Defense Gene Expression Analysis of *Arabidopsis thaliana* Parasitized by *Orobancha ramosa*

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ABSTRACT

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The infection of *Arabidopsis thaliana* roots with the obligate parasite *Orobancha ramosa* represents a useful model for a study of the molecular events involved in the host plant response to a parasitic plant attack. To avoid analysis problems due to the subterranean development of *O. ramosa*, we developed two in vitro co-culture systems: *O. ramosa* seedlings infesting *Arabidopsis* plantlet roots and callus tissues. We were then able to investigate the expression patterns of some host plant genes selected among genes known to be involved in metabolic pathways and resistance mechanisms activated during several plant-pathogen interactions including ethylene, isoprenoid, phenylpropanoid, and jasmonate

biosynthesis pathways, oxidative stress responses, and pathogenesis-related proteins. Molecular analyses were carried out using polymerase chain reaction amplification methods allowing semiquantitative evaluation of transcript accumulation during early (first hours) and late (15 days) stages of infestation, in whole roots or parts close to the parasite attachment site. In *A. thaliana*, *O. ramosa* induced most of the general response signaling pathways in a transient manner even before its attachment to *A. thaliana* roots. However, no salicylic acid-dependent defense is observed because no activation of systemic acquired resistance markers is detectable, whereas genes, co-regulated by jasmonate and ethylene, do display enhanced expression.

Additional keywords: broomrape, in vitro culture.

Orobancha sp. (broomrape) is an obligate root-parasite completely devoid of chlorophyll and consequently dependent on its host for a heterotrophic supply of organic resources and energy. It is one of the most important agricultural pests in several major cropping systems (faba bean, sunflower, tobacco, and tomato) in Europe, Africa, and Asia (34). Infestation inevitably results in reduced growth and yield losses, even before parasite emergence (19). Chemical control methods of *Orobancha* spp. are difficult to apply because both host and parasite are flowering plants (11,13). Indeed, whatever the crop, none of the developed control methods is wholly successful. For this reason, new control methods, which are more selective and active at the early stages of subterranean development of *Orobancha* spp., have to be developed. This has been approached through the engineering of new resistant cultivars, but this resistance was rapidly overcome (32). Consequently, a better understanding of mechanisms involved in the host-parasite interaction is needed.

Although many physiological and biochemical studies have been carried out on both partners, most molecular studies have been focused on the parasite (6,12,30,37). Nevertheless, dissection of the molecular response in the host has been initiated. In 1996, Westwood et al. (50), using a β -glucuronidase (GUS) strategy, demonstrated the activation of tomato *hmg2* promoter in transformed tobacco as early as 1 day after root penetration by *O. aegyptiaca*. This enhanced expression is spread in the cortical and vascular tissues of the host, around the penetration site, and persists on infested roots for 4 weeks (52). This could be considered as a defense reaction because tomato *hmg2* encodes a 3-hydroxy-3-methylglutaryl CoA reductase (HMGR), a protein involved in isoprenoid biosynthesis pathway, and is activated

specifically during defense responses associated with phytoalexins and sesquiterpenes production. Similarly, the basic *PR-1* gene promoter from tobacco is induced after *O. aegyptiaca* attachment on transformed tobacco roots (21). Using a differential display strategy, Gowda et al. (18) identified several genes whose expression is induced in marigold roots during attempted parasitism by *Striga* spp. One of these, *NRSA-1*, encodes a protein exhibiting significant homology to disease resistance proteins from other plant species. Recently, in the case of the incompatible reaction of *Lycopersicon esculentum* in response to *Cuscuta reflexa* attack, a subtractive suppression hybridization experiment revealed the over-expression of some host genes. Among these, *LeAqp2* exhibits homology to aquaporin-like genes (48). Thus, to date, few molecular approaches have been undertaken, which is very little in view of the complexity of patterns of defense against parasitic plants. In addition, none used fully genetically characterized host plants.

It has been demonstrated that several mechanisms of resistance to *O. cumana* may or may not occur simultaneously in the same genotype of sunflower (23). This clearly illustrates the need for an accurate kinetic study of the gene expression pattern in host roots, from the earliest contact with *Orobancha* seed to the installation of a functional haustoria. In this preliminary study, the chosen degree of resistance of the host plant was not as important a feature as good genetic knowledge. Using in vitro culture methods (15,49), *A. thaliana* recently has been shown to be parasitized by *O. aegyptiaca* and *O. ramosa*. This powerful genetic tool should open up new horizons in host-parasitic plant interaction research based on molecular approaches.

