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Elucidation of biocontrol mechanisms of *Trichoderma harzianum* against different plant fungal pathogens: Universal yet host specific response

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ABSTRACT

In the present study, different transcripts of *Trichoderma harzianum* ThHP-3 were evaluated for their response against four fungal pathogens *Fusarium oxysporum*, *Colletotrichum capsici*, *Colletotrichum truncatum* and *Gloesercospora sorghi* using RT-qPCR. The time course study of *T. harzianum* transcripts related to signal transduction, lytic enzymes, secondary metabolites and various transporters revealed variation in expression against four fungal pathogens. In a broader term, the transcripts were upregulated at various time intervals but the optimum expression of *cyp*3, *abc*, *nrp*, *tga*1, *pmk, ech42* and *glh20* varied with respect to host fungi. Additionally, the expression of transcripts related to transporters/cytochromes was also observed against *Fusarium oxysporum* after 96 h whereas transcripts related to secondary metabolites and lytic enzymes showed significant difference in expression against *Colletotrichum* spp. from 72 to 96 h. This is first study on transcriptomic response of *T. harzianum* against pathogenic fungi which shows their host specific response.

1. Introduction

The ability of *Trichoderma* strains to colonize different substrates and diverse environmental habitats make them cosmopolitan fungi [1–3]. The mutual interaction of *Trichoderma* with plants and ability to compete against plant pathogenic fungi in plant rhizosphere is of vital importance in agriculture [4–6]. The production of lytic enzymes [7–12] and secondary metabolites [13,14] by *T. harzianum* are largely considered responsible for targeting and destroying cell walls of phytopathogenic fungi [15]. The metabolic arsenal of *T. harzianum* has played an important role in its development as an ecofriendly alternate to the synthetic fungicides and its commercialization as biocontrol agent (BCAs) [16–19]. The ability of BCAs to recognize and mediate molecular events in the presence of a potential host is of paramount importance for deploying its weapons against its prey host. At molecular level, *Trichoderma* spp. are known to exhibit different transcriptomics response at different stages of interaction against their hosts [3–19].

In recent studies, a number of efforts have been made on understanding the molecular mechanisms involved in host recognition to effective execution of antagonistic behavior of BCAs [18,20]. The transcriptomes of *Trichoderma* expressed under stimulated mycoparasitic conditions involving host cell wall of pathogenic fungi such as *Sclerotinia sclerotiorum* [20], *F. solani* [5] and other stress conditions have played an important role in identification of different transcripts [3,6,21–26]. The plant pathogenic fungi such as *Fusarium oxysproum*, *Colletotrichum* spp and *Gloeosercospora sorghii* are known to cause vascular wilt [27], anthracnose [28] and zonate leaf spot [29] in various agricultural crops.

So far in a number of studies, either single or two pathogenic fungi have been used for understanding transcriptomics response of BCAs. The expression of *T. harzianum* genes in presence of deactivated mycelium of four pathogenic fungi will help in developing better understanding of various molecular events. The present study therefore involves the use of autoclaved mycelium of four plant pathogenic fungi as a carbon source in medium to support the growth and to understand the expression of various transcripts of *T. harzianum* which are supposed to be transcribed during mycoparasitism.

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Table 1
Primers pairs for quantification of different genes expressed in *T. harzianum* ThHP-3 using RT-qPCR.

Functions	Gene	Primer	
		Forward (5-3′)	Reverse (5-3′)
Housekeeping genes	*act1*	GACATTTACGGCGGAGATGAG	AGGACGGCAGGATTGATTTG
Signal transduction	*tga1*	GAGACTGTCAACCGAATG	TGAGGAAGAGAATGATGGA
	tmkA	GAAGTGGAGTTGCCGTTA	CCGTATGCGTTGGAGATT
Lytic enzymes	*glh*-19	TGAGGTTATTACGGAGAT	CATAGGCAACAATGAGAT
	ech42	CATCAATCTGGTGGCATA	CGTCCTTGTTCATGGTAT
	asp protease	CTCACACCTTCTCAATAC	GTTAATCCAAGCGTAGTA
	α-1,3-*glu*	GGAACCTCATTGATGGATAT	GGAATGGTGCCAACATAT
	β 1–4, *glu*	GGTGTGACTTATGTGGAT	TTACTCCTTCGCAATCAA
	glh-13	AGGCTTCAACCAAGAGAT	CCATCCAAGTACCACAGT
	glh-27	CGATATGGATGCTCTATTCA	TTCTGGACAGTGGTTCTA
	asp6281	CTCACACCTTCTCAATAC	GTTAATCCAAGCGTAGTA
	asp	CCGTTACAACTGTCTTCAC	CTTCGTCTCCTCCATCTC
	pepM22	TTCCGCACATCACTTGAA	GTCCCTCCACTCTACATATAC
	srp	ACCAAGATACTGCCAACA	TACCAACGCTGAGAACAT
	peptidase M22	TTCCGCACATCACTTGAA	GTCCCTCCACTCTACATATAC
Metabolites related	*cytochrome* P450 CYP2	CCGAATAGTCCATGAGTTA	GAATACAGAGATGATGAAGTG
	cytochrome P450 CYP3/CYP5/CYP6	CTGGACTATGTGATACCT	CGAATACGAAGAGTGAAC
	cytochrome P450 CYP4/CYP19/CYP26	AACGCTCATTACGCTCTT	TGGTCTCTTCTTCATCTTAATCA
	nrp	AAGGCTTCATGGATCATTATATC	CTGTCATCTGTGGCGTAT
	Multidrug/pheromone exporter, abc	GAGGAGCAGGTGATTAAG	CTTGTTACCAACCAGTGT

