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Research Papers

Transcriptomic analysis of *Sporisorium reilianum* in response to the strigolactone analogue GR24

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Summary. A suppression subtractive hybridization (SSH) approach was used to generate cDNA libraries representing genes differentially expressed in the haploid cells of the maize head smut pathogen *Sporisorium reilianum* exposed to GR24, a strigolactone analogue. Strigolactones are present in root exudates and have been known to trigger germination of parasitic plant seeds and signal mycorrhizal fungi to connect to root systems forming mutualistic relationships. Cell respiration increased within 1 h after GR24 addition, but decreased after 5 and 8 h. All induced cells were used to construct a cDNA library, which contained 1440 clones. The cDNA ESTs were deposited on macro-array membranes and hybridized with P^{32} cDNA probes obtained from mRNA isolated from *S. reilianum* yeast cultures exposed or not to 100 nM GR24 for three time intervals. A total of 678 ESTs were identified as differentially expressed during three time-courses in response to GR24. A set of 36 candidate genes were analyzed by qRT-PCR that presented an induction of the genes at 1 h, confirming the hybridization data. Induced genes mostly affect catalysis functions (45%) like cell respiration (27%), and cell signaling (26%). Although the biological significance of this perception remains hypothetical, these results indicate that strigolactones could have a wider biological influence in the rhizosphere than previously recognized.

Key words: transcriptomic, strigolactones, qRT-PCR, macroarray, SSH.

Introduction

Plant roots release a wide range of compounds which are involved in complex communication processes in the rhizosphere. These compounds include sugars, polysaccharides, amino acids, aliphatic acids, aromatic acids, fatty acids, sterols, phenolic derivates, enzymes, vitamins, plant growth regulators and other secondary metabolites (Uren, 2000; Dakora and Donald, 2002). Several of these molecules were described as involved in plant-microbe interactions (Bais *et al.*, 2004). There is no doubt that sugars and amino acids are potential microbe stimuli, but knowledge on secondary metabolites, which trigger microbe responses in the rhizosphere, is relatively limited (Nelson, 1991; Harsh *et al.*, 2006).

Several examples illustrate that communication between plants and micro-organisms before infection can be key steps in the interaction process. For instance, flavonoids are involved in the legume-Rhizobium communication (Peters *et al.*, 1986; Peters and Long, 1988). Strigolactones are another group of rhizosphere compounds that act as important signals for arbuscular mycorrhiza (AM) fungi (Akiyama *et al.*, 2005). These compounds induce hyphal branching from germinated spores of *Gigaspora margarita* at concentrations of less than nanomolar. Strigolactones were first identified as inducers of germination of seeds of parasitic weeds reviewed by Bouwmeester *et al.* (2003). The double incidence of these molecules is intriguing and several reviews relate on their benefits *vs* harmful effects (Akiyama

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and Hayashi, 2006; Humphrey *et al*., 2006; Paszkowski, 2006; Bouwmeester *et al*., 2007).

The fact that phylogenetically divergent organisms like parasitic weeds and AM fungi are sensitive to strigolactones raises the question of the range of influence of these molecules. It has been suggested that strigolactones are specific signals for AM fungi as these molecules have no branching effect on other fungal species (Steinkellner *et al*., 2007). In AM fungi, strigolactones can rapidly trigger O_2 consumption and cause rapid increases in the NADH concentration, NADH dehydrogenase activity, and the ATP content of fungal cells (Besserer *et al*., 2006; Besserer *et al*., 2008).

