African Journal of Biotechnology Vol. 10(70), pp. 15739-15743, 9 November, 2011
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB11.393
ISSN 1684–5315 © 2011 Academic Journals

Full Length Research Paper

Effect of GR24, a synthetic analogue of strigolactones, on gene expression of solopathogenic strain of *Sporisorium reilianum*

S. K. Sabbagh

Department of Plant Protection, Faculty of Agriculture and Institute of Plant Biotechnology, University of Zabol, Iran.
E-mail: sk.sabbagh@uoz.ac.ir.

Accepted 12 October, 2011

***Sporisorium reilianum* f.sp *zeae*, a basidiomycetous fungus belonging to Ustilaginaceae, is the causal agent of maize head smut. Its pathogenicity is initiated by fusion of two compatible sporidia which give rise to the formation of a dikaryotic pathogenic hyphe. In addition, pathogenic dimorphic diploid strains called solopathogens can be formed where no mating occurs. Strigolactones are the trace molecules in plant root exudates perceived by some fungi at subnanomolar concentrations that have been implicated in inducing spore germination in fungi. Cell respiration in fungal cells was measured through polarography and fluorescence assay. GR24, a synthetic analogue of strigolactones, induced a burst of cell respiration 1 h after adding GR24 (10^{-7}M) that gradually decreased at 5 and 8 h after addition of GR24. Quantitative polymerase chain reaction (qPCR) analysis showed that transcription levels of genes involved in cell respiration and a 12 kDa heat shock protein were up-regulated 1 h after the addition of GR24 to a culture of the solopathogenic strain but no influence was observed on the other pathogenicity genes and on the culture morphology. These results suggest that strigolactones influence the rhizosphere and play a role in plant-microbe interactions**.

Key word: GR24, strigolactones, *Sporisorium reilianum*, solopathogen root exudates.

INTRODUCTION

Sporisorium reilianum f.sp *zeae* (Kühn) Langdon and Fullerton, a soilborne basidiomycetous fungus belonging to Ustilaginaceae, is the causal agent of maize head smut. The pathogen infects the host plant at the seedling stage, supposedly through the roots. The infection is systemic, and the disease symptoms become apparent only after the onset of flower development when the fungal sori replace male or female inflorescences (Martinez et al., 2000). Pathogenicity is initiated by fusion of two compatible sporidia which give rise to the formation of a dikaryotic pathogenic hyphe. Interestingly, the pathogen can become solopathogenic, whereby strains are able to switch from the saprotrophic sporidial stage to parasitic hyphe without mating (Ehrlich, 1958; Holton et al., 1968; Puhalla, 1968; Sabbagh et al., 2010). Solopathogenic strains are able to infect the host plant at the seedling stage by penetrating the roots (Sabbagh et al., 2010). The infection is systemic but disease symptoms not become apparent until flowering, whereby the reproductive tissues are replaced with smut sori. Plant roots release a wide range of compounds into the rhizosphere, some of which can be involved in plant-microbe interactions (Bais et al., 2004; Dakora and Donald, 2002; Uren, 2000). Little is known regarding secondary metabolites that trigger the microbe responses in the rhizosphere (Bais et al., 2006; Nelson, 1991). Strigolactones are carotenoid derivatives of root exudates that control numerous biological processes (Gomez-Roldan et al., 2007). These compounds are thought to be the principal plant-derived signals that promote seed germination of the parasitic plants Striga and Orobanche (Bouwmeester et al., 2007; Cook et al., 1972). Strigolactones are also important signals for arbuscular mycorrhizal fungi (AMF) (Akiyama and Hayashi, 2006; Besserer et al., 2006) which induce spore germination of *Gigaspora margarita* at below nanomolar concentration (Besserer et al., 2006). Other effects of strigolactones on fungi have been reported (Martinez et al., 2001; Sabbagh

et al., 2008; Steinkellner et al., 2007). However, nothing is known about the role of strigolactones in interactions between the root and soil-inhabiting pathogenic fungi. The fact that highly phylogenetically divergent organisms like parasitic weeds and AMF are sensitive to strigolactones raises the question of the spectrum range of influence of these molecules. Bessere et al. (2006) showed that strigolactones rapidly trigger O_2 consumption of the AMF. Reactive oxygen species (ROS) produced by specific NADPH oxidases (Nox) can serve both defense and differentiation signaling roles in multi-cellular organisms (Takemoto et al., 2007). In the filamentous fungus *Podospora anserine*, two NADPH oxidase isoforms are required for sexual reproduction and ascospore germination (Malagnac et al., 2004). Yap1 gene in *Ustilago maydis*, involved in H_2O_2 detoxification is required for virulence. This fungus, using its Yap1-controlled H_2O_2 detoxification system, copes with early plant defense responses (Molina and Kahmann, 2007).

