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Role of acetosyringone in the accumulation of a set of RNAs in the arbuscular mycorrhiza fungus *Glomus intraradices*

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Summary. Plant root exudates contain a range of low molecular weight metabolites that trigger many of the structural and physiological changes associated with the progression and establishment of mycorrhizal symbiosis. Here, the physiological response triggered by acetosyringone (AS) was studied in *Glomus intraradices*. Incubation of *G. intraradices* spores with AS resulted in an overall increase in hyphal respiration. A *G. intraradices* cDNA library was then screened with a total cDNA probe obtained from the AS-treated spores and mycelium. cDNAs from genes induced in AS-treated *G. intraradices* were assigned to different functional categories, such as protein synthesis, membrane transport, signal transduction, and general metabolism, but without further information regarding their function or identity. A cDNA coding a fragment of a histidine kinase was also induced by AS, suggesting a two-component mediated response to the metabolite. In addition, the differential accumulation of a cruciform DNA-binding protein mRNA, termed as *GiBP1*, was also observed. Time-course experiments demonstrated the rapid accumulation of *GiBP1* within 2 h of AS induction. These results indicate the presence of a set of fungal genes that are induced by AS. These findings are discussed in terms of the possible molecular events that follow the exchange of signals between mycorrhizal symbionts. [**Int Microbiol** 2008; 11(4):275-282]

Key words: *Glomus intraradices* · arbuscular mycorrhiza · acetosyringone · gene differential expression · macroarrays

Introduction

The formation of arbuscular mycorrhizae (AM) reflect the mutualistic associations between plant roots and fungi of the order Glomeromycota [31]. AM is the most widespread form of symbiosis with vascular plants, occurring in ~80% of these plants [16]. The establishment of a functional mycorrhizal symbiosis may require the exchange of signals between partners, in addition to the bidirectional transfer of nutrients [33]. Several compounds released by the roots of vascular plants, such as flavonoids, are hypothesized to play a role in plant-fungal cross-talk and thus in AM formation [40]. AM and symbioses with other fungi, such as *Trichoderma*, enhance the absorption of nutrients such as phosphate from soil and improve the plant's resistance to biotic and abiotic stresses [8,34,35,37]. The structural and physiological modifications that occur in the symbionts strongly suggest changes in the gene expression profile of both partners. Accordingly, the coordinated expression of homologous genes in both the plant and the fungi has been demonstrated, at least in the case of nitrate reductase [20]. However, the mechanism through which this coordination is achieved is poorly understood.

It has been shown that diffusible factors produced by the fungus and perceived by the plant, termed Myc factors, regulated similar downstream gene products, such as the Nod factors of rhizobia [9,10,21]. Nod factors increase mycorrhizal colonization and stimulate lateral root formation.

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Genetic analysis has shown that this response requires the "common" symbiotic genes DMI1 and DMI2 [26]. However, it is not known whether the products of these genes mimic the symbiotic mycorrhizal factors of legumes and non-legumes. In this regard, a metabolite from the root exudates of *Lotus japonicus* was identified as a sesquiterpene strigolactone, a potent elicitor of hyphal branching—the earliest response during the establishment of AM—in AM fungi [1]. However, pure strigolactone, when applied to isolated spores, does not induce extensive branching, as observed during in vivo symbioses, thus suggesting that additional factors secreted by the plant are involved in the late developmental progression of AM. Indeed, this is likely to be the case and it has been proposed that the additional secondary metabolites exuded by the root, such as phenolic compounds, have a role in the early communication events of endomycorrhizal symbionts [16]. Among the responses in the fungal symbiont, an increased metabolic rate is the most obvious one and is observed by the higher growth rate of branching mycelia. In plants, acetosyringone (3´,5´-dimethyl-4´-hydroxyphenone; AS) has been identified as a major extracellular phenolic metabolite, particularly in tobacco cell suspensions, and its bioactive properties have been shown to influence early events in plant bacterial pathogenesis [4]. Furthermore, AS is produced by plant tissues in response to wounding. In *Agrobacterium tumefaciens*, AS induces virulence factors, involved in T-DNA transfer, through a two-component signaling pathway. Moreover, a complete symbiotic interaction was demonstrated in a two-component axenic culture comprising *A. rhizogenes*-transformed hairy roots and AM [3]. In this system, we observed that the addition of AS enhances hyphal growth and spore production in *Glomus intraradices*; therefore, by analogy with the recognition of AS in bacterial and fungal organisms, it is possible that a two-component system is involved in the establishment of AM.