Based on these results, we tested the effects of GR24 on cell respiration of the plant pathogenic soil fungus: *Sporisorium reilianum* (Basidiomycota, Ustilaginales), which causes head smut of of maize. This disease is present in most areas where maize is frequently grown. Teliospores present in sori of infected corn are disseminated by wind. When temperature and soil moisture are optimal (Téféri *et al*., 1989), the teliospores germinate in the soil as a four-celled metabasidium (Ingold, 1994). Each metabasidium produces a large number of haploid sporidia. Compatible sporidia fuse and form dicaryotic infection hyphae. The morphological events of the fusion between compatible haploid sporidia have been described in *Ustilago maydis* (Snetselaar, 1993).We focused on *S. reilianum* as it is a soil pathogen infecting maize only via host roots (Martinez *et al*., 2000). As a dimorphic fungus, the pathogen is easy to manipulate in its sporidium form for assays in liquid medium. This species is also closely related to *Ustilago maydis*, the genetic phytopathogenic model for which the annotated genome is available (Kamper *et al*., 2006). Our objective was to describe the early response of *S. reilianum* cells to GR24.

Materials and methods

Fungal strain and cultural conditions

A haploid strain of *S. reilianum* (CRM) isolated in our laboratory was used in this study. This strain was cultivated in potato dextrose broth (PDB) in shake culture (100 rpm) at 24°C until cultures reached mid-log phase (19 h). The *Escherichia coli* K-12 derivative $DH5_{\alpha}$ was used for cloning. Bacteria were grown in Luria-Bertani (LB) medium supplemented with 2% Ampicillin, and incubated overnight at 37°C with shaking at 245 rpm.

GR24 preparation

The strigolactone analogue GR24 (Chiralix, Nijmegen, NL) was prepared as a concentrated solution at 10 mM in 100% acetone and then diluted with water to 100 μM (1% acetone/H_2O). Ten μL of this solution used as treatment, and 10 μL 1% acetone used as control were added to 10 mL of the fungal cultures (10^7 cells mL^{-1}) in PDB medium.

Measurement of cell respiration

Measurements of respiration were performed using polarography and fluorescence assay. Polarography was performed using a Clark electrode according to the protocol used by Besserer *et al*. (2006). Fluorescent assay was carried out at three time points 1, 5 and 8 h in presence GR24 solubilized in acetone, in comparison to the control 0.01% acetone. From the 10 mL cell culture, an aliquot of 100 μL (10^7 cells mL^{-1}) was analyzed at each time interval by adding 20 μL of CellTiter-Blue™ reagent (CTBR Promega, Madison, WI, USA). Cell respiration was measured on 96 well optical reaction plates at 25°C by recording fluorescence with an excitation wavelength of 560 nm and an emission wavelength of 590 nm using a Fluoroskan (FL600 bio-tek, Winooski, VT USA). Stimulated and control cells were collected from cultures by centrifugation and kept at -80°C for further RNA extraction.

RNA isolation and reverse transcriptase polymerase chain reaction (RT-PCR)

Total RNA from *S. reilianum* cells treated and control were isolated using a standard phenol-chloroform procedure and were purified using an RNA purification Kit (Promega). Total RNA was quantified using a Nanodrop ND-100 spectrophotometer and RNA quality was assessed by 1% agarose gel electrophoresis stained by ethidium bromide. The integrity of the RNA was evaluated with an Agilent 2100 bioanalyzer (Agilent Technologies, Santa Clara, USA).

First-strand cDNA was synthesized from total RNA using Superscript First-strand Synthesis System (Superscript II RNAaseH, Invitrogen). Fifty-100 ng of the total RNA each from the non-stimulated

and GR24 stimulated cells were used to prepare double-strand cDNA using SmartTM PCR cDNA Kit (BD Bioscience, Clontech, Palo Alto, CA, USA) according to manufacturer's protocol.

Suppressive subtractive hybridization (SSH)

All of the SSH experiments were performed with the haploid *S. reilianum* strain M. SSH was carried out using double stranded cDNA derived from the cells treated by GR24 as the tester and acetone as the driver. At 1 h, 5 h and 8 h time points, more than 90% of the cells were in the exponential growth phase. A cDNA subtraction kit was used as recommended by the manufacturer. Products from the secondary PCRs of the SSH procedure were inserted into pGEM-T using a T/A cloning kit (Promega). *Escherichia coli* strain DH5 alpha was used for propagating the library. Clones were plated on LB medium supplemented with X-gal (100 μg mL^{-1}), IPTG (60 μg mL^{-1}) and Ampicillin (50 μg mL^{-1}) and re-grown in 96-well plates at 37°C for 24 h and stored at -80°C.

