Response of Putative Pathogenicity-related Genes in *Tilletia indica* Inciting Karnal Bunt of Wheat

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Karnal bunt of wheat (*Tilletia indica*) is an important internationally quarantined disease from food security point of view. For understanding host specificity and host-pathogen interaction, putative pathogenicity-related genes were analysed in *Tilletia indica* in response to host factor at different time points. Highest radial mycelia growth (3.4 cm) was recorded in media amended with susceptible host factor followed by resistant host (2.6 cm) and control (2.0 cm) at 30 days after incubation significantly. Fourteen homologous sequences of putative pathogenicity-related genes, viz. *TiPmk1*, *TiKss1*, *TiHog1*, *TiHsp70*, *TiKpp2*, *TiCts1*, *TiHos2*, *TiChs1*, *TiPrf1*, *TiSid1*, *TiSsp1*, *TiSte20*, *TiUbc4* and *TiUkc1*, were identified in *T. indica* by *in silico* analysis. Some of the pathogenicity-related genes were highly expressed significantly in *T. indica* in response to susceptible host factor as compared to resistant host factor. *TiPmk1, TiHog1*, *TiKss1* were found highly upregulated up to 26-fold (3 days), 20-fold (3 days) and 18-fold (4 days), respectively, significantly in presence of susceptible host factor. The *TiCts1* and *TiChs1* showed transcripts up to 26-fold (4 days) and 20-fold (3 days) in the presence of susceptible host factor. Further, the *TiUbc4* and *TiUkc1* were found upregulated up to 20-fold and 7-fold at 8 days and 3 days post incubation. This study provided the insight on expression of putative pathogenicity-related genes in *T. indica* which will help in understanding the infection mechanism and basis for further functional genomics approach.

Keywords: *Tilletia indica*, *Triticum aestivum*, host factor, pathogenicity-related genes, qRT-PCR

Introduction

Wheat is one of the most important cereal crops of India and worldwide. In recent years, wheat grain quality has been seriously affected due to re-emergence of Karnal bunt disease in India (Gurjar et al. 2016). The disease was first reported from Karnal, Haryana, India (Mitra 1931) and since then, it has been occurring in all major wheat-growing states of north-western India, as well as in other parts of the world like Pakistan, Nepal, Afghanistan, Mexico, Syria, Brazil, USA, Iran and South Africa. Karnal bunt of wheat became an important disease after the introduction of dwarf wheat varieties in North-West

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India, where it was of minor importance earlier (Nagarajan et al. 1997). *T. indica* has become a major sanitary and phytosanitary issue in the wake of the recent Agreement of Agriculture stipulated by the World Trade Organization. Currently, 77 countries have restricted on importing wheat from areas where the disease occurs (Bonde et al. 2004). The wheat importing countries have imposed strict quarantine measures and insist on a zero tolerance limit (Singh and Gogoi 2011). The disease poses an economic threat to the wheat industry due to reduction in grain quality rather than yield (Tripathi et al. 2013). The *T. indica* fungus has a pathogenesis process which infects the host plant at boot stage (S2, Zadok's stage 49) by dikaryotization between compatible mating types. At the maturity of wheat grain, teliospores are liberated during harvesting/threshing to the soil surface or dispersed on or in the grain and the disease cycle begins again (Dhaliwal and Singh 1989). *Tilletia indica* pathogen is a soil, air and seed borne pathogenic fungus (Carris et al. 2006). Teliospores of *T. indica* survive in soil up to 5 years. The germination of teliospores is sensitive to temperature and light conditions. Fresh teliospores have a period of dormancy. Exposure of spores to a temperature of −196 °C in liquid nitrogen for 15 minutes also provides higher germination (Bansal et al. 1983). The pathogen is genetically highly diverse because of its genetic recombination or mating behaviour between two compatible allantoid sporidia just before infection (Aggarwal et al. 2010).