In this study, we analyzed the physiological and molecular responses of *G. intraradices* to AS. For this purpose, an array of cDNAs from the fungus was probed with labeled-cDNAs of the induced tissue. The induced or repressed RNAs, encoding proteins of different functional categories, were characterized. We also characterized the mRNA of the induced cruciform DNA-binding protein, and time-course experiments showed its rapid accumulation. Physiological analysis performed with AS and branching factor (BF) indicated that these compounds induce the expression of genes related to mitochondrial activity in the fungus, an increase in respiration, and mitochondrial reorganization. These findings are discussed in terms of the possible molecular events that follow the exchange of signals at the molecular level during the early stages of mycorrhizal symbiosis.

Materials and methods

Biological material. *Glomus intraradices* was isolated in our laboratory from soil collected in the agricultural region of Texcoco, Mexico, where maize is usually grown. About 6 kg of soil was separated through a 200-µm mesh, and spores were then dissected using a stereomicroscope. The separated spores were sterilized with 2% (w/v) chloramine T for 10 min, and rinsed thrice for 10 min each in a solution of 2% (w/v) streptomycin sulfate and 1% (w/v) gentamycin sulfate [7]. Chloramine treatment and antibiotic rinses were repeated twice. The fungal spores were then placed onto previously transformed *Agrobacterium rhizogenes* carrot hairy roots. Once the mycorrhiza developed on the hairy roots, the resulting spores were transferred in new medium onto fresh roots contained in a two-compartment Petri dish, as described previously [36]. These axenic cultures were maintained with liquid M medium present in the second compartment, as described [24]. The isolated fungus was identified by amplification followed by sequencing of its rDNA ITS region, as described below. AS (3´5´-dimethoxy-4-hydroxyacetophenone, Sigma-Aldrich) was used at a final concentration of 200 µM in the induction and incubation experiments.

Cytology. The respiratory rate was assessed by measuring the mitochondrial density in *G. intraradices* hyphae. MitoTracker Green (Molecular Probes, Eugene, Oregon) was employed as follows: After 3–4 days of germination in M liquid medium, germinating spores of *G. intraradices* (30 spores per treatment) were incubated with the dye (1 µM) for 1.5 h at 28°C in the dark, under 2% CO_2. During the staining process, the germinated spores were treated with 200 µM of AS (final concentration in 0.001% (v/v) acetone) or with 0.001% (v/v) acetone (control). After treatment, the germinated spores were washed with M medium, mounted onto glass slides, and observed by confocal microscopy (SPC-SP5, Leica, Mannheim, Germany) [laser (405 diode, UV), 405 nm, laser (argon, visible), 458 nm, laser (argon, visible) (power), 20%, laser (HeNe 543, visible), 543 nm laser (HeNe 633, visible), 633 nm laser (MP, MP), 810 nm laser (405 diode, UV), 405 nm, emission, 503.1–560.7 nm], using a HCX PL APO lambda blue 63.0 × 1.40 OIL UV objective. For the negative control, spores were preincubated with 2 mM NaCN for 4 h to inhibit respiratory activity before staining [5,39]. An average of 60 images of the hyphal segments, selected from the tip of the hypha towards the spores, from either AS-treated or control germinated spores, were measured in 12 independent experiments and processed by image analysis with the Leica Application Suite, Advantage Fluorescence Lite 1.8.0 build 1346, 2005–2007 (Mannheim, Germany).

In each hyphal segment, the number of bright spots/µm^2 was calculated in every image as an estimate of the mitochondrial density, with the value distribution of pixels used to measure the fluorescence mean value or "fluorescence density." Based on these fluorescence densities, mitochondrial biomass rather than mitochondrial activity was determined. Histograms were used to represent the fluorescent spot density, in arbitrary units, as a function of ROI (resonance object inference) in the presence of AS or under control conditions. The histograms represent the frequency (%) distribution of 12 experiments.

Fungal RNA purification. Two-month old mycelia from the second compartment were collected and immediately frozen in liquid nitrogen. Total RNA was extracted using a previously described modified method [23]. Briefly, mycelia and spores were ground with a mortar and pestle in liquid nitrogen. The frozen powder was thoroughly mixed with 3 ml of 8 M guanidinium chloride and 3 ml of TE-saturated phenol, chloroform, isoamylic alcohol (25:24:1) and then centrifuged at 10,000 ×*g* for 45 min. The aqueous phase was extracted with chloroform (1:1) and centrifuged at 10,000 ×*g* for 5 min. The RNA was precipitated by the addition of 6 µl of 5 mg linear acrylamide (Ambion)/ml, 0.7 volumes of ethanol, and 0.2 volumes of 1 M acetic acid. This mixture was incubated for 2 h at –80°C and then centrifuged at 10,000 ×*g* for 30 min. The pelleted RNA was rinsed with 70% ethanol, briefly dried, and then dissolved in sterile deionized water for further use. Poly $(A)^+$ RNA was obtained by affinity chromatography (Qiagen,

Santa Clarita, CA) and used as the template for the synthesis of cDNA using a SuperScript III RNase H-Reverse Transcriptase (Invitrogen, Carlsbad, CA).