Probe labeling

Total RNA prepared from treated (GR24) and untreated (acetone) were reverse-transcribed and used as probes for expression profile analysis. The reverse transcription reaction was performed in a 20 μL solution as follows: 1 μL oligo dT_{18} (100 μM), 10 μg total RNA and distilled water up to 8 μL, were heated at 65°C for 5 min, quick chilled on ice and collected by brief centrifugation. Then, 4 μL 5× first strand buffer, 2 μL 0.1 M DTT, 1 μL 10 mM dNTP Mix (10 mM each of dATP, dTTP, dGTP), 1 μL RNasin (40 units μL^{-1}), 3 μL [32P] dCTP (10 mCi μL^{-1}) and 1 μL (200 units) Superscript® III Reverse Transcriptase (Invitrogen ™) were added, and the tubes mixed by gentle incubation at 42°C for 1 h. The probes were denatured in a heat block at 100°C for 5 min, followed by 5 min on ice and then used for hybridization.

Macroarray hybridization

All clones of the cDNA library were PCR amplified in a 50 μL reaction by using Master mix kit (Promega), using oligonucleotide primers SP_6 (5'-ATTTAGGTGACACTATAG-3') and T_7 (5'-TAATACGACTCACTATAGGG-3). The amplified PCR fragments were verified by 1% agarose gel electrophoresis for multiple bands and PCR products in an estimated DNA concentration of 40 ng μL^{-1}. PCR products were denaturized in 50% dimethyl sulfoxide (DMSO) and were mixed with 5 μL bromophenol blue, then DNA was spotted onto nylon filter (Merk Millipore, Billerica, UK) using a BioGrid Robot (BioRobatics, Cambridgeshire, UK) equipped with a 0.2 mm pin tool. Each membrane was then treated with 10% SDS for 3 min, denatured by a 0.5 M NaOH/1.5 M NaCl solution for 5 min, neutralized with a solution of 1.5 M NaCl/0.5 M Tris HCl (pH 7.5) 1 mM EDTA for 5 min and rinsed with two × SSC (one × SSC: 0.1 M NaCl, 0.01 M Na citrate, pH 7.0) for 5 min. The spotted DNAs were fixed on the filter by UV crosslinking, or incubating for 2 h at 80°C. The filters were pre-hybridized for 16 h at 65°C in 20 mL of Church solution (1% BSA, 1 mM EDTA, 0.25 M Na_2HPO_4–NaH_2PO_4 and 7% SDS). The denatured probes were then added to the solution, and hybridization carried out overnight at 65°C.

Detection and quantification of hybridization signals

Washed macroarrays were exposed to a Fuji Film Imaging plate (Tokyo, Japan) for 18 h. Radioactive images were scanned with a Amersham Biosciences Optical Scanner Storm 840 (Little Chalfont, UK), with resolution of 50 μm. Quantification of the signal intensity was performed with ImagQuant 5.0 software. The signal intensity of each spot was obtained by the following steps: the mean value of the signal intensity of the negative control (water) was subtracted from the signal intensity of each spot. We then normalized the signal intensities against the intensity of β-tubulin as a housekeeping gene. The uncentered Pearson correlation coefficient r was calculated using Microsoft Excel. Changes in mRNA expression in excess of 1.5-fold between the two samples were considered for these experiments as differentially expressed sequence.

