1*) cellulose synthase, (*2*) *hsr203J*, (*3*) peroxidase, (*4*) glutathione S-transferase, (*5*) chalcone synthase, (*6*) dehydrin-like protein, (*7*) β-glucanase, and (*8*) ripening-related protein genes in cDNA from *P. sativum* infected roots and *O. crenata* nodules. A control *O. crenata* expressed gene (*9*) was used. No amplification products appeared using cDNA from *O. crenata* demonstrating that transcripts detected are of *P. sativum* origin.

Table 2. Tubercle number and % necrotic tubercles of *O. crenata* on pea roots at 35 days post inoculation in Petri dish assays

Data shown as mean ± s.e. Values are mean of 10 replicates in two independent experiments

Accesión	Tubercle no. per plant[A]	Necrotic tubercles (% of total no.)
Messire	59 ± 5.66	9.42 ± 2.10
Ps624	6 ± 1.73	53.82 ± 5.20

[A]Included the S2 (crown-roots start to develop), S3 (bud <1 cm), and S4 (first development of floral spike) developmental stages according to ter Borg *et al*. (1994).

Discussion

In the present work, a molecular approach to compare the expression patterns of some defence-related genes known to be expressed in response to parasitic plant infection was addressed by the RT-PCR strategy. Two pea genotypes differing in their sensitivity to *O. crenata* were selected and used to monitor the gene expression patterns from the earliest contact with *Orobanche* radicles to the well developed parasite tubercle.

The role of the hypersensitive-like reaction (HR) in resistance of legumes to *O. crenata* has been debated in the past on the basis of the appearance of necrotic lesions during the interaction with vetch (Goldwasser *et al*. 1997) or chickpea (Rubiales *et al*. 2003). Pérez-de-Luque *et al*. (2005*b*), have shown that unsuccessful penetration of *O. crenata* seedlings during the initial steps in the interaction and the necrosis of the established tubercles cannot be attributed to cell death in the host. In this study, we evaluated transcript accumulation of the *hsr203J* gene, usually employed as a molecular marker of the hypersensitive response (Gopalan *et al*. 1996; Pontier *et al*. 2001). Induction of *hsr203J* was detected in both infected genotypes at 15dpi during the first contacts between host and parasite. However, the up-regulation was maintained throughout all the experiment only in infected Messire plants when no significant death or darkened tubercles were observed. This casts doubt on the active role of *hsr203J* as a resistance mechanism. In this sense, some authors have suggested that *hsr203J* would be a negative regulator of the HR (Tronchet *et al*. 2001; Nasir *et al*. 2005). This protein might function as a scavenger for ROS-derived compounds (Tronchet *et al*. 2001) produced by an oxidative burst following parasite penetration into host roots. Oxidative stress, in the absence of HR, has already been shown during the *A. thaliana–O. ramosa* interaction (Vieira Dos Santos *et al*. 2003). It may be hypothesised that oxidative stress is induced by the penetration of *Orobanche*, generated upon the cell-wall degradation of the host cells during the compatible reaction. According to this model, Messire probably responds to the infection by both a detoxification mechanism involving *gst* and induction of *hsr203J* implicated in cell protection.