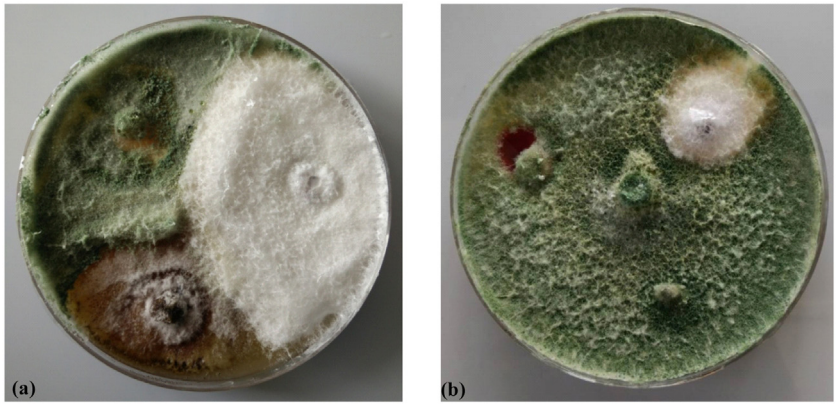

Fig. 1. Confrontation assay of *T. harzainum* ThHP-3 against *F. oxysporum* **(a)** and *C. truncatum* **(b)** on PDA plate showing antagonistic potential of ThHP-3 after 5 and 15 days of post inoculation.

2. Material and methods

2.1. Fungal isolates

The biocontrol isolate ThHP-3 identified as *Trichoderma harzianum* with 18S Genbank accession number KP064223 was used for the present study [11,12] and fungal pathogens *C. capsici*, *F. oxysporum*, *G. sorghi* and *C. truncatum* were procured from Department of Plant Pathology CSK-HPKV Palampur, Himachal Pradesh India.

2.2. Confrontation assay

The antagonistic activity of *T. harzianum* isolate was evaluated against *C. capsici*, *F. oxysporum*, *G. sorghi* and *C. truncatum* using dual culture assay on potato dextrose agar (PDA) [30]. The mycelia bit of pathogenic fungi were inoculated on PDA Plates 48 h prior to the inoculation of *T. harzianum* ThHP-3.

2.3. Sample collection

For mycelia collection, a spore suspension of 5×10^6 spores mL^{-1} of the *T. harzianum* was inoculated to 100 mL Czapek-Dox broth (CDB) containing sucrose- 5 g, $NaNO_3$-3.0 g, K_2HPO_4–1 g, $MgSO_4$-0.5 g, KCl-0.5 g, $FeSO_4$–10 mg L^{-1}; pH 5.5 supplemented with 1% autoclaved mycelium of four different plant pathogenic fungi in 250 mL conical flasks and CDB medium with only sucrose was used as control [10]. The cultures were incubated at 28 °C for 4 days in an orbital shaker with constant shaking at 180 rpm and samples were collected at regular time intervals. The mycelial samples were harvested by vacuum filtration and stored at −80 °C until further use.

2.4. Total RNA isolation and cDNA synthesis

The total RNA was isolated using Trizol reagent (Life Technology) and quantified using Qubit RNA estimation kit (Life Technologies) and analyzed on denaturing formaldehyde agarose gel electrophoresis. The total RNA was treated with DNase I (Invitrogen) for the removal of any contaminating DNA before cDNA synthesis. The cDNA synthesis was performed in a 40 μL reaction volume containing 2 μg of total RNA using a mixture of oligo$(dT)_{10\text{-}18}$ and random primers in 3:1 ratio using verso cDNA synthesis kit (Thermo Scientific). For RT-qPCR, cDNA was diluted 5 folds and then used as a template.