Sequence analysis and annotation

Selected cDNA clones were screened using PCR with plasmid primers (T_7, SP_6). Plasmid minipreps of clones containing inserts of 300–400 bp or more were made using Minipreps Kit (Promega) as described by manufacturer's protocol. Prior to sequencing, all plasmids were checked for concentration and presence of an insert by *Ras1* digestion and electropho-

resis on agarose gels. The 5′-end DNA sequencing was conducted at the Toulouse Genopole (France) using the T_7 primer, Big Dye Terminator chemistry (Foster City, CA, USA) and an ABI3700 sequencer (Applied Biosystems). Selected cDNA clones were also sequenced from the 3′- end using the T7 primer by gene express institute (Paris, France). The cDNA sequences were edited onto our interface databank script, then screened for quality and were trimmed of cloning vector using CROSSMATCH program. DNA sequences were then queried using the NCBI standalone BlastAll program (Alschul *et al.*, 1997) against the BLASTN (nucleic acid), TBLASTX (translated protein query search), *U. maydis* genome, Swissprot, version6 UniProt and UniRef100. Sequence similarities above 50% with E values less than $1E^{-10}$ were considered as statistically significant positive matches (data not presented). Sequences that matched to hypothetical proteins or having low similarity to a known protein were considered as unknown genes. Gene ontology was performed using the accession number of Uniref protein blast results.

Primer design

Primers used in this study were designed with Primer3 program (http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi). The primers were checked for secondary structures with oligoanalyser software (http://www.idtdna.com/analyzer/Applications/OligoAnalyzer/Default.aspx).

The primers used in this study are summarized in the Table 2.

RT-qPCR

Reverse transcriptase-quantitative polymerase chain reactions (RT-qPCR) were carried out in optical 384-well plates using a lightcycler ABI PRISM 7900 HT sequence detection system and PCR MasterMix for Syber Green Assays (Perkin Elmer Applied Biosystem), according to the manufacturer's protocol in 10 μL reaction volumes. Each gene amplification was prepared in triplicate. Two biological repetitions (treatment and control) were carried out. Triplicates were validated with technical error under 0.5 CT. The optimal baseline and threshold values were determined using automatic CT function available with the SDS 2.2 software (Applied Biosystems).

Results

GR24 increases cell respiration of *Sporisorium reilianum*

Prior to isolation of the transcript, the eliciting effect of GR24 on cell respiration was monitored using both methods (see Materials and methods, above). A burst of cell respiration was observed at 1 h post addition of GR24. This induction decreased at 5 and 8 h to 5% and 1 % of induction, respectively (Figure 1).

Hybridization of macroarray

Differential hybridization of probes for three-time point stimulation independently with 1440 cDNA clones arrayed on high-density nylon filters yielded 678 positively hybridizing clones. For comparative purposes, signal intensity was compared to signals from known human desmin clones. To reduce background noise and select genes of interest, clones which did not present a clear pattern of differential expression during three time point stimulation were filtered out using ANOVA analysis (*P* value cut off >0.5). Only duplicate genes with *P*-values <0.5 were considered as reproducible. Clones with ratio value >1.5 were selected as up-regulated and clones with ratio value <0.3 were considered as down-regulated (data not presented). We considered genes as differ-

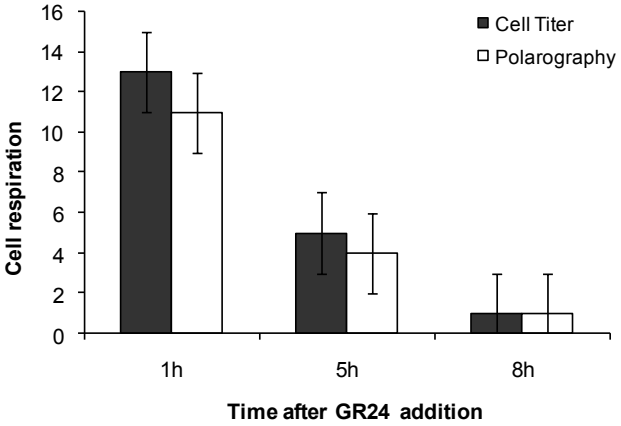

Figure 1. Cell respiration measured by using polarography method and CellTiterBlue reagent. Oxygen consumption was measured using polarographic method and CellTiterBlue reagent. Values correspond to the ratio of slopes ($100 \times (s_{GR}\text{-}s_{Ct})/s_{Ct}$) obtained on cells elicited or not with GR24 during three independent experiments.