Identification of the AM isolate by rDNA ITS analysis. Genomic DNA was extracted from mycelia and spores with the DNeasy Plant MiniKit (Qiagen) and then used as a template to amplify the ITS regions by PCR, with the internal transcribed spacer (ITS) 1 and ITS4 primers, as previously described [39]. The amplified 773 bp fragment was cloned into the pDRIVE vector (Qiagen), sequenced, and then blasted against extant databases [2]. Highest homology (97%) was observed with *G. intraradices* strain JJ22 [18] when clone Gi001 (containing 18S ribosomal RNA gene, partial sequence; ITS1, 5.8S ribosomal RNA gene and ITS2, complete sequence; and 25S ribosomal RNA gene, partial sequence) from *G. intraradices*, was compared against the database. Clone Gi001 was deposited in GenBank with the accession no. EF488829.

Construction of a *Glomus intraradices* cDNA library. A cDNA library was constructed from 2-month-old mycelia grown in vitro with *A. rhizogenes*-transformed carrot roots in the second compartment, free of plant material, following the manufacturer's instructions (Clontech, Mountain View, CA). The amplified library contained 10^9 independent plaque-forming units, among which 87% contained inserts. The recombinant λTriplEx2 phages were zapped into pTriplEx2 plasmid derivatives according to the manufacturer's instructions (Clontech).

Reverse Northern blot screening. A total of 1046 randomly selected recombinant clones of *G. intraradices* cDNA library were blotted in duplicate onto Hybond N+ nylon membranes (Amersham Biosciences). Positive controls consisted of vectors containing rDNA 18S and 28S, or a *G. intraradices* tubulin PCR fragment. Plasmid DNA was denatured with solution I (0.2 N NaOH and 1% SDS), fixed to the membranes with UV, and neutralized. The membranes were then prehybridized with Church buffer (0.5M Na_2HPO_4, 7% SDS, 1% BSA, 1 mM EDTA) and hybridized at 65°C overnight against [α-^{32}P] dCTP-labeled cDNA, synthesized from RNA obtained from untreated and AS-treated mycelia. Membranes were then washed sequentially with 2× SSC for 15 min at 65°C and exposed to Hyperfilm MP high-performance autoradiography film (Amersham Biosciences) for 15 days.

Isolation of full-length gene *GiBP1*. A clone encoding a cruciform DNA-binding protein with homology to that of *Glomus versiforme* was selected for further characterization from the collection of the genes up-regulated in the presence of AS. The full-length cDNA was obtained by using 5′ RACE [30]. PCR was carried out with Takara Ex Taq polymerase (Takara, Japan). The obtained DNA fragments were cloned into pDRIVE (Qiagen), sequenced, and analyzed.

Detection of transcripts by linear RT-PCR. The accumulation of the selected mRNAs was assessed by linear RT-PCR. Total RNA was treated with RNase-free RQ1 DNase (Promega) before cDNA synthesis. The number of cycles and the amount of template used were normalized for every experiment. The primer sequences employed were: 18S rDNA: forward: 5′-TAACAGTCCAAATTCCCCGGAACA-3′, reverse: 5′-CGAGG AACAATTGGAGGGCAA-3′; *Gihik1*: forward: 5′-CAGGCATAATCCAG GGTCAATTCTGT-3′, reverse: 5′-AATCACTTGGCGCAGACGAGAGTC -3′; beta-tubulin: forward: 5′-GCTGGTCCTTTTGGACAACTAT-3′, reverse: 5′-GTAAAGTACCCATACCAGCTCCAGT-3′; and *GiBP1*: forward: 5′-AACATGTCTGAACCATCCAGAGTC-3′, reverse: 5′-ATGTT TGGGAG CTTCTTTCTTTGC-3′. All the primers were synthesized by Sigma-Genosys. The RT-PCR products were quantified by densitometric analysis of ethidium-bromide-stained gels using Image Quant software (Bio-Rad, Hercules, CA). RT-PCR analyses were performed in duplicate on independent samples.

Sequence analysis. Sequences were compared against different databases using both BLASTN and BLASTP algorithms [2].