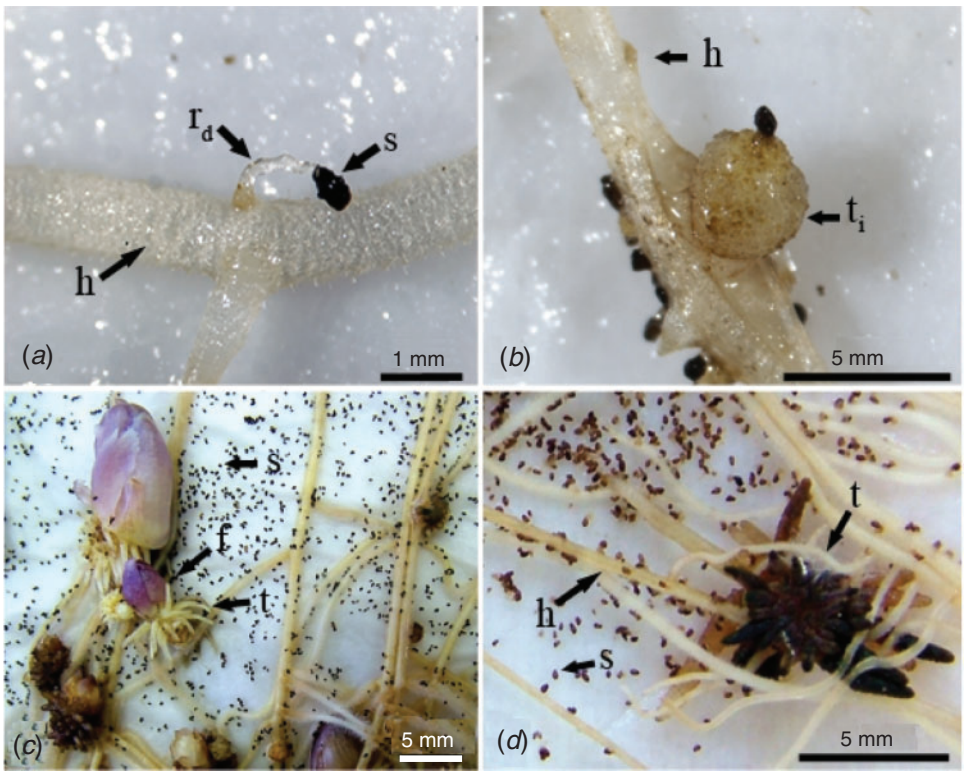

Fig. 2. Parasitisation process in the dish system. (*a*) Radicles (r_d) of germinated *O. crenata* seeds (s) contacting with susceptible Messire roots (h), 15 dpi. (*b*) Initial stages of tubercle formation (t_i) in Messire roots, 21 dpi. (*c*) Developed *O. crenata* tubercles (t) in Messire roots showing (f) initial floral spike formation, 35 dpi. (*d*) Necrotic *Orobanche* tubercle in incompletely resistant Ps624 roots (h), 35 dpi.

Table 3. Gene expression patterns in *P. sativum* roots on the basis of real-time RT-PCR experiments
Values shown indicate average relative expression ratio to control (average data from two independent experiments with three technical replicates). Bold text indicates statistically significant induction ($P \leq 0.05$)

Target gene	Ps624			Messire		
	15 dpi	21 dpi	35 dpi	15 dpi	21 dpi	35 dpi
			Defence and cell rescue			
Cellulose synthase	0.13	1.30	**5.45**	1.25	0.35	**10.11**
hsr203J homologue	**5.00**	1.42	1.70	**13.90**	**4.69**	**22.32**
Peroxidase	**5.27**	**6.47**	n.d.	1.20	**1.26**	n.d.
			Glutathione metabolism			
Glutathione S-transferase	1.60	**2.10**	1.22	**20.49**	1.55	**50.44**
			Phenylpropanoid synthesis			
Chalcones synthase	3.32	0.78	0.84	**15.37**	**0.49**	0.82
			Response to stress			
Dehydrin-like protein	0.82	**11.34**	**22.39**	**51.87**	**0.67**	**1302.92**
Glucanase	1.39	**2.92**	2.51	**2.08**	**4.38**	1.77
Ripening-related protein	**2.16**	4.07	0.85	0.58	5.57	n.d.