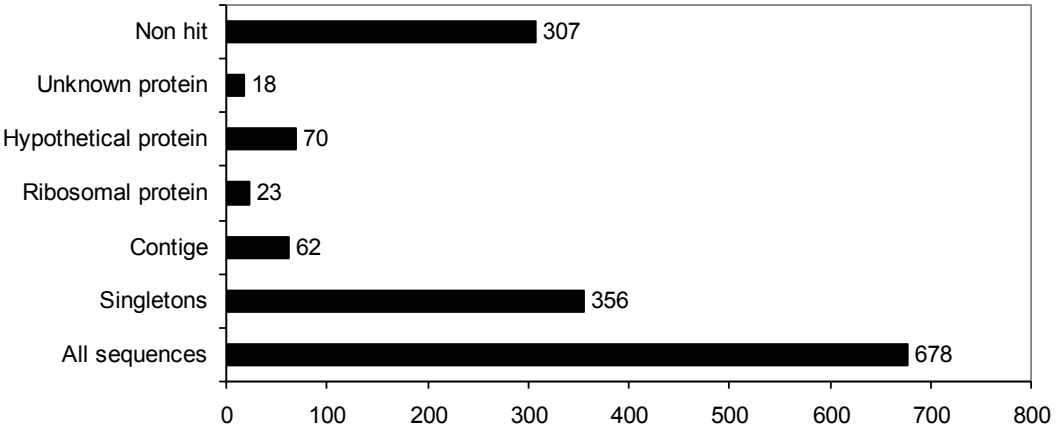

Figure 2. Blast results of 678 ESTs from *S. reilianum* obtained by macroarray experiment.

entially expressed whose expression level was 5-fold above background.

cDNA sequence analysis and gene ontology (GO)

Sequencing reaction was performed on the 678 clones obtained from the macroarray approach. Clones with cDNA inserts shorter than 80 pb, vector alone and clones that produced low quality sequences were eliminated. Finally 418 were qualified, and then contigs were performed by CAP3 software. Between these qualified sequences, 356 were singleton and 62 contigs contained multiple sequences. The average EST number in each contig was 5.2 ESTs. The average nucleotide number was 393 for singletons and 604 pb for contigs. The BLAST searches were carried out on 418 uni-genes against all sequences available in different gene banks. General sequence analysis of the SSH library showed that 23 sequences (5.5%) corresponded to ribosomal protein, 70 (17%) sequences to hypothetical protein, 18 sequences (4.5%) to unknown protein and 307 ESTs (73%) did not show any homology with sequences deposited in databases (Figure 2). Functional annotations (Gene ontology) of the 70 hypothetical proteins showed that 18% corresponded to anabolism and 72% to catabolism functions. All data was stored on a MySQL server that can be accessed through a web interface.

Based on the examination of the highest matches of sequence similarity, 70 putative functional identities were obtained, among which 36 unigenes with E-value $<10^{-20}$ were selected as candidate genes. Gene ontology analysis of the 36 selected genes showed that the genes belonged to six different categories: 22.2% to cell respiration (8 EST, CR), 19.4% to amino-acid and carbohydrate metabolism (7 EST, AACM), 13.8% to ubiquitin (5 EST, UQ), 13.8% to elongation factor (5EST, EF), 5.5% to calcium binding protein (2 EST, CbP), and 8.5% to heat shock and HESB-like proteins (3 EST, HsP). Based on this functional analysis, 16.6% (6 EST, NM) did not match with any GO in gene ontology research (Table 1).