Results

Growth rate of *Glomus intraradices* during early stages of development. Mycelia grown in axenic cultures on M media were incubated with AS as described above. A time-course experiment at 0, 48, and 72 h was carried out in which the germinating spores were incubated with AS. Spore germination was observed in all treatments; however, branching by AS-treated spores was more extensive than that of the untreated controls (Fig. 1). As a positive control, a parallel experiment was carried out with spores growing on strigolactone (already identified as a branching factor), which, as expected, stimulated hyphal growth and ramification (Fig. 1). Mycelium grew normally after AS treatment. In all treatments, the mycelia covered the compartment such that spores were produced after 2 months of incubation. The treated spores were pigmented like those of the control and were able to colonize newly formed carrot roots. In independent experiments, the developing mycelia differentiated to spores 4–7 days after AS treatment; however, this result was not consistently observed. Nonetheless, in agreement with this finding, we observed a molecular response, the induction of specific transcripts, (described below) after just 2 h of AS addition. The observed induction was only obtained when the spores were temporarily incubated in the presence of AS; by contrast, when the mycelia were continually incubated with fresh AS, added periodically, hyphal development and cellular lysis occurred infrequently.

Change in the respiratory rate in growing mycelium of *Glomus intraradices*. Spores collected for physiological analysis were allowed to germinate and mitochondrial activity was monitored as described. After 3 days, the respiratory activity of emerging mycelium from AS-treated AM fungi was greater than that of the untreated controls, as shown in the fluorescence-based assay, and was associated with an increase in both respiration and the overall metabolic rate. In addition, the mitochondria displayed morphological changes, including an increase in length, which is characteristic of metabolically active cells (Fig. 2). As the negative control, the mycelium was treated with NaCN to eliminate respiratory activity, in which case there was no fluorescent signal associated with respiration. To quantify these observations, the fluorescence density was evaluated in five areas of 100-μm^2 segments of the emerging hyphae in both the control and AS-treated fungi (Fig. 3). Fluorescence intensity, observed from the tip toward the spore along the hyphae, differed notably between control and AS-treated spores. These results confirmed that the respiratory rate of AS-treated germinating spores of *G. intraradices* is significantly increased. Similar to the observed response at the molecular level

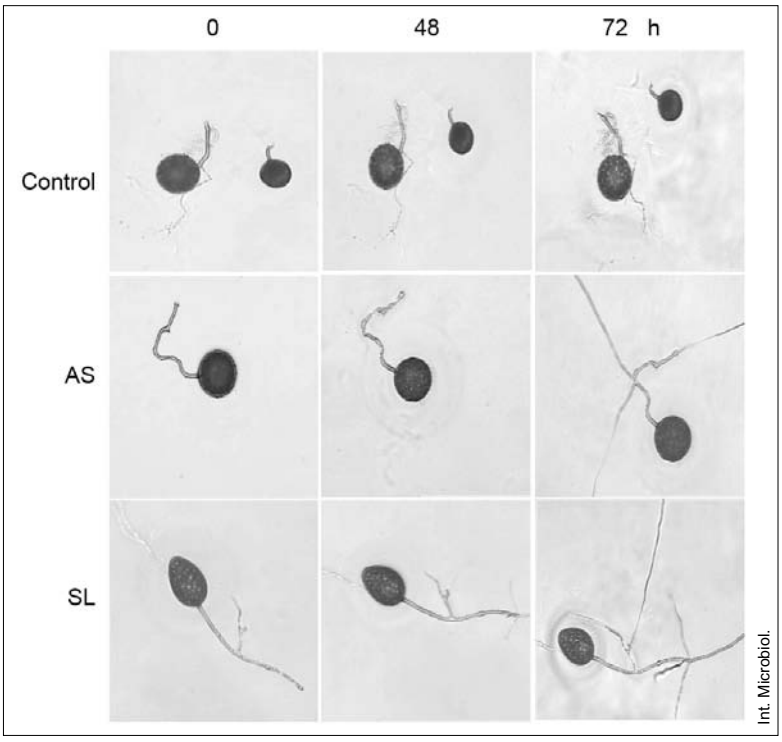


Fig. 1. Morphology of the germinating spores in a time-course incubation with acetosyringone (AS) or strigolactone (SL). Spores were germinated in the presence of AS or SL and hyphal growth was estimated after 48 and 72 h. Hyphal length was higher in spores incubated with AS than in control spores.

(induction and/or repression of a set of genes), the response occurred within 1.5 h after addition of the stimulus.