S. reilianum is closely related to *Ustilago maydis*, the genetic phytopathogenic model in which an annotated genome is available (Kamper et al., 2006) and this annotated genome has been used to identify the genes in a solopathogenic strain of *S. reilianum* that are induced in response to GR24 (Sabbagh, 2008). Solopathogenic strains of smut fungi in their sporidial (diploid) form are easily manipulated for *in vitro* assays. Martinez et al. (2001) reported that the fractions of root exudates could induce physiological transition from haploid spordial stage to mycelial form in a strain of *S. reilianum* on artificial medium. The objective of this study was to test the effect of GR24 on metabolic and defense gene expression, cell respiration and inducition of changes in morphology in solopathogenic strain of *S. reilianum* (Sabbagh et al., 2010).

MATERIALS AND METHODS

Fungal strain and cultural condition

The haploid strain SRZN of *S. reilianum* was used to produce fuzzy form of solopathogenic strain according to Sabbagh et al. (2008, 2010). Fungal strains were cultured in PDB and incubated at 24°C on a rotary shaker at 100 rpm for 19 h or when cultures reached mid-log phase.

Induction of cells by GR24

The strigolactone chemical analogue GR24 (Chiralix, Nijmegen, NL) was prepared as a concentrated solution at 10^{-2} M in 100% acetone. It was diluted with sterile distilled water to 10^{-4} M (1% acetone/H_2O). The amount of 10 µl from this solution was added to 10 ml of the *S. reilianum* culture containing 10^7 cells ml^{-1} grown in potato dextrose broth (PDB). The control consisted of 10 ml of the same fungal culture with similar concentration to which was added 10 µl of 1% acetone. The effect of strigolactone analogue (GR24) generating a solopathogenic strain of *S. reilianum* from the haploid strain was monitored *in vitro* in potato dextrose agar (PDA) culture containing charcoal according to Kirk et al. (2001).

Measurement of cell respiration

Cell culture was supplemented by 10 µl of GR24 at a concentration of 10^{-4} M and 1% acetone in 10 ml of fungal culture (10^7 cells ml^{-1}) in PDB for treatment and control, respectively. At 1, 3 and 5 h post-supplementation, cell respiration was measured. 20 µl of CellTiter-Blue™ reagent (CTBR Promega, Madison, USA) was added to 100 µl actively growing cell culture. Cell respiration was measured using 96-well-optical reaction plates at 25°C using a Fluoroskan FL600 (Bio-tek, Vermont, USA). Fluorescence was recorded with an excitation wavelength of 560 nm and an emission wavelength of 590 nm. Treated and control cells were collected from cultures by centrifugation and kept at -80°C for RNA extraction.

GR24 induction of morphological changes in *S. reilianum*

100 fresh cells of SRZN haploid strain of S. *reilianum* were placed in a Petri plate containing PDA and charcoal. The fungal strain was also cultured on 10 ml PDB medium (10^7 cells ml^{-1}). Both cultures were supplemented with 10 µl of GR24 at a concentration of 10^{-4} M. The effect of GR24 on the morphological characteristics of the haploid strain of *S. reilianum* was investigated in both media. Physiological transition (haploid to solopathogeny) was followed up for one week.

RNA methods

Total RNA from solopathogenic strain (SRZC1) cells (treated and control) were isolated using a standard phenol-chloroform procedure, and was purified using a RNA purification Kit. Total RNA was quantified using a Nanodrop (ND-100) spectrophotometer and RNA quality was assessed by 1% agarose gel electrophoresis stained by ethidium bromide. First-strand cDNA was synthesized from total RNA using Superscript First-strand Synthesis System (Superscript II RNAaseH, Invitrogen). Amount of 50 to 100 ng from the total RNA of both stimulated cells by GR24 and control were separately used to prepare double-strand cDNA using SmartTM PCR cDNA Kit (Bioscience, Clontech, USA) according to the manufacturer's protocol. The effect of GR24 in expression of some genes involved in cell respiration, transcription (ribosomal protein), and genes responsible for the previous (ras1, rop1) steps of pathogenicity were analysed.