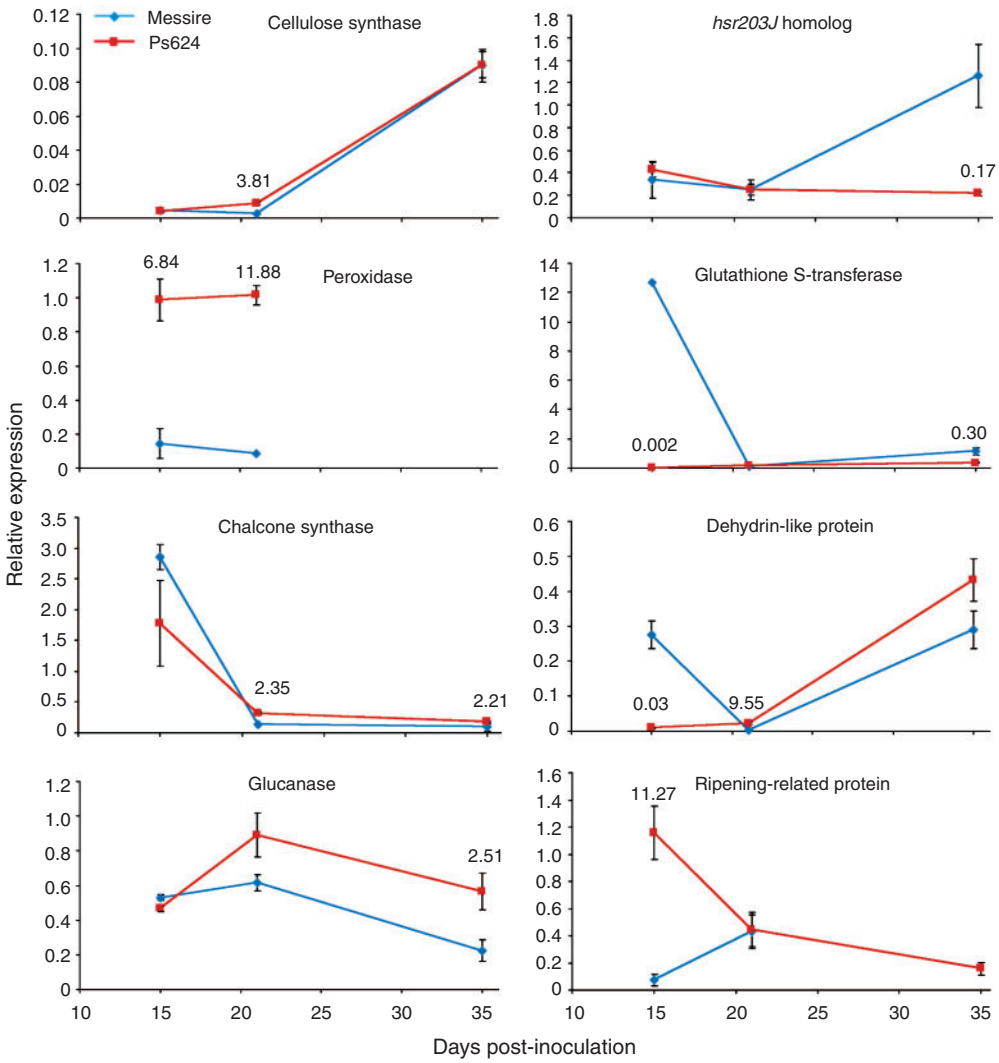

Fig. 3. Transcriptional changes in parasitised *P. sativum* roots. A distribution of the normalised expression [(Ps624 inoculated/*ef-1α*) *v.* (Messire inoculated/*ef-1α*)] is shown. Ratios between the two infected genotypes (statistically significant, $P \leq 0.05$) are presented.

However, comparative analysis of regulated genes revealed quantitative and qualitative differences in the gene expression profiles between the two infected genotypes (Fig. 3). Genes identified with more than a 2-fold change in expression in the resistant compared with susceptible genotype were tentatively classified as associated with the molecular resistance response. An early induction at 15 dpi was detected for a ripening-related protein with a domain for Bet v I allergen belonging to a protein family group including the pathogenesis-related protein of the PR-10 group (Moiseyev *et al*. 1997). Inducible expression, RNase activity, and ligand-binding activities have linked Bet v I allergen to plant defence as well as to abiotic stress (Samac and Graham 2007). A recent work, using a proteomic approach to investigate the *M. truncatula–O. crenata* interaction, led to the identification of Bet v I allergen associated with resistance (M. A. Castillejo, unpubl. data). Another observation at 15 dpi was the accumulation of peroxidase transcripts, which persisted at 21 dpi. There is strong evidence supporting the implication of peroxidases in plant resistance to parasitic plants (Goldwasser *et al*. 1999; Vieira Dos Santos *et al*. 2003; Ángeles Castillejo *et al*. 2004). The formation of protein cross-links of the cortical cell walls has been suggested to be involved in resistance (Echevarría-Zomeño *et al*. 2006; Pérez-de-Luque *et al*. 2006*a*). Thus, the peroxidase activity induction observed is likely to be implicated in cell-wall reinforcement through oxidative cross-linking of structural proteins conferring mechanical barriers to the invading parasite.

Combined with the physical barriers, the induction of several genes mediating other mechanisms of resistance takes place after the vascular connections have been established. Since *Orobanche* must overcome such activated barriers, this could explain first, the delayed development of the few established individuals and finally, the death of the tubercles. In this sense, there was a notable up-regulation of the *dhl* gene in Ps624 (11.34-fold induced, the most strongly *Orobanche*-induced gene in this genotype at 21 dpi). Dehydrins are members of a protein family expressed during dehydration stress and have been identified in a range of species including pea (Roberton and Chandler 1992). Although their specific role remains a challenging area for further study, this protein might comprise part of the alterations in host metabolism necessary to overcome the water deficiency caused by the parasite.