Identification of *Glomus intraradices* mRNAs misregulated by AS. Initial screening of 1046 cDNA clones allowed the identification of 31 that were induced by AS and five that were down-regulated; these clones were sequenced and the sequences compared with those in extant databases. Table 1 shows the identified sequences as well as their levels of induction. Genes whose products were potentially involved in protein synthesis, membrane transport, signal transduction, and general metabolism were identified in this set. Clones with a cutoff *E*-value > 0.03 were considered to be without identity [25], and thus potentially representative of new genes involved in the establishment or maintenance of the condition induced by AS. Among the clones reflecting increased levels of mRNA accumulation in response to AS, a significant proportion corresponded to ribosomal RNA and to those encoding proteins. This may be related to the probable increase in the overall metabolic rate and in protein synthesis during the growth and differentiation of mycelia in response to AS. Indeed, 28S ribosomal RNA and an H^+-ATPase encoding transcript were up-regulated 1.1 and 4.8 times, respectively. The H^+-ATPase belonged to the membrane transport category and showed the highest degree of induction. A cruciform DNA-binding protein, termed *GiBP1* and similar to a *G. versiforme* homologue [6], showed a 3.2-fold induction over control levels. A cDNA with high homology to a *M. truncatula* clone, similar to a serine/threonine protein kinase from *A. thaliana*, was found to be similarly up-regulated (1.8-fold). Different genes for structural components of ribosomes were overexpressed after AS treatment, such as ribosomal proteins and RNA. Clones without assigned function (1A7, 1B10, 1C9, 1C12, 1D11, 1H10, 4H9, 9B7, 10B6, 10B10, 11E6) and without identity (1A12, 1B9, 1B12, 1F12, 4E7, 5C7, 7A9, 7C8, 9C5, and 11G4) were also up-regulated (Table 1).

The expression pattern of the gene coding for the cruciform DNA-binding protein was further characterized. Expression of this gene was described to be associated with sporulation in *G. versiforme* [6].

Characterization of the full-length *GiBP1* cDNA. The *GiBP1* clone obtained from the *G. intraradices* cDNA library was 664 bp in length, including the 3´ untranslated region. Using 5´ RACE, the cDNA was completed. The ORF encodes a protein of 99 amino acids (deposited in GenBank under accession no. EF488828). The deduced amino acid sequence has 93% similarity with a putative cruciform DNA-binding protein from *G. versiforme* [6], 42% similarity with HMP1 from *U. maydis* [12], and 38% similarity with a cruciform DNA-recognition protein from *A. bisporus* [13]. The protein contains six putative phosphorylation sites, suggesting post-translational regulation. It also contains the Pfam domain, *CsbD*, a transmembrane region present in stress-response proteins, whose expression in prokary-

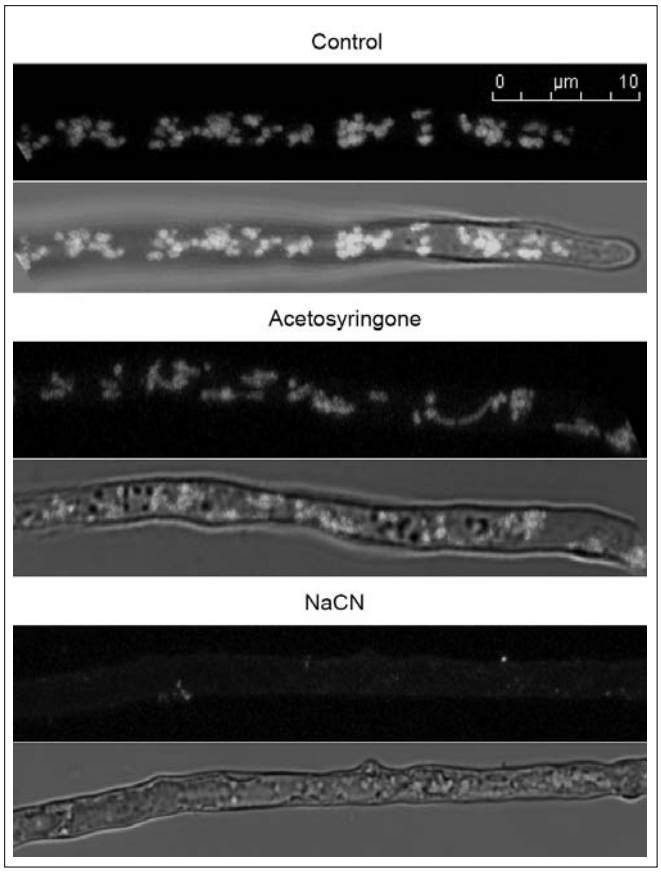


Fig. 2. Effect of AS on mitochondrial density and shape in the hyphae of germinating spores. Staining with MitoTracker Green shows the control (upper panel), AS-treated (middle panel), and negative control, NaCN-treated (lower panel) hyphae. In each panel, the confocal image of mitochondria-associated fluorescence is presented, with the corresponding transmitted-light image shown below. The bar shown refers to all panels.

otes is mediated by Sigma B [29]. The *Pfam* domain is restricted to but widely present in prokaryotes and eumycota. In vitro, the *U. maydis* homolog HMP1 has the capacity to bind to DNA oligonucleotides that mimic Holliday junctions; it also displays some of the features of HMG (high mobility group) proteins, such as small size and solubility in diluted acid [22]. Despite its affinity for DNA, *U. maydis* HMP1 apparently does not have a role in genetic recombination [12].