The aim of this present work was to develop a sterile, in vitro *A. thaliana*-*O. ramosa* co-culture system in order to dissect the molecular events involved in the response of the host plant to parasite contact and attack. This system avoids problems inherent to the subterranean development of *O. ramosa*, such as uprooting from pots and possible wound induction, as well as difficulties in the

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appreciation of infestation timing. The choice of the parasitic species turns on *O. ramosa*, a parasite we have used for carbon metabolism studies, and whose impact on some crop cultures (rape seed, hemp, and tomato) is more than worrying in France (7).

MATERIALS AND METHODS

Plant material. *O. ramosa* L. seed were collected from an infested *Brassica napus* (rapeseed) field in Poitou-Charente, France. *A. thaliana* ecotype Wassilewskaja (Ws) seeds were obtained from H. Höfte (INRA, Versailles) and used throughout the experiment. *A. thaliana* dark-grown calli were provided by H. Höfte (INRA, Versailles) and maintained in solid culture Gamborg medium (Sigma, Saint Quentin Fallavier, France) at a constant temperature of 25°C and subcultured every 15 days. Average approximate callus weight was 100 mg.

***A. thaliana*–*O. ramosa* in vitro co-culture.** Actively growing *A. thaliana* roots were infested by *O. ramosa* seedlings in a sterile hydroponic system (Fig. 1). This consisted of a Magenta box filled with Shimura medium (43) and an inclined plastic plate covered by a 60-µm nylon miracloth (a 100-µm filter did not allow the attachment of the parasite). The liquid medium progressed by capillarity along the nylon filter, on the top of which 10 surface-sterilized seeds of *A. thaliana* were placed. Boxes were placed for 15 days in a growth chamber at 21°C under 100 µmol quanta PAR m⁻² s⁻¹ condition with a 12-h photoperiod. After 7 days, the Shimura medium was replaced by Gibeaut medium (14), allowing a better root development. Before infestation, *O. ramosa* seeds were surface sterilized in sodium hypochlorite (12%) and preconditioned during a week in darkness at 21°C in petri dishes containing glass fiber filter papers moistened with sterile distilled water. Three days before infestation, germination of seed was induced in petri dishes containing glass fiber filter papers moistened with GR24, an analog of strigol, a natural germination stimulant, at 1 ppm (22). Applying approximately 150 *O. ramosa* germinated seeds close to the root systems, covered by a black tissue, resulted in ≈30 attached parasites. Magenta boxes also were covered with a black plastic sheet, except the lid, so that any shoots could grow under light from above the box. Gibeaut medium was renewed as needed. These experimental conditions allowed a root growth maintained throughout the experiment and did not lead to a wound reaction during the plant material dissection or a microorganism contamination. The developmental stages of *O. ramosa* are defined following Labrousse et al. (23): stage 1, broomrape attachment to the host root; stage 2, tubercle formation; stage 3, ad-

ventitious roots on the tubercle; stage 4, underground shoot growth; and stage 5, emerged broomrape. Using this experimental device, attached parasites (stage 1) were not observed before the seventh day of co-culture. One week later, stage 2 and, soon after, stage 3, were observed. Most of the haustoria grew and developed a stem whose development into a flowering stem was not observed, probably because of nutritional default of the pathosystem after some weeks.

***A. thaliana* callus infestation.** In all, 100 surface-sterilized germinated seeds of *O. ramosa* were placed on each callus surface. When deposited on calli, none of the broomrape-germinated seeds were able to fix on this tissue. Calli were incubated for various periods, after which the seeds were removed and the callus tissue harvested and immediately immersed in liquid nitrogen.