Quantitative polymerase chain reaction (qPCR)

Reverse transcription quantitative polymerase chain reaction (RT-qPCR) was carried out in optical 384-well plates using a lightcycler ABI PRISM 7900 HT sequence detection system (Perkin Elmer/Applied Biosystem, Foster City, USA) and PCR MasterMix for Syber Green Assays (Applied Biosystems, Foster City, USA), according to the manufacturer's protocol. The amplifications were performed using the following concentration: 8 µl of Syber Green PCR MasterMix (Applied Biosystems), 10 µM of each oligonucleotide primers (final concentrations) and 2 µl of cDNA template in 10 µl reaction volume. Each gene amplification was prepared in triplicates. Two biological repetitions were carried out. Triplicates were validated with technical error under 0.5 CT. The amplification conditions were: 50°C for 2 min, 95°C for 10 min, 40 cycles at 95°C for 15 s, and 60°C for 1 min. Melting curve analysis was performed after each reaction, to exclude non-specific amplifications, with the thermal cycle at 95°C for 15 s, 60°C for 15 s and 95°C for 15 s. The optimal baseline and threshold values were determined using automatic CT function available with the SDS 2.2 software (Applied Biosystems).

Table 1. Effect of GR24 10^{-7} M on cell respiration of Solopathogenic SRZN strain of *Sporisorium reilianums*.

Strain	Redox potential (celltiter-blue)		
	1 h	2 h	3 h
Solopathogenic strain SRZN	+13%	+5%	+ 1%

Oxygen consumption was measured using Cell titer Blue reagent on cell stimulated by GR24 at three time point (1 h, 3 h and 5 h). Values correspond to the ratio of slopes (100 x (f_{GR}-f_{Ct})/f_{Ct}) for Celltiter-blue respectively.

RESULTS

Increases of cell respiration in solopathogenic strain

The eliciting effect of GR24 on cell respiration was monitored by measuring redox potential. A 13% increase in burst of cell respiration over the untreated controls (+13 %) was observed at 1 h post-supplementation of GR24 (Table 1) that decreased to 5 and 1% at 3 and 5 h.

GR24 did not induce morphological changes in *S. reilianum*

No transition from haploid to solopathogenicity due to treatment with GR24 was observed in our experimental condition, suggesting that this compound has no role in the generation of fuzzy strain.

Influence of GR24 on gene expression

The effect of GR24 on gene expression of solopathogenic strain was investigated using qPCR. Data acquired from solopathogenic strain cells induced by qRT-PCR showed that 1 h post supplementation of GR24, transcription of Cytc1 (2.7 fold) and prib60S (3.24 fold) were up-regulated, but not the other genes and on other times (data not shown).

DISCUSSION

S. reilianum, the causal agent of the maize head smut, is a soil borne pathogen which infects its host via roots. In this study, a solopathogenic strain of the pathogen was used. The strain is capable of infecting maize roots without showing symptoms on the aboveground parts of corn. We demonstrated that GR24 was capable of effecting physiological changes of solopathogenic strain of *S. reilianum* but did not change the morphological habit of the fungus. Some root exudates have been shown to act as plant signals in pathogens before infection (Nelson, 1991). In our previous work, the effect of GR24 on haploid strain of *S. reilianum* was investigated by analysis of transcriptomes expressed at different times and different concentrations of GR24. The majority of EST were expressed following GR24 treatment of *S. reilianum* related to genes involved in cell respiration (Sabbagh, 2008). We were interested in investigating the effects of GR24 on some genes involved in cell respiration and pathogenicity of *U. maydis*, a fungus phyllogeniticaly related to *S. reilianum* (Sabbagh, 2008). At 1 h post supplementation of GR24, all genes involved in cell respiration were induced. Cell respiration increased a bit at 5 h, and was null at 8 h post supplementation of GR24. No induction of genes involved in cell respiration was observed in those times, but an induction of actin and putative 12 kDa heat shock protein was observed. The quantitative analysis of cells induced at 8 h post supplementation of GR24 did not show any increase in candidate genes transcripts. Recently, Steinkellner et al. (2007) tested the effect of strigolactone on the microconidial germination of *Fusarium oxysporum* f.sp. *lycopersici*. Martinez et al. (2001) reported that the fractions of root exudates could induce physiological transition (haploid to mycelium) of haploid strain of *S. reilianum* on artificial medium. They found that strigolactones do not affect the microconodial germination of the fungus. So, based on their preliminary data, they suggested that strigolactones are specific signals for AMF but are not general plant signals for fungi. It is possible that these molecules exert their influence at specific penetration sites or infection stages on fungal cells after spore germination and before penetration into root. In our experiment, the percent of 10^{-7} M of the synthetic strigolactone analogue GR24 induced an increase in cell respiration. Interestingly, the GR24 is also known to stimulate hyphal branching of AMF (Besserer et al., 2006; Lpez-Rez et al., 2008). It can also affect nodule formation in alfalfa (Soto et al., 2010). It can be understood that GR24 could influence cell respiration and induce expression of some genes involved in cell development. It has no effect on genes of pathogenicity pathway, as shown in *U. maydis*. Despite this observation, this molecule could be useful either to induce growth of mycorrhizal fungi for industrial production or as a pharmacological hormone to aggravate mycorrhizal fungi in soil with pathogenic organism such as fungi and bacteria.