However, the accumulation of *dhl* transcripts cannot explain the necrosis of *O. crenata* tubercles. Two main factors have been suggested to be involved in resistance: vessel occlusion (Pérez-de-Luque *et al*. 2005*b*, 2006*b*) and/or accumulation of toxic compounds such as phenolics (Sherghini *et al*. 2001; Echevarría-Zomeño *et al*. 2006). Peroxidases are known to be involved in the cell-wall modification discussed above, but also appear to be implicated in this later form of resistance. The peroxidases polymerise polysaccharides and polyphenols to produce stable vascular occluding gels (Crews *et al*. 2003). Recent reports have related vessel occlusion in pea resistance to high peroxidase activity (Pérez-de-Luque *et al*. 2005*a*; Mabrouk *et al*. 2007). The increase in abundance of transcript for peroxidase at 21 dpi (11.88-fold difference, the strongest difference between infected genotypes at this time-point) seems to highlight the important role that this enzyme plays in defence against *Orobanche*. Curiously, three genes not up-regulated in the resistant genotype showed higher relative expression values in Ps624 when the two genotypes were compared. Chalcone synthase is located in the phenylpropanoid pathway leading to synthesis of phenolic compounds or phytoalexin production. The derived products may confer mechanical and chemical barriers to *Orobanche*, suggesting the important role of the phenylpropanoid pathway in the elicited defence (Griffitts *et al*. 2004; Echevarría-Zomeño *et al*. 2006; Pérez-de-Luque *et al*. 2006*a*; Lozano-Baena *et al*. 2007). Cellulose synthases are responsible for the biosynthesis of one of the principal polysaccharides of the cell wall and the role in defence of β-glucanases has been pointed out in releasing oligosaccharide elicitors (Esquerré-Tugayé *et al*. 2000). Increased levels of cellulose synthase and β-glucanase have been detected in *Medicago* and pea resistant to *O. crenata*, respectively (Ángeles Castillejo *et al*. 2004; M. A. Dita, pers. comm.). Although no up-regulation was observed in infected plants, high expression levels in Ps624 might suggest that a higher constitutive level for some transcript expression in the incompletely resistant genotype could help the plant in more rapidly priming defence reactions against pathogens.

All these mechanisms are based on the assumption that the host recognises the pathogen and reacts against it. The induction of *dhl* or *gst* genes as early as 15 dpi in the susceptible genotype demonstrates that the parasite is apparently detected and defensive mechanisms are activated. But this raises the question of why such a response is too slow or ineffective to prevent *Orobanche* development. This has been related to a delayed response due to reduced input into the plant signal recognition system (Tao *et al*. 2003) or an active process of defence-gene supression (Caldo *et al*. 2004). So far, there is no convincing evidence that parasitic plants suppress the response of the host. The perceived signal input is greater in incompatible reactions (unsuccessful attachment, penetration, and darkening of established tubercles) and therefore the output signal is greater. The observation that gene activation in the host does not mount an effective defence against *Orobanche* might indicate that the invasion is recognised only partially.

Conclusion and remarks

This work describes a first transcriptional approach with the aim of studying gene expression patterns in *P. sativum* after infection with the parasitic plant *O. crenata*. The complexity of resistance to parasitic plants in legumes is a consequence of the coordinated induction of several mechanisms. Following invasion of *P. sativum* tissues by *O. crenata*, a range of defence mechanisms are triggered to restrict its growth. Induction of defence genes in host plants underlies the perception of the parasite by the host, even in the case of a compatible reaction. But gene activation in this case is not sufficient to result in host resistance. If the transcript inductions observed are expressed as functional proteins, the defence response comprises reinforcement of cell walls, activation of pathogenesis-related proteins, and the phenylpropanoid pathway. Up-regulation of genes involved in these mechanisms combined with high constitutive expression determines a more effective defence against the parasite. Further experiments are needed to understand the biological function of genes involved in the basic mechanisms governing resistance to

parasitic plants. Understanding the function of genes plays an essential role in the characterisation of disease processes and this will be of great importance in directing pea breeding programs and developing resistant crops.

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