Reverse transcription-polymerase chain reaction analysis of gene expression. Total RNA was isolated from up to 100 mg of *A. thaliana* roots using the RNeasy Mini kit (Qiagen, Hilden, Germany) and treated by DNase I enzyme (Sigma). First-strand cDNA was synthesized from 300 ng of total RNA using the SuperScript II reverse transcription (RT) enzyme according to the manufacturer's instructions (Invitrogen, Carlsbad, CA). Independent polymerase chain reactions (PCR) using equal aliquots (2 µl) of cDNA samples were performed using gene-specific primers (Table 1). The choice of the primer sets was made in order that an amplification of genomic DNA gave a product containing an intron, allowing a direct confirmation that RT-PCR products had an mRNA origin. Annealing conditions were determined empirically. Preliminary experiments demonstrated that the amount of PCR product increased with increasing numbers of cycles, indicating that the reaction components were not a limiting factor.

SMART amplification. In all, 50 ng of total RNAs extracted as described above were reversed-transcribed using the SMART PCR cDNA Synthesis kit (Clontech, Palo Alto, CA), following manufacturer's instructions. Following Gonzalez et al. (17), aliquots of 1 µl from 10-fold dilution of each of the two SMART cDNA populations (infested roots and control) were amplified in 50-µl reactions using specific primers for two internal controls (*ubq5* and *ef-1α*). Normalization of the bands obtained after a cycle in the exponential stage was conducted by densitometry. Amplifications of each of the differentially expressed genes then were performed at exponential phase (newly determined for each tested gene) using the normalized template values and their specific primers.

RESULTS

Gene expression pattern before parasite attachment on roots. The occurrence of transcripts, encoding for several defense-related proteins, before attachment of broomrape seedlings on *A. thaliana* roots was assessed by RT-PCR and is summarized in Figure 2. RNAs used for reverse transcription were extracted from whole roots from 1 h to 7 days after placing *O. ramosa* seed near them. Three time points (0, 1, and 7 days) were done on non-infested roots. With the exception of the expected *thi2.1*, all the RT-PCR reactions resulted in a single product at the size predicted for a properly spliced mRNA (Table 1). Amplifications of three genes exhibiting constitutive expression (*ef-1α*, *act1*, and *rps23*) were used as positive controls of the quality of both extracted RNA and RT-PCR reactions. An early transient induction, which persisted only a few hours, was observed after 1 h for the genes *pal1*, *gst1*, and *aos*. After 2 h, *lox1* and *c4h* also exhibited transient enhanced expression. The gene *acc2* was the only case of an mRNA accumulation induced and maintained throughout the experiment with a marked increase at 7 days after infestation. Gene *lox2* was the latest gene to be expressed (after 1 day). Of the transcripts examined, only the *PR-3* mRNA response was not enhanced. As concerns *PR-1* and *asa1*, no cDNA amplification was obtained, although genomic amplification was successful (data not

Fig. 1. Experimental device for in vitro co-culture of *Arabidopsis thaliana*–*Orobanchaceae ramosa*; b., magenta box; l., lid; m., medium; p., plastic plate covered by nylon; r., host root; s., parasite seedling; sh., host shoot.

shown). When infested or control roots were compared without black tissue protection, no such difference in the expression of all the studied genes was observed (data not shown). The highest differential expression was obtained for the *thi2.1* assay, starting from barely any mRNA accumulation in noninfested roots and increasing up to a maximum after only 1 h. However, the size of the amplified product was higher (800 bp) than expected (104 bp). Sequencing revealed that this corresponded to an *A. thaliana* sequence encoding an unknown protein (GenBank Accession No. NP-568531). A protein BLAST search indicated that this unknown protein also shares high similarities with *Solanum tuberosum* putative membrane proteins, for which mRNA accumulation (*pon1* and *pon2*) is induced during cell necrosis. Hybridization between the *thi2.1*-specific primers and the unknown sequence was made possible due to high sequence homologies.

Gene expression pattern after parasite attachment on roots.

The mRNA accumulation of 20 defense-related genes was compared in *A. thaliana* root tissues noninfested and infested by *O. ramosa* after 15 days. Root fragments of 5 mm, adjacent to the

parasite penetration site, were dissected for RNA extraction. A SMART strategy made possible the study of gene expression in a very small tissue sample. The differential expression between both conditions then was determined by semiquantitative PCR on a normalized template of uninfested and infested SMART cDNA samples (Fig. 3). Normalization was accomplished and verified using primers from *ubq5* and *ef-1 α* genes (28) (the same intensity in PCR reactions stopped at the exponential phase). Among the 20 studied genes, four different expression patterns could be distinguished: no expression detected in any condition (*gst1*, *PR-1*, *PR-2*, *PR-5*, *PR-8*, *asa1*, and *pai1*) despite the primers being able to amplify genomic DNA; induced expression (*acc2*, *hmg2*, *c4h*, *4cl*, *lox1*, *lox2*, *aos*, *PR-3*, *thi2.1*, and *pdf1.2*); constitutive expression (*pal1* and *aoc*); and repressed expression (*hmg1*). The most significant differences in expression were observed for the induced genes *acc2*, *thi2.1*, and *aos* and the repressed gene *hmg1*. It should be noted that, contrary to the previous experiment, *thi2.1* RT-PCR product size corresponded to that expected (104 bp).