REFERENCES

Akiyama K, Hayashi H (2006). Strigolactones: Chemical signals in fungal symbionts and parasitic weeds in plant roots. Ann. Bot. 97: 925-931.

Bais H, Park S, Weir T, Callaway R, Vivanco J (2004). How plants communicate using the underground information superhighway. Trends Plant Sci. 9: 26-32.

Bais H, Weir T, Perry L, Gilroy S, Vivanco J (2006). The Role of Root Exudates in Rhizosphere Interactions with Plants and Other organisms. Annu. Rev. Plant Biol. 57: 233-266.

Besserer A, Puech-Pag V, Gomez-Roldan V, Jauneau A, Roy S, Portais J, Roux C, Bécard G, Séjalon-Delmas N (2006). Strigolactones Stimulate Arbuscular Mycorrhizal Fungi by Activating Mitochondria. PLoS Biol. 4: p. 226.

Bouwmeester HJ, Roux C, Lopez-Raez JA, Bécard G (2007). Rhizosphere communication of plants, parasitic plants and AM fungi. Trends Plant Sci. 12: 224-230.

Cook CE, Whichard LP, Wall ME, Egley GH, Coggon P, Luhan PA, McPhail AT (1972). Germination stimulants. 2. The structure of strigol - a potent seed germination stimulant for witchweed (*Striga lutea* Lour.). J. Am. Chem. Soc 94: 6198-6199.

Dakora FD, Donald AP (2002). Root exudates as mediators of mineral acquisition in low-nutrient environments. Plant Soil, 245: 35-47.

Ehrlich HG (1958). Nuclear behavior in mycelium of solopathogenic line and in cross of two haploid lines of Ustilago maydis (DC)Cda. Mycologia, 50: 622-627.

Gomez-Roldan V, Roux C, Girard D, Bécard G, Puech V (2007). Strigolactones: Promising plant signals. Plant Signal. Behav. 2: 163-164.

Holton CS, Hoffmann JA, Duran R (1968). Variation in the Smut Fungi. Ann. Rev. Phytopathol. 6: 213-242.

Kamper J, Kahmann R, Bolker M, Ma LJ, Brefort T, Saville BJ, Banuett F, Kronstad JW, Gold SE, Muller O, Perlin MH, Wosten HAB, de Vries R, Ruiz-Herrera J, Reynaga-Pena CG, Snetselaar K, McCann M, Perez-Martin J, Feldbrugge M, Basse CW, Steinberg G, Ibeas JI, Holloman W, Guzman P, Farman M, Stajich JE, Sentandreu R, Gonzalez-Prieto JM, Kennell JC, Molina L, Schirawski J, Mendoza-Mendoza A, Greilinger D, Munch K, Rossel N, Scherer M, Vranes M, Ladendorf O, Vincon V, Fuchs U, Sandrock B, Meng S, Ho ECH, Cahill MJ, Boyce KJ, Klose J, Klosterman SJ, Deelstra HJ, Ortiz-Castellanos L, Li W, Sanchez-Alonso P, Schreier PH, Hauser-Hahn I, Vaupel M, Koopmann E, Friedrich G, Voss H, Schluter T, Margolis J, Platt D, Swimmer C, Gnirke A, Chen F, Vysotskaia V, Mannhaupt G, Guldener U, Munsterkotter M, Haase D, Oesterheld M, Mewes HW, Mauceli EW, DeCaprio D, Wade CM, Butler J, Young S, Jaffe DB, Calvo S, Nusbaum C, Galagan J, Birren BW (2006). Insights from the genome of the biotrophic fungal plant pathogen Ustilago maydis, 444: 97-101.