RT-q PCR

RT-qPCR was used to independently assess the validity of the variation in gene expression detected by macroarray hybridization. Primers were designed (Table 2) and tested against cDNA from GR24-induced haploid cells of *S. reilianum* before quantitative mRNA assay. Amplification was carried out in triplicates. Amplification results were treated using Sequence Detection System software (S.D.S) version 2.2 (Applied Biosystems). At 1 h post addition of GR24, the relative changes in transcript level determined by RT-qPCR and macroarray hybridization were similar and the majority of candidate genes were over expressed.

It must be pointed out that five of the genes induced at 1 h post addition of GR24 correspond to cell respiration (Table 2), in relation to the observed induction of cell respiration measurement at 1 h. This increase of cell respiration is less at 5 h, and nil at 8 h. At these time intervals, no respiration gene induction

Table 1. Gene annotation for 36 candidate genes issuing from Macroarray experiments.

Name of gene	Gene annotation	Category[a]
Srz1-P11E9	Small ubiquitin-related modifier (SUMO)	UQ
Srz2-P11D11	Succinate dehydrogenase/fumarate reductase	CR
Srz3-P12A9	DNA binding protein	AACM
Srz4-P12C4	Hsp70 protein	HsP
Srz5-P12C7	Actin	EF
Srz6-P12D9	ATPase	AACM
Srz7-P12F8	*Ustilago maydis* 521 hypothetical protein	NM
Srz8-P13A7	Cytochrome-c oxidase	CR
Srz9-P15B1	Rho GTPase; RhoA	AACM
Srz10-P15G10	CbP	CbP
Srz11-P1F12	Actin	EF
Srz12-P2B3	Annexin;	EF
Srz13-P2D1	Isocitrate dehydrogenase NADH	CR
Srz14-P5A8	Uncultured Prochlorococcus marinus clone	NM
Srz15-P5C7	NADH:ubiquinone oxidoreductase	UQ
Srz16-P5F1	Cytochrome C oxidase	CR
Srz17-P5F10	Ubiquitine	UQ
Srz18-P5F12	Succinate dehydrogenase/fumarate reductase	CR
Srz19-P5G5	N-acetyltransferase activity	AACM
Srz20-P6C3	Na+/proline symporter	AACM
Srz21-P8A1	*Ustilago maydis* 521 hypothetical protein	AACM
Srz22-P1C8	Aldolase	NM
Srz23-P1C9	Dihydrosphingosine phosphate lyase	AACM
Srz24Co3	HESB-like domain-containing protein	HsP
Srz25C6	*Sporisorium reilian*um translation elongation factor1-alpha (tef1) gene	EF
Srz26Co7	*Ustilago maydis* translation elongation factor EF1-alpha (tef1) gene	EF
Srz27Co15	Ubiquitin	UQ
Srz28Co16	*Ustilago maydis* 521 hypothetical protein	NM
Srz29Co18	Succinate deshydrogenase	CR
Srz30Co25	EF-hand, calcium binding motif	CbP
Srz31Co26	Histone H2A [*Ustilago maydis* 521]	NM
Srz32Co29	12 kda heat shock protein (glucose and lipid-regulated protein)	HsP
Srz33Co32	NAD/FAD-utilizing enzyme apparently involved in cell division	CR
Srz34Co33	Glycosyl hydrolases family	CR
Srz35Co37	*Ustilago maydis* 521 hypothetical protein	NM
Srz36Co48	Ubiquitin-conjugating enzyme E2	UQ

[a] UQ, Ubiquitine; CR, Cell Respiration; AACM, Amino-Acid and Carbohydrate Metabolism; HsP, Heat shock Protein; EF, Elongation Factor; CbP, Calcium binding Protein; NM, not matching.

Table 2. Relative expression of candidate genes from cells treated by GR24 during 1h.