It is difficult to manage the disease effectively through cultural practices and fungicide application. The best approach to manage the disease is through resistant cultivars. Till now, wheat varieties with durable KB resistance have not been developed. Screening the resistant wheat varieties using many pathogenic isolates of *T. indica* is difficult; thereby identification of resistant and susceptible breeding lines is difficult. Recent studies have indicated that, host defense responsive genes, viz. puroindoline protein PINB (AT_KB2), β-1, 4-glucanase (AT_KB3), and chitinase (AT_KB7) were highly upregulated in the resistant cultivar (HD 29), whereas the down-regulation of these genes were observed in the susceptible (WL711) cultivar (Tripathi et al. 2013). Till now, genes involved in virulence of *T. indica* have not been studied but few putative pathogenesis-related genes have been characterized in other fungi mainly *Ustilago maydis* which is a model pathogenic fungus for host-pathogen interaction studies. Pathogenesis-related genes, viz. mating and pathogenic development (*Pmk1*, *Kss1*, *Kpp2*, *Ste20*); morphology, appressorium development and multi-stress tolerance (*Hog1*); protein folding and heat stress tolerance (*Hsp70*); cell separation (*Cts1*); virulence (*Hos2*); dimorphic behaviour sexual development (*Chs1*), siderophore biosynthesis and pathogenicity (*Sid1*), the mobilization of storage lipids in spores (*Ssp1*), filamentous growth morphogenesis (*Ubc4*) and pigment formation (*Ukc1*) were isolated and characterized in *Ustilago maydis*. *Tilletia indica* and *Ustilago maydis* are in the same fungal taxonomic group of Basidiomycota and smut and bunt fungi have many similarities. Response of putative pathogenesis genes in *T. indica* is much needed to understand the infection mechanism of Karnal bunt and for inform any further functional genomics approach. Therefore, we aimed to determine the expression pattern of putative pathogenesis-related genes in *T. indica* in the presence of host factors (WH 542) and HD 29) using qPCR assay.

Materials and Methods

Isolation of Tilletia indica *culture and plant material*

The *Tilletia indica* culture was isolated and established from the teliospore inoculum of infected Karnal bunt of wheat seed (KB-1) using the single spore technique described by Warham (1987). Further, monoteliosporic fungal culture was maintained in agar slants and incubated at 16 ± 2 °C temperature in biological oxygen demand (BOD) incubator under alternate light and dark conditions for further study. Two wheat cultivars, resistant (HD 29) and susceptible (WH 542) were collected from Division of Genetics, ICAR-Indian Agricultural Research Institute, New Delhi, India. Cultivars were grown in a growth chamber at 24 °C/18 °C (day/night cycle), 60% relative humidity and a photoperiod of 16 h (212 µmol m^{-2} s^{-1} photon flux density). Wheat plants were raised up to the boot leaf stage of wheat spike which is most congenial stage for *Tilletia indica* infection in wheat crop.

Host factors preparation

Acetone extracts were prepared from resistant (HD29) and susceptible (WH542) cultivars of wheat from tissues of boot leaf stage (Zadok's 49, S2). Spikes (50 g) were ground in liquid nitrogen to a fine powder using pestle and mortar. Ground plant tissues suspended in cold acetone, in the ratio of 1 g of sample in 10 ml of acetone. The suspension was then agitated in cold condition for 5 hours and filtered through muslin cloth to remove larger debris and stored at 4 °C in air tight bottles. Acetone was then evaporated at room temperature using rotary evaporator. Finally, the obtained dried material was resuspended in 1/10th of the volume of sterilized distilled water and filtered by 0.22 µ filter before adding onto the PDA agar plates (Rana et al. 2001).

Radial mycelial growth of T. indica, *minimal media and isolation of total RNA and cDNA*

For fungal mycelia growth, host factor of resistant and susceptible cultivars were spread on PDA plates. Then, mycelial bit (2 mm) of *T. indica* fungus was inoculated on the PDA plates, and then plates were kept in BOD incubator at 18 ± 1 °C to measure radial growth of fungus up to 30 days. Mycelial growth was measured after 0, 4, 8, 15, 20 and 30 days after incubation with two replications and statistically analysed using unpaired *t*-test.