TABLE 1. Primers used for reverse transcription-polymerase chain reaction (RT-PCR)^a

Gene	PCR primers	PCR product size (bp)	Annealing condition (°C)	Literature
<i>thi2.1</i>	5'-GAAGGTGTATGCAAGTGAGTGG 5'-CAGTGCTCATTGGTAGCATCAG	104	60	47
<i>pdf1.2</i>	5'-GGTGGAAAGCACAGAAGTTGT 5'-AATACACACGATTTAGCACC	202	53	38
<i>PR-1</i>	5'-GTAGGTGCTCTTGTCTCTCC 5'-CACATAATTCCCACGAGGATC	419	57	40,46
<i>PR-2</i>	5'-CGAGCTCGTGCTCGATGTT 5'-GTGTAGCTGAAGTAAGGGTA	377	55	8,40
<i>PR-3</i>	5'-GTGGATGGGCTACAGCACC 5'-AATAGCAGCAACGAGGAGG	544	52	39,42
<i>PR-5</i>	5'-GATGGAGGATTTGAATTGAC 5'-AGTTAGCTCCGGTACAAGTG	559	53	40,46
<i>PR-8</i>	5'-CAATATCGAGCTTGGCTCTC 5'-CTGCGATTGAAGTGGTCCAC	529	61	42
<i>gst1</i>	5'-CAGCCACTAGAAGAGTTCTCAT 5'-CTTGAAGTCTCCATCTTCAAAGG	155	58	53
<i>pal1</i>	5'-CAGCTAGAGCCGGTGTGAATG 5'-CCGAATATTCCGGCGTTAAGGA	172	55	8,33
<i>c4h</i>	5'-ATGGGTCAGCGTAACCTAGT 5'-AAGCTCTGAGCTAATCGACT	442	57	5
<i>4cl</i>	5'-GAGTAGTCATCGTCTGCATC 5'-GTCTCCGAAGACTTCGCAAT	468	57	25
<i>asa1</i>	5'-CTGTGACCGTTACTGGATAC 5'-GACATCTGAGAACCAGGCTC	482	57	35
<i>pai1</i>	5'-GATGTGTGGCATCACATCAG 5'-TTCTGGATACCGTCTGTACC	811	59	26
<i>lox1</i>	5'-GGATGGACTCACTGTGAAGAG 5'-GTGTCCTGCTTGCATAAGTCTT	121	55	4,31
<i>lox2</i>	5'-GTAGAGAGTCCTTGTGAGTCT 5'-GAGACCTCTCGACCAAGTTATG	277	60	4,39
<i>aos</i>	5'-CGATCCATCGGAGCCTAAAC 5'-GGCTTCAACGAGAATCTCAC	429	61	24
<i>aoc</i>	5'-CATCTAACAGTCCAGGTCTC 5'-TCCTCGTAAGTGAGATAAGG	399	56	nr
<i>acc2</i>	5'-GATCATCCAAATGGGCTTTGCA 5'-GTCACCTCTTCCACCTCTAGCTT	206	58	27
<i>hmg1</i>	5'-CAATGCTGTGAGATGCCTGT 5'-TCATCGGTAAGATACTCAAG	449	54	10
<i>hmg2</i>	5'-TTGTCAGCGCTGTGTTTCATC 5'-GCAGACATTAGTGAAGCTC	289	56	10
<i>ef-1α</i>	5'-GTGAGCACGCTCTCTTGCT 5'-GAGACTCGTGGTGCATCTCA	458	55	29
<i>ubq5</i>	5'-GTGGTGCTAAGAAGAGGAAG 5'-GATCAAGCTTCAACTCCTTCT	252	55	3
<i>act1</i>	5'-GGCGATGAAGCTCAATCCAAAC 5'-GTCACGACCAGCAAGATCAAGA	391	55	1
<i>rps23</i>	5'-GCWGACAAGBMBTACAAGAAG 5'-TGNCCTTACGNCYAAATCCAG	260	54	nr