Name	Left primer	Right primer	Gene annotation	FC[a]
Srz1-P11E9	ATACGCCGACCATCAAAGAG	CGAGGTACCACCAAGCTCTC	Ubiquitin-related modifier (SUMO)	1.9
Srz2-P11D11	CGGAGAGGGATGATGTCAAT	ACAGCACGGTAGTCGAGCTT	Succinate dehydrogenase/fumarate reductase	4.2
Srz3-P12A9	TACTGATGCGTCTCGCAGTT	AACTTTGACGACCTCGTTGG	DNA binding protein	2.3
Srz4-P12C4	GTGTATGCGGTTAGGGGATG	GACAGGGCACTCTCCAAAAA	Hsp70 protein	3.8
Srz5-P12C7	AGTGCTTCTAAGCGCTGGTC	AGGATTTGCAGGAGCAGCTA	Actin	10.6
Srz6-P12D9	AGCTGGTCATGAGCGACTTT	TCGATGTGCTTCTCGATGTC	ATPase	2.5
Srz7-P12F8	GGTGGTGACGAGGTTCTTGT	GACGAGCATAGGTCCGAGAA	*Ustilago maydis* 521 hypothetical protein	3.1
Srz8-P13A7	GTGGCATACCACCAAGACCT	GGACGAATCCATTTCTGGAC	Cytochrome-c oxidase	1.5
Srz9-P15B1	AACACGAGAGGAAAGCGTGT	CTCGTCTCCCTCAACGACTC	Rho GTPase; RhoA"	1.8
Srz10-P15G10	GAAAGAGGTGCGAACGAGAC	AAGCTGTTCACGACACATCG	Calcium binding protein	2.3
Srz11-P1F12	AGTGCTTCTAAGCGCTGGTC	AGGATTTGCAGGAGCAGCTA	Actin	9.7
Srz13-P2D1	GGCGGACTACGTTTACAAGC	ACGCCATCTTCAAGACCATC	Isocitrate dehydrogenase NADH	2.9
Srz17-P5F10	GTCAAGACCCTCACGGGTAA	CTGGATCTTGGCCTTGACAT	Ubiquitine	7.3
Srz18-P5F12	CGGAGAGGGATGATGTCAAT	ACAGCACGGTAGTCGAGCTT	Succinate dehydrogenase/fumarate reductase	2.6
Srz19-P5G5	ATCGCTGAACAGCACCAAG	ATAGAGCGATGGCTTTGACG	N-acetyltransferase activity	1.7
Srz20-P6C3	CGTTTGCTCCCTCTTTTCCT	AAGCTTTCCGAAACAAAGCA	Na+/proline symporter	2.7
Srz27Co15	GTCAAGACCCTCACGGGTAA	ATGTCAAGGCCAAGATCCAG	EF-hand, calcium binding motif	5.6
Srz31Co26	ATGATGCCTGATGTTGCGTA	GCAGTCGTCTCCTTTCTCGT	Histone H2A [*Ustilago maydis* 521]	4.5
Srz32Co29	GTTTGATTGCGAGTGGAACA	CAGCATGGACATCGACTCAT	12 kda heat shock protein (glucose and lipid-regulated protein)	2.7
Srz33Co32	TTTGATTTCCAGTCGGTTCC	GACTTGAGTGCAGCCAATGA	NAD/FAD-utilizing enzyme apparently involved in cell division	2.9

[a] FC, Fold Change.

was observed, but a 2-fold induction of actin (Srz5) and a 2.8-fold induction of putative 12 kDa heat shock protein (Srz32) were observed (Table 2). The quantitative analysis of the cell induces for 8 h did not show any increase in transcription of the candidate genes.