For gene expression analysis, ten percent host factors (resistant and susceptible) were added in 100 ml minimal media in 250 ml conical flask. The *T. indica* mycelial bit were inoculated in supplemented minimal media (0.4 mM NaCl, 2.0 mM KH$_2$PO$_4$, 0.3 mM (NH$_4$)$_2$HPO$_4$, 0.6 mM CaCl$_2$, 0.6 mM MgSO$_4$, 3.6 mM FeCl$_3$, 0.2 mM thiaminehydro chloride, 0.1% (w/v) trypticase peptone, 1% (w/v) glucose, 5% (w/v) malt extract, 2 mM KCl, 1 mM H$_3$BO$_3$, 0.22 mM MnSO$_4$·H$_2$O, 0.08 mM ZnSO$_4$, 0.021 mM CuSO$_4$, pH 5.8) and then incubated at 18 ± 1 °C in BOD incubator under light and dark conditions up to 15 days. Then, fungus mat harvested at different time points, collected and filtered

through muslin cloth, followed by washing with sterilized distilled water and immediately stored in –80 °C freezer. The different time points were considered for total RNA isolation.

For total RNA isolation, about 0.2 g fungal tissue was crushed in liquid nitrogen without letting it thaw. The powdered material was transferred to a new tube; immediately 1 ml RiboZol™ reagent (Amresco, USA) was added and the mixture was vigorously shaken in order to homogenize the sample, and incubated for 10 min at room temperature. Two hundred microlitre of chloroform was added per ml of RiboZol™ reagent and the tube was vigorously vortexed for 30 sec and incubated at room temperature for 10 min. Following centrifugation (13,000 rpm; 15 min; 4 °C), the upper aqueous phase was transferred into a fresh RNase free tube. The RNA of the aqueous layer was precipitated by mixing with 0.7 volumes of isopropyl alcohol; the mixture was incubated for half an hour at 4 °C and centrifuged (12,000 rpm; 10 min; 4 °C). The supernatant was discarded and the RNA pellet was washed twice with 75% ethanol by dislodging the pellet from the surface of the tube with vigorous shaking followed by centrifugation at 10,000 rpm for 5 min at 4 °C. Finally, the RNA pellet was dried for 10 min and dissolved in an adequate volume of DEPC-treated water, before cDNA was synthesized. The cDNA was constructed as follows: 1 µg total RNA was used for cDNA synthesis. The reaction was performed using Verso cDNA synthesis kit (Thermo Fisher Scientific, USA): 20 µl reaction (5X cDNA synthesis buffer, 5 mM dNTPs mix, RNA primers (500 ng/µl anchored oligo dT; 400 ng/µl random hexamer), RT enhancer, reverse transcriptase, RNA) was performed using PCR cycling program at 42 °C for 30 min (cDNA synthesis), 95 °C for 2 min.

Table 1. Pathogenicity-related genes used for qPCR assay

Genes	Functions	References
MAP kinase *Pmk1*	Mating and pathogenic development	Mayorga and Gold 1999
MAP kinase *Kss1*	Mating and pathogenic development	Muller et al. 2003
MAP kinase *Hog1*	Pathogenicity and stress tolerance	Zheng et al. 2016
Hsp70	Heat stress tolerance	Holden et al. 1989
MAP kinase *Kpp2*	Mating and pathogenic development	Muller et al. 1999
Chitinase *Cts1*	Cell separation	Langner et al. 2015
Hos2	Virulence and mating-type genes	Elias-Villalobos et al. 2015
Chitin synthase *Chs1*	Mating, virulence and dimorphic	Xoconostle-Cazares et al. 1996
Prf1	Sexual development	Hartmann et al. 1999
Sid1	Siderophore biosynthesis	Mei et al. 1993
Dioxygenase *Ssp1*	Mobilization of storage lipids	Huber et al. 2002
MAP KKKK *Ste20*	Role in mating and pathogenicity	Smith et al. 2004
MAPKK kinase *Ubc4*	Filamentous growth	Andrews et al. 2000
Protein kinase *Ukc1*	Morphogenesis and pathogenicity	Durrenberger and Kronstad 1999

In silico *identification of putative pathogenicity-related genes*

The fourteen putative pathogenicity related genes in *T. indica* (Table 1) were identified from *Ustilago maydis* based on putative functions. These putative genes were explored by BLASTn search in the *Tilletia horrida* genome using the *U. maydis* genes as query using *in silico* approach. The nucleotide sequences were retrieved from NCBI Genbank and BLAST analysed with *Tilletia horrida* draft genome (Wang et al. 2015). The most homologous regions of genes were identified for real time-PCR primers and then primers (Table 2) were designed using IDT primer design tool. The designed primers were synthesized from GCC Biotech Pvt., India.