Differential expression of *GiBP1* during different stages of AS treatment. Linear RT-PCR was carried out to analyze *GiBP1* transcript accumulation, which began 1 h after the addition of AS, and reached the highest accumulation level at 4 h. Time-course experiments performed 0, 1, 2, 3, 4, 8, 24, and 48 h allowed detection of the *GiBP1* transcript (Fig. 4A, upper panel), which was normalized with 18S RNA (Fig. 4A, lower panel). Furthermore, parallel assays, in which 18S RNA was used to normalize cDNA concentrations, showed a similar pattern of induction, confirming the findings obtained with β-tubulin RNA (data not shown).

Accumulation of a histidine kinase transcript in the presence of AS and strigolactone. Since AS is sensed by a histidine kinase, part of a two-component system in *Agrobacterium tumefaciens* [15], we cloned a PCR fragment of a homologous gene in *G. intraradices* and tested its inducibility by AS or the *G. intraradices* branching factor strigolactone. Linear RT-PCR was carried out to analyze *GiHK1* transcript accumulation (Fig. 4B). Induction of the normalized cDNA was observed within 24 h of incubation with either compound. Time-course experiments carried out for 24, 48, or 72 h allowed detection of the *GiHK1* transcript in all cases. Similar induction of a histidine kinase transcript was observed in *A. thaliana* in response to environmental signals [11]. Further analysis is required to assign an in vivo function to this potential signal transductor in *G. intraradices.*

Discussion

Numerous plant metabolites have been hypothesized to act as signals that trigger endomycorrhizal symbiosis, and thus alterations in general metabolism as well as differential gene regulation. Among these signals, the phenolic sesquiterpene lactone strigolactone has been shown to be the much sought-after branching factor [1]. Other compounds, such as cytokinin analogs, have also been implicated in the signaling process in plants [19,32]. AS is one such analog, but it also induces the transfer of DNA from *Agrobacterium* to plants [38]. Analysis of the gene-expression profile of endomycorrhizal fungi following the addition of AS may shed light on the biochemical processes underlying the morphological changes occurring prior to and after the onset of symbiosis.

Germinating spores incubated with AS displayed only a slight increase in hyphal growth and ramification compared with the control. However, the rate of respiratory activity was a significantly increased in AS-treated spores, evidenced by a change in mitochondrial density. Interestingly, the continuous application of AS was deleterious for the fungus, indicating that the inducer must be sensed transiently to trigger a response. The increase in respiration was observed throughout the entire extent of the hyphae, most notably in the tip. To quantify these observations, the fluorescence density was evaluated in five 100-μm^2 segments of the emergent hyphae in both control and AS-treated cells.

The induction of specific transcripts occurred after just 2 h of AS addition and is in agreement with early differentiation, occurring in the pre-contact interaction phase. The increase in respiratory activity, as measured in fluorescence-based assays,