Discussion

Sporisorium reilianum, as many soil microorganisms, is likely to perceive root exudates as a precursor to infection of its maize host (Martinez *et al.*, 2001). Here, we investigated the effect of GR24, a strigolactone analogue, on the transcriptome of haploid cells of this fungus. Strigolactones are root exudate molecules which are involved in interactions between plants and soil organisms. These molecules not only induce germination of seeds of parasitic weeds but are also involved in the endomycorrhizal symbiosis (Bouwnmester *et al.*, 2007). Arbuscular mycorrizal fungi respond to these molecules by active branching of hyphae (Akiyama *et al.*, 2005) and induction of mitochondrial respiration (Besserer *et al.*, 2006). Steinkellner and *et al.* (2001) reported that strigolactones had no effect on branching of different filamentous soil fungi or on germination of microconidia of *Fusarium oxysporum*. However, we have observed that GR24 induces cell respiration of *Ustilago maydis* (unpublished results).

In the present study we investigated the genes involved in the respiratory response with an SSH approach; and the candidate genes which could participate in the previous step of interaction of *S. reilianum* with host roots were also identified. At 1 h post addition of GR24, most of the 36 selected genes revealed by SSH were confirmed by qRT-PCR. No induction occurred at time 5 and 8 h, which could be due to aging of sporidium cultures after adding GR24. In our test conditions assays in 10 mL of liquid medium, the log phase of the cultures was short and steady state was rapidly reached in 12 h. In these conditions the time point 8 h corresponds to an aging culture. Among the genes induced at 1 h, several genes involved in cell respiration, cell wall development, cellular growth, heat shock protein, HESB-like protein and actin biosynthesis were up-regulated.

The respiratory response of *S. reilianum* to strigolactones is still speculative. It could be proposed that these molecules participate to the perception of a putative host plant in their environment, activating the global metabolism of these fungi, leading to active invasive growth, meeting and mating of compatible strains in the soil and further infection of roots. On the other hand, an activation of 13 to 20% of cell respiration 1 h after adding of GR24 to the cells present in a rich medium and harboring active metabolism in absence of external inducers could be considered as an oxidative burst. Previously, we demonstrated that at high concentration (10 μM), GR24 inhibited cell redox potential (Sabbagh, 2008). The increase of cell respiration estimated by redox potential measurement with the CellTiter Blue assay confirmed by RT-qPCR could be related to an increase of free oxygen ions in mitochondria, then inducing the production of NAD(P)H by NAD(P)H oxido-reductase. These enzymes have been proposed to carry out a number of physiological functions including cell proliferation and differentiation (Takemoto *et al.*, 2007). Based on gene annotation data (Table 1), Srz13P2D1 revealed high homology to isocitrate dehydrogenase NADH and Srz15P5D7 to NADH:ubiquinone oxidoreductase sequences. In the gene expression analysis by qRT-PCR, the variation level of Srz13P2D1 was 2.9 fold, while Srz15P5D7 did not show any variation in the induced cells in response to this compound. In this case, strigolactone is considered as a stressful factor that should be detoxified by the cells, and could participate in plant defense. It is also relevant to an increase in cell respiration. Members of Hsp70 family are strongly up-regulated by heat stress and toxic chemicals, particularly heavy metals such as arsenic, cadmium, copper and mercury (Ritossa, 1996).

Increase of gene expression level of Srz4-P12C4 EST related to Hsp70 protein could be due to detoxification activity of fungi encountering this molecule as a toxic chemical substance.

The biological incidence of strigolactones on the pathogenesis of *S. reilianum* is still to be defined. The use of maize mutants deficient in strigolactone production will be very useful to assist this definition. Maize mutants y9 and vp5, impaired in the biosynthesis of carotenoids, have been described as reduced producers of strigolactones (Matusova *et al.*, 2005). These mutants are less infected by mycorrhizal fungi than wild types, and addition of GR24 complements their phenotype (Gomez-Roldan *et al.*, 2007). However, these mutations on carotenoid biosynthesis are pleiotropic, plants are albinos and dwarf. Mutants directly targeted to strigolactone biosynthesis could be more interesting for testing the incidence of these molecules on smut pathology. To date, such plant mutants are not available.

In conclusion, the increase of cell respiration genes induced by GR24 in *S. reilianum* leads us to propose that strigolactones could have a general physiological effects on soilborne fungi and be implied in rhizosphere organization.

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