^a *thi2.1*, thionin; *pdf1.2*, defensin; *gst1*, glutathione s-transferase; *pal1*, phenylalanine ammonia-lyase; *c4h*, cinnamate-4-hydroxylase; *4cl*, 4-coumarate-coenzyme A ligase; *asa1*, anthranilate synthase alpha subunit; *pai1*, phosphoribosylanthranilate isomerase; *lox*, lipoxygenase; *aos*, allene oxide synthase; *aoc*, allene oxide cyclase; *acc2*, 1-aminocyclopropane-1-carboxylate synthase; *hmg*, 3-hydroxy-3-methylglutaryl-coA reductase; *ef-1 α* , elongation factor 1-alpha; *ubq5*, ubiquitin; *act1*, actin; *rps23*, ribosomal protein S23; nr, not reported.

Early time-course expression pattern on infested callus. In order to assess the usefulness of *A. thaliana* callus in the study of molecular responses to parasitic attack, the expression pattern of four genes (*pal1*, *c4h*, *PR-3*, and *gst1*), was analyzed during the first 24 h of contact with *O. ramosa* seedlings (Fig. 4). The *act1* control exhibited a constitutive expression, whereas the four other genes studied were up-regulated. While the expression of *pal1* and *c4h* continued to increase for 24 h, *gst1* expression, which was highly induced during the first 8 h, decreased at later times. The highest *PR-3* mRNA accumulation was observed after 16 h. A control was done on cell callus tissues treated only by GR24 (the molecule used to induce broomrape seed germination) for 8 h. For all primer pairs used, no difference between GR24-treated and untreated calli was observed.

DISCUSSION

The main goal of this work was to carry out a methodological study of the gene expression of *A. thaliana* roots in response to the presence of, and then to infestation by, *O. ramosa* seedlings. Thanks to an in vitro experimental device, a response was demonstrated from the first hour following germinated seed contact with *A. thaliana* roots. Several genes of the jasmonate (*lox*, *aos*, *aoc*, *PR-3*, and *thi2.1*) and of the ethylene (*acc2*) pathways were induced successively. Interestingly, this happened both before and after the attachment of *O. ramosa* on the host roots, which did not occur before the seventh day of contact. On the other hand, genes regulated by salicylic acid (*PR-1*, *PR-2*, and *PR-5*) and camalexin (*asa1* and *pai1*), were not activated by *O. ramosa*. It should be born in mind that camalexin is currently the sole phytoalexin evidenced in *A. thaliana* (41).

Methodological aspects. *A. thaliana* was grown in vitro, allowing the development of root infestation by broomrape to be followed step by step and to eliminate wounding the roots. This culture method was realized under sterile conditions, so the observed reactions were defense-specific to *O. ramosa* parasitism and not to bacteria or fungi contaminations. Until now, all the screening of resistance of *A. thaliana* ecotypes (51) has been done

in polypropylene bags. It was shown that, when fixed, *Orobancha* spp. could flower. This does not exclude the occurrence of defense reactions, whether partial or weak, which obviously could not be linked to an incompatible reaction. The chosen ecotype Ws does not differ a priori from the other ecotypes studied until now. Two experimental models were compared: roots and, in order to obtain more mRNA, *A. thaliana* calli. Calli differ from roots because they never are parasitized by *O. ramosa* seedlings. However, modifications of gene expression were observed after 8, 16, and 24 h of contact, but these differ to some extent from those of parasitized roots. Indeed, *pal1* is activated in calli after 8 h, but only during the first 2 h in roots. Expression of *PR-3* does not vary in roots, whereas it increases after 16 h in callus. On the other hand, *gst1* and *c4h* present the same pattern of expression in both models. However, the number of studied genes being limited, it is at present not possible to validate definitively the callus model, and the study of other defense genes shown to respond to parasitism in roots is needed. Callus fresh weight is of ≈ 100 mg, which is far more than the biomass obtained from roots segments. Callus would thus allow analysis requiring more RNA, as Northern blot, or more molecules, as enzymology or ethylene measurements. While simplified, the callus system seems interesting, but still remains to be validated for the study of *A. thaliana*-*O. ramosa* interaction. However, in plant pathology, numerous studies are performed using callus or cell cultures. mRNA was extracted from root fragments of 5 mm cut on both side of an attachment site in order to avoid a parasite material contamination and at a maximum distance of 1 cm from the haustorium in order to avoid a dilution of the gene response, which could be weak. This poses the question of the eventuality of a gene expression at a greater distance from the haustorium. But in a preliminary experiment, the use of the whole root system led to a large dilution of the response for the genes studied after *O. ramosa* attachment. Moreover, Griffiths et al. (20) observed a very localized expression of *hmg2* and *hmg1* in tomato roots parasitized by *O. aegyptiaca*. However, *A. thaliana* roots are very small and contain very small amounts of RNA; therefore, it is necessary to amplify mRNAs. Therefore, RT-PCR and SMART amplification were performed