Semi quantitative RT-PCR and quantitative PCR

Putative pathogenicity-related genes (*TiPmk1, TiFus3/Kss1, TiHog1, TiHsp70, TiKpp2, TiCts1, TiHos2, TiChs1, TiPrf1, TiSid1, TiSsp1, TiSte20, TiUbc4* and *TiUkc1*) were amplified by using PCR in the presence of cDNA sythesized from total RNA extracted from

Table 2. Primers used in semi-quantitative and quantitative PCR analysis

Genes	Primers sequences
TiPmk1	F: 5'AACTTATGGAGACCGACATGC3' R: 5'CGTTGAGTAAGAGGTTTGAGGG3'
TiKss1	F: 5'ACAATTCAGAGCCCACAGG3' R: 5'CGATAATCTCTGCCAGGGAAG3'
TiHog1	F: 5'TCGTACTTTTGCCAGGTCAG3' R: 5'TCTGAAGATCTGCGACTTTGG3'
TiHsp70	F: 5'TCAATACCAAGAGTCAGCGG3' R: 5'GACTCGTATTCCCAAGGTTCAG3'
TiKpp2	F: 5'TCATGACCGAATACGTTGCC3' R: 5'ATCTCGACCAGAATGCAGC3'
TiCts1	F: 5'ACTCCCAGTCCACGTCTAG3' R: 5'ACATACTCTCCCCATTTCGC3'
TiHos2	F: 5'GACTGTCCCGTCTTTGATGG3' R: 5'AAAGCTTCCTGCACACCGT3'
TiChs1	F: 5'CAGAACTTCGAGTACAAGATCAGTAACA3' R: 5'TTCTCCCTTGAAGTAGGATGCA3'
TiPrf1	F: 5'CCATCAAGGACTCTTCCGC3' R: 5'TTCTTGATGTTGAGCTCCTCG3'
TiSid1	F: 5'GTAACCATGATCTTCCGAGCCA3' R: 5'CGGACGACGGAGTAGTTGGT3'
TiSsp1	F: 5'CAACCAGCACTCCTCTTATCTCGAT3' R: 5'CGGTTGAGGGTGGCATCAT3'
TiSte20	F: 5'AGTACCTCCACAATGAAGGCA3' R: 5'TCTCCTTTGGCCATCTCGAT3'
TiUbc4	F: 5'TTGCACAATGTTGGGATGCT3' R: 5'ATCGGCGCTGGATCGTTC3'
TiUkc1	F: 5'GTGGATGAAGCCGAGCTTGT3' R: 5'CGCTTCAGAAGAGCGAGATGTT3'

T. indica treated with host factors. The following program was used in PCR (BioRad system, 96-wells plates): initial denaturation at 95 °C for 10 min, 40 cycles of denaturation 95 °C for 30 sec, annealing 60 °C for 30 sec, extension at 72 °C for 30 sec and final extension at 72 °C for 2 min. *RPS17* was used as internal control which showed equal expression at all time points.

Pathogenicity genes were analysed for gene expression level at different time points with two biological replicates using two-step qPCR. The qPCR conditions were standardized for the ideal Tm using gradient PCR for each selected gene. The following cycles were used in the real time PCR thermal cycler: pre-incubation at 95 °C for 10 min, denaturation 95 °C for 15 sec, annealing and extension at 60 °C for 1 min, 40 cycles of amplification. The threshold (C_T) values were automatically calculated; the transcript levels were normalized against internal control *RPS17* gene expression in case of *T. indica* and the fold changes were calculated based on the non-treated control. The SYBR green was used as a detection dye. Each 20 µl reaction were mixed well and the real time-PCR analysis was carried out. After 40 cycles, the specificity of the amplifications was verified by melting from 60 to 95 °C, resulting in melting curves. Gene expression in *T. indica* were normalized by subtracting the mean reference gene i.e. *RPS17* C_T value from their C_T value (ΔC_T). The fold change values were calculated using the expression, where $\Delta\Delta C_T$ represents ΔC_T condition of target gene $-\Delta C_T$ control gene. The fold expression was calculated according to the $2-\Delta\Delta C_T$ method mentioned by Schmittgen and Livak (2008) and statistically analysed using unpaired *t*-test.