Kirk PM, Cannon PF, David JC, Stalpers J (2001). Ainsworth and Bisby's Dictionary of the Fungi. 9th ed. CAB International, Wallingford, UK.

Lpez-RJez JA, Charnikhova T, Gmez-RoldJn V, Matusova R, Kohlen W, De Vos R, Verstappen F, Puech-Pages V, Bécard G, Mulder P, Boumeester H (2008). Tomato strigolactones are derived from carotenoids and their biosynthesis is promoted by phosphate starvation. New Phytologist, 178: 863-874.

Malagnac F, Lalucque H, Lepe`re G, Silar P (2004). Two NADPH oxidase isoforms are required for sexual reproduction and ascospore germination in the filamentous fungus Podospora anserina. Fungal Gene. Biol. 41: 982-997.

Martinez C, Jauneau A, Roux C, Savy C, Dargent R (2000). Early infection of maize roots by *Sporisorium reilianum* f. sp. zeae. Protoplasma, 213: 83-92.

Martinez C, Buée M, Jauneau A, Bécard G, Dargent R, Roux C (2001). Effects of a fraction from maize root exudates on haploid strains of *Sporisorium reilianum* f. sp. zeae. Plant Soil, 236: 145-153

Molina Lz, Kahmann R (2007). An Ustilago maydis Gene Involved in H_2O_2 Detoxification Is Required for Virulence. Plant Cell, 19: 2293-2309.

Nelson EB (1991). Exudate molecules initiating fungal response to seed and roots. In: The rhizosphere and plant growth. Keister DL, Cregan PB, Eds.; Kluwer: Dortrecht: pp. 197-209.

Puhalla JE (1968). Compatibility reactions on solid medium ans interstrain inhibition in *Ustilago maydis*. Genetics, 60: 461-474.

Sabbagh SK (2008). Adaptation à la pénétration racinaire de deux Ustilaginaceae parasites du maïs: Ustilago maydis et Sporisorium reilianum: Analyse microscopique et transcriptomique. PhD thesis. Université Toulouse/France.

Sabbagh SK, Naudan M, Roux C (2008). Isolation of a natural solopathogenic strain of Sporisorium reilianum f.sp. zeae (Ustilaginaceae, Basidiomycetes). Commun. Agric. Appl. Biol. Sci. 73: 101-108.

Sabbagh SK, Diagne-Leye G, Naudan M, Roux C P (2010). Solopathogenic strain formation strongly differs among Ustilaginaceae species. FEMS Microbiol. Lett. 305:121-127.

Soto MJ, FernJndez-Aparicio M, Castellanos-Morales V, Garcيa-Garrido JM, Ocampo JA, Delgado MJ, Vierheilig H (2010). First indications for the involvement of strigolactones on nodule formation in alfalfa (*Medicago sativa*). Soil Biol. Biochem. 42: 383-385.

Steinkellner S, Lendzemo V, Langer I, Schweiger P, Khaosaad T, Toussaint JP, Vierheilig H (2007). Flavonoids and Strigolactones in Root Exudates as Signals in Symbiotic and Pathogenic Plant-Fungus Interactions. Molecules, 12: 1290-1306.

Takemoto D, Tanaka A, Scott B (2007). NADPH oxidases in fungi: Diverse roles of reactive oxygen species in fungal cellular differentiation Fungal Genetics Biol. 44: 1065-1076.

Uren N (2000). Types, amounts and possible functions of compounds released into the rhizosphere by soil grown plants. In: The Rhizosphere: Biochemistry and Organic Substances at the Soil Interface, ed. Pinton R, Varanini Z and Nannipieri P. New York: Marcel Dekker: pp. 19-40.