Fig. 2. Early changes in gene expression of *Arabidopsis thaliana* roots in response to *Orobancha ramosa* attack. Agarose gels showing reverse transcription-polymerase chain reaction results for the indicated genes; h, hours after infestation; d, days after infestation; NI, noninfested roots.

and, while semiquantitative, they were seen to be suitable to study the *A. thaliana*–*O. ramosa* interaction. The SMART technology is used increasingly when only small amounts of mRNA are available (9,16). In this study, results from RT-PCR were shown to be consistent and reliable for the following reasons: (i) control, obtained at least from two or three genes, always were seen to be identical (ii) assays with genes for jasmonate and ethylene pathways showed regular increase or decrease along the kinetic study; and (iii) repetitions done on distinct RNA isolations have led to the same results.

Defense gene expression. The gene expression pattern of *A. thaliana* parasitized by *O. ramosa* for 15 days suggests that *O. ramosa* parasitism has more in common with pathogens than with wounding alone in *A. thaliana* (Table 2). Indeed, four genes (*pdf1.2*, *PR-3*, *lox1*, and *hmg2*) that are not activated by wound (39) are activated in *A. thaliana* roots after infestation by *O. ramosa*. However, it should be mentioned that the parasite penetrates the host roots and then induces wound symptoms by cell destruction. Depending on the nature of the pathogen which affects *A. thaliana*, activated metabolic pathways are different. Generally, one, or even two, of the four defense pathways (camalexin, Salicylic acid, jasmonate, and ethylene) are activated (45). In response to *A. thaliana* infestation by *O. ramosa*, the biosynthesis pathways of camalexin and salicylic acid do not seem to be activated. In-

deed, the *asa1* and *pai1* genes, known to be involved in camalexin synthesis, do not exhibit enhanced expressions. In the same way, no mRNA accumulation is observed for three of the salicylic acid pathway markers, *PR-1*, *PR-2*, and *PR-5*. This suggests that no systemic acquired reaction or phytoalexin synthesis is triggered during the infestation of *A. thaliana* by *O. ramosa*. On the other hand, the *acc2* gene, which encodes for the ACC synthase, is induced, suggesting an ethylene synthesis. Furthermore, genes of the jasmonate pathway (*lox* and *aos*), as well as genes known as markers of this biosynthesis pathway, are significantly expressed. This indicates that *A. thaliana* responds to *O. ramosa* parasitism by an induction of both ethylene and jasmonate pathways. In *A. thaliana*, the same kind of defense mechanism is induced by two other pathogens, *Botrytis* and *Erwinia* spp. (36,44).

In *A. thaliana*, *hmg1* is expressed as a housekeeping gene in all organs. The expression of *hmg2* is only detected in plantlets, inflorescences, roots, and tissues where numerous cell divisions take place. It seems also that this gene is involved in the synthesis of isoprenoids specific to particular cell types or at certain development stages (2). In the present study, expression of the *hmg1* and *hmg2* genes also was investigated: *hmg1* is repressed while *hmg2* is activated. The expression pattern of *hmg2* is in agreement with its already defined pattern of expression because numerous divisions take place in the attachment site of *Orobancha* spp. (23).