Results

Radial mycelial growth of Tilletia indica

Mycelia growth of *T. indica* (KB-1 isolate) media amended with host factors showed clear differentiation at 8 days after incubation (DAI) (Fig. 1) as compared to control. Radial growth was found significant (*p*-value < 0.002), i.e. 1.3 cm, 1.1 cm, 0.95 cm in SHF, RHF and control, respectively, at 8 DAI. Finally, radial mycelial growth was recorded significantly as 3.4 cm, 2.6 cm, 2.0 cm in SHF, RHF and control, respectively, after 30 DAI. Highest mycelial growth (3.4 cm) recorded in the susceptible host factor (WH542) (Fig. 2).

The most homologous regions of pathogenicity-related genes, viz. *TiPmk1, TiFus3/ Kss1, TiHog1, TiHsp70, TiKpp2, TiCts1, TiHos2, TiChs1, TiPrf1, TiSid1, TiSsp1, TiSte20, TiUbc4* and *TiUkc1* (Table 1) were identified based on *in silico* approach. Differential expression pattern of putative pathogenicity related genes (*TiPmk1, TiFus3/Kss1, TiHog1, TiHsp70, TiKpp2, TiCts1, TiHos2, TiChs1, TiPrf1, TiSid1, TiSsp1, TiSte20, TiUbc4* and *TiUkc1*) showed in the presence of host factor using PCR from synthesized cDNA. Comparatively, most of the genes in *T. indica* were highly expressed in response to susceptible factors (WH542) rather than resistant host factors (HD29) at different time points (0, 1, 3, 4, 8 and 15 days).
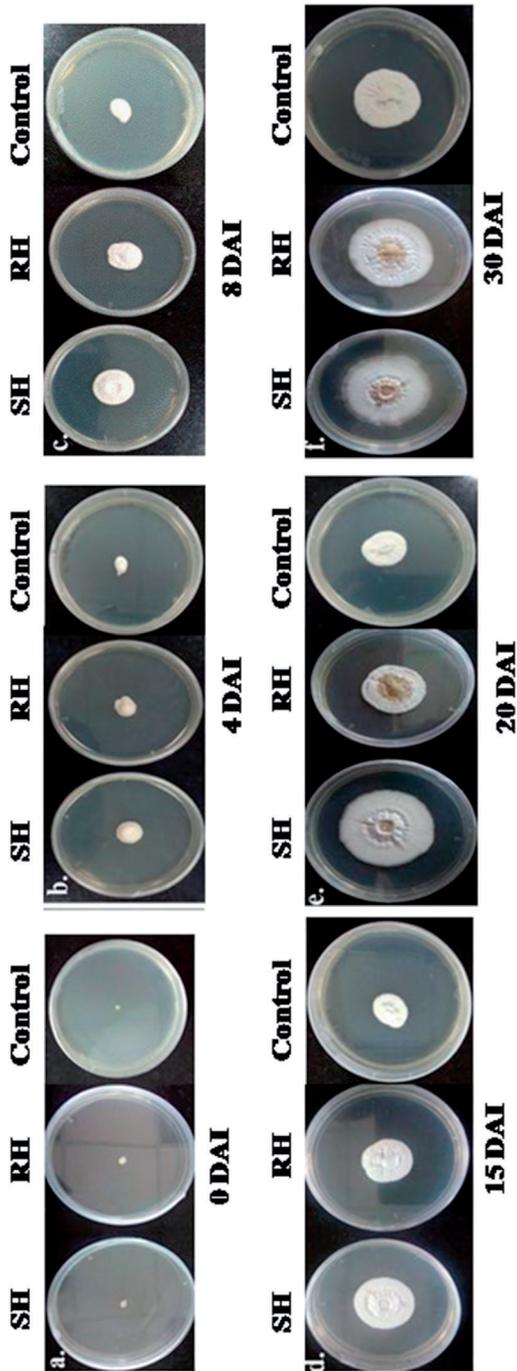
Figure 1. Response of host factors on *T. indica* growth. *T. indica* mycelial growth on agar media supplemented with boot stage extract of wheat at different time points as follows: a. 0 day b. 4th day c. 8th day d. 15th day e. 20th day f. 30th day. [SH – susceptible host factor (WH542); RH – resistant host factor (HD29); Control – without host factor]
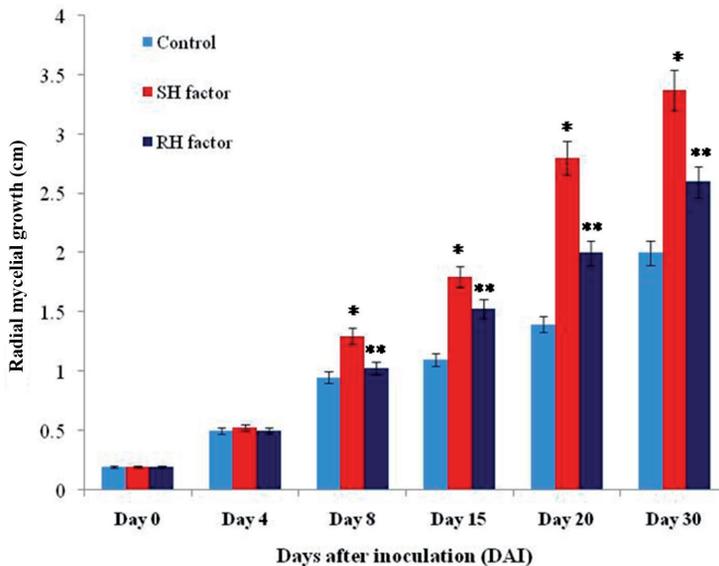
Figure 2. Graph showing radial growth of fungus and days after incubation. The data are shown as mean ± standard error. [SH – susceptible host factor (WH542); RH – resistant host factor (HD29); Control – without host factor; *significant in susceptible host factor; **significant in resistant host factor]