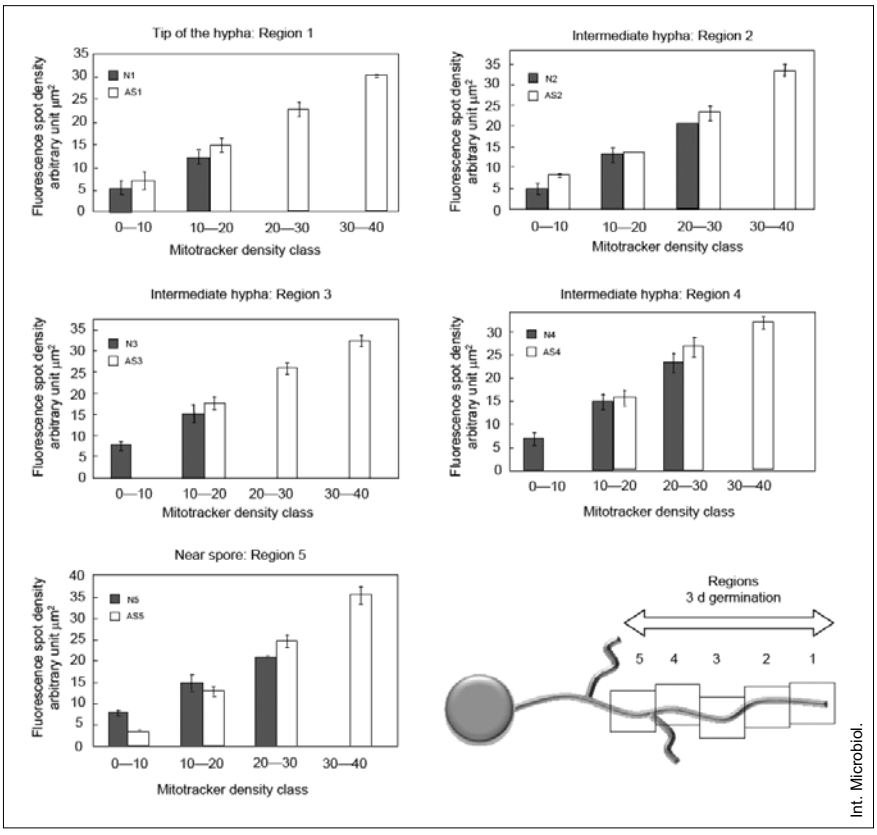
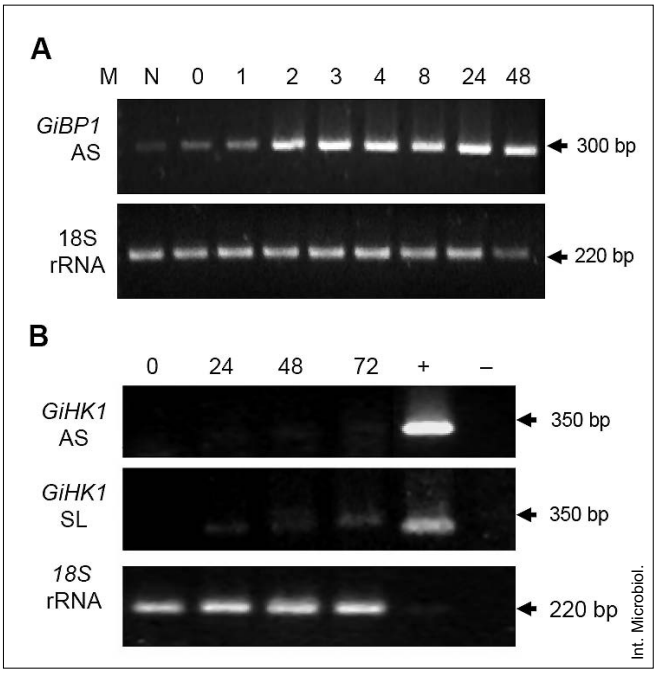


Fig. 3. Quantification of the respiratory rate in AS-treated cells vs. that in the control. The cellular response of germinating spores of Glomus intraradices stained for 1.5 h with Mito-Tracker Green and treated or not with 200 µM of AS. Based on the mean values increment, four classes were identified with respect to the hyphal structure on different areas (100 µm²). In each area, the fluorescence spot density of the hyphal structure was found to be higher in the classes with 20–40 units.



suggests an increase in the overall metabolic rate, which may be necessary for the progression of symbiosis. Spores obtained from the AS treatment were able to colonize new roots, indicating that the treatment did not affect their viability.

Several clones from the library with homologs in databases likely code for proteins with different functions, such as DNA binding, membrane transport, signal transduction, and general metabolism. Several of the obtained RNAs encode proteins whose function and identity are as yet unknown.

Clone 1C6 is highly similar to a gene encoding an H^+-transporting ATPase, which is the main ion pump in the plasma membrane. The enzyme plays a central role in the physiology and bioenergetics of fungi and plants [14]. A similar function, i.e., the generation of a membrane potential that drives the translocation of cations, amino acids, and sugars, may also be invoked in *G. intraradices*.

The function of the protein product of clone 4E8, which encodes a metallothionein also present in *Ostrea edulis* (AJ306366), may be similar to that of most metallothioneins, i.e., regulating the metal concentration within cells, and more

Fig. 4. Time course of the accumulation of mRNAs after incubation with plant metabolites. (**A**) *GiBP1* in AS-treated cells. The linear RT-PCR products of GiBP1 mRNA obtained from AS-treated mycelium harvested at different times are shown; N, normal condition without AS, and 0, 1, 2, 3, 4, 8, 24, and 48 h after AS addition. (**B**) Gihk1 in AS and SL-treated cells. Treated cells were harvested at 0, 24, 48 and 72 h. Transcript accumulation elicited with AS is shown in the upper panel, with the SL-induced transcripts shown below. Total RNA was isolated from Glomus intraradices, reverse transcribed, standardized based on 18S rRNA concentration, and PCR-amplified using specific primers for 20 cycles. PCR products were visualized after gel electrophoresis. The expected sizes (in bp) are indicated by the black arrow.