Fig. 3. A, Changes in gene expression of *Arabidopsis thaliana* roots after 2 weeks of infestation by *Orobancha ramosa*. Agarose gels showing semiquantitative reverse transcription-polymerase chain reaction (RT-PCR) results for the indicated genes; *ubq5* and *ef-1α* were used as controls to normalize the amount of template in the SMART cDNA samples from noninfested (NI) and infested (I) roots. B, PCR amplification on genomic DNA for the indicated genes; MW, molecular weight marker in base pairs.

On the other hand, the repression of *hmg1* is more intriguing because it is considered as a housekeeping gene. In the tomato-*O. aegyptiaca* interaction (20), the parasite induces an alteration of gene expression (activation of *hmg2* and repression of the squalene synthase involved in the general metabolism), mobilizing the host energy toward the production of defense compounds. In the *A. thaliana*-*O. ramosa* pathosystem, the same kind of reaction could be expected: activation of *hmg2* for defense compound synthesis like isoprenoids and repression of the housekeeping *hmg1* gene, reducing the energy expense.

An early response is detected in *A. thaliana* plantlets attacked by *O. ramosa*, even before parasite attachment on host roots. This indicates that a signal emitted by *O. ramosa*-germinated seed is recognized by *A. thaliana* which, while susceptible, activates de-

fense reactions against its aggressor. Secretion of pectolytic molecules by *Orobanchae* germination was suggested in a previous study (29) and may be involved in this process. However, to our knowledge, this kind of signal, which triggers early defense molecular reactions, never has been demonstrated before this study. The nature of this signal molecule or molecules remains to be determined.

In conclusion, this work describes a first approach to the *Arabidopsis*-*Orobanchae* interaction, studied by expression analysis of some genes known to be involved in defense reactions. Most of these exhibit rapid transient changes in their expression patterns as has been observed already in other plant-pathogen interactions (e.g., bacteria, fungi). However, strategies such as differential display or suppression subtractive hybridization would allow the isolation and characterization of genes specifically involved in this interaction. As yet, using RT-PCR, we have fortuitously detected a gene encoding for a protein whose function is still unknown in *A. thaliana*.

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Fig. 4. Early changes in gene expression of *Arabidopsis thaliana* callus in response to *Orobanchae ramosa* attack. Agarose gels showing reverse transcription-polymerase chain reaction results for the indicated genes; h, hours after contact; GR24, callus treated only with GR24.

TABLE 2. Inducible *Arabidopsis thaliana* defense genes

Gene	Induction by ^a					
	W	P/E	SA	JA	<i>Orobancha ramosa</i>	
					Early	Late
Pathogenesis-related proteins						
<i>thi2.1</i>	Yes	Yes	No	Yes	nd	Yes
<i>pdf1.2</i>	No	Yes	No	Yes	nr	Yes
<i>PR-1</i>	No	Yes	Yes	No	nd	nd
<i>PR-2</i>	nr	Yes	Yes	nr	nr	nd
<i>PR-3</i>	No	Yes	nr	Yes	No	Yes
<i>PR-5</i>	nr	Yes	Yes	No	nr	nd
<i>PR-8</i>	No	Yes	No	Yes	nr	nd
Oxidative stress						
<i>gst1</i>	Yes	Yes	nr	No	Yes	nd
Aromatic metabolism						
<i>pal1</i>	Yes	Yes	nr	Yes	Yes	No
<i>c4h</i>	Yes	nr	nr	nr	Yes	Yes
<i>4cl</i>	Yes	Yes	nr	nr	nr	Yes
Tryptophan pathway						
<i>asa1</i>	Yes	Yes	No	nr	nr	nd
<i>pai1</i>	nr	Yes	No	nr	nr	nd
Fatty acid signalling and metabolism						
<i>lox1</i>	No	Yes	nr	Yes	Yes	Yes
<i>lox2</i>	Yes	Yes	Yes	Yes	Yes	Yes
<i>aos</i>	Yes	nr	nr	nr	Yes	Yes
<i>aoc</i>	Yes	nr	nr	Yes	nr	No
Signal, regulatory, functions, others						
<i>acc2</i>	Yes	nr	nr	nr	Yes	Yes
<i>hmg1</i>	nr	nr	nr	nr	nr	rep
<i>hmg2</i>	No	nr	nr	nr	nr	Yes

^a W, wounding; P/E, pathogen or elicitor; SA, salicylic acid; JA, jasmonic acid; nd, not detected; nr, not reported (39); rep, repression.

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