Table 1. *Glomus intraradices* genes misregulated in the presence of acetosyringone

Clone	Accession no.	Length (bp)	Similarity	Identity score	Accession no.	E value[b]	AS/N[b]
1C10	EF488828	300	Cruciform DNA-binding protein *G. versiforme*	283/300	AF034574	1e-126	3.2
1A5[a]	EL930622	375	rDNA 28S *G. intraradices*	349/371	AY373433	1e-154	1.1
2G1[a]	EL930627	304	rDNA 18S *G. intraradices*	292/303	AY635831	2e-140	1.0
1D12	EF488829	71	*rDNA 28 S G. intraradices*	68/68	DQ273828	5e-26	1.6
1H11	EL930634	390	Cyt. oxidase *O. sativa*	62/80	AC091246	6e-04	3.5
1C8	EL930623	476	Hypothetical *P. yoelii*	64/83	XM_721909	8e-04	4.2
1C6	EL930630	179	H^+-ATPase *Z. mays*	23/23	BT016177	0.008	4.8
8B4	EL930635	304	CYP514 *D. discoideum*	39/45	XM_1134512	0.002	1.2
1A7	EL930637	171	EST *G. intraradices*	172/172	BM027222	8e-08	2.6
1B10	EL930638	613	*G. intraradices*	18/30	AU098243	8e-10	1.8
1D11	EL930641	86	*G. intraradices*	78/78	BM439344	9e-23	3.4
1H10	EL930642	351	*G. intraradices*	286/286	BI451994	4e-121	1.8
4G9	EL930633	527	*M. truncatula* S/T kinase	41/47	BQ137824	1e-04	1.9
10A7	EL930644	476	*G. intraradices*	460/469	BM027118	0.0	0.9
10B6	EL930645	207	Hypothetical *U. maydis*	19/31	XM_753667	1e-05	1.4

[a]Clones used to normalize cDNA concentrations.

[b]*E*: Expected value from BlastX. *AS/N*: the ratio between normal and AS-incubated cells from the normalized density values for each clone (Array vision Evaluation 8.0). *N*: normal condition without AS, *AS* acetosyringone.

specifically, may participate in the uptake, transport, and metabolism of zinc in biological systems. This protein has also been associated with heavy-metal tolerance in *G. intraradices* AJ574700. The presence of different isolates of *G. intraradices* in heavy-metal-polluted sites indicates that AM fungi are able to survive under this condition by using an avoidance strategy [28].

The full-length cDNA of the *GiBP1* clone, coding for a cruciform DNA-binding protein, was obtained, which allowed a more detailed comparison with its putative homologs. The protein is similar to a variety of DNA-binding proteins, such as HMP1 from *U. maydis* [9], SHS9 from *A. bisporus* [13], and Gv1 from *G. versiforme* [6]. Based on the function of *U. maydis* HMP1, it is possible that the *GiBP1* protein is involved in inducing or maintaining DNA structure, but not directly in genetic recombination. It has been suggested to represent a new class of HMG-like architectural proteins, probably involved in the regulation of expression through maintenance of the higher-order structure of DNA [12]. SHS9 from *A. bisporus* has the same properties as HMP1; however, it is up-regulated in the sporophores of *A. bisporus*, suggesting a role in sporulation [13].

In this study, we identified those mRNAs up-regulated in response to AS, a structural analog of a plant cell wall compound involved in *Agrobacterium*-mediated DNA transfer. During symbiosis, the root of the plant and AM fungi communicate with each other to coordinate the growth and differentiation that occur in response to stimuli coming from the other symbiotic partner [17,27]. Such intercellular communication is based on the secretion and perception of chemical signals, which in turn activate a developmental program in the other symbiont, leading to AM formation. AS is sensed by a histidine kinase membrane receptor/transductor in *A. tumefaciens* [15]; the fact that a *G. intraradices* histidine kinase transcript (*Gihk1*) is induced by AS and strigolactone suggests that a two-component pathway is involved in the early development of AM. Therefore, the function of AS and its structural analogs function in nature may be to act as signaling molecules that, along with strigolactone, activate root–fungus communication prior to the establishment of AM symbiosis. Further studies are necessary to understand the role of these compounds in mycorrhizal symbiosis.

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