Relative expression analysis of MAP kinases, metabolism genes and other virulence genes

Different identified MAP kinase pathway genes (*TiPmk1*, *TiKss1*, *TiKpp2*, *TiSte20* and *TiHog1*) play different role in mating and pathogenic development, morphology, appressorium development and multi-stress tolerance in fungi. Relatively, *TiPmk1* gene have shown significantly up-regulation up to 26-fold in transcripts at 3 days in SHF as compared to RHF (13-folds). *TiPmk1, TiKss1, TiHog1, TiKpp2* and *TiSte20* genes were found significant up regulation in response to SHF (WH542) up to 26-fold (3 days), 18-fold (4 days), 20-fold (3 days), 9-fold (15 days) and 15-fold (3 days) respectively (Fig. 3A, Table S1*). In response to RHF, the relative expression of genes were also found upregulated up to 7.04-fold (8 days), 13.34-fold (15 days), 2.12-fold (8 days), 9.11-fold (8 days) and 9.52-fold (3 days), respectively. Chitin metabolism related function genes (*TiCts1* and *TiChs1*) were differentially expressed in presence of host factors. *TiCts1* and *TiChs1* were found upregulated up to 26-fold (4 days) and 20-fold (3 days) (Fig. 3A) in response to SHF. The relative expression of these genes was found upregulated up to 10-fold (8 days) and 3-fold (8 days), respectively, in response to RH factors. Comparatively, *TiCts1* and *TiChs1* genes were highly expressed in *T. indica* at 3 and 4 DAI in SHF.

Expression of the filamentous growth, morphogenesis and pigment formation genes in *T. indica,* i.e. *TiUbc4* and *TiUkc1,* respectively, were analysed in response to wheat host

*Further details about the Electronic Supplementary Material (ESM) can be found at the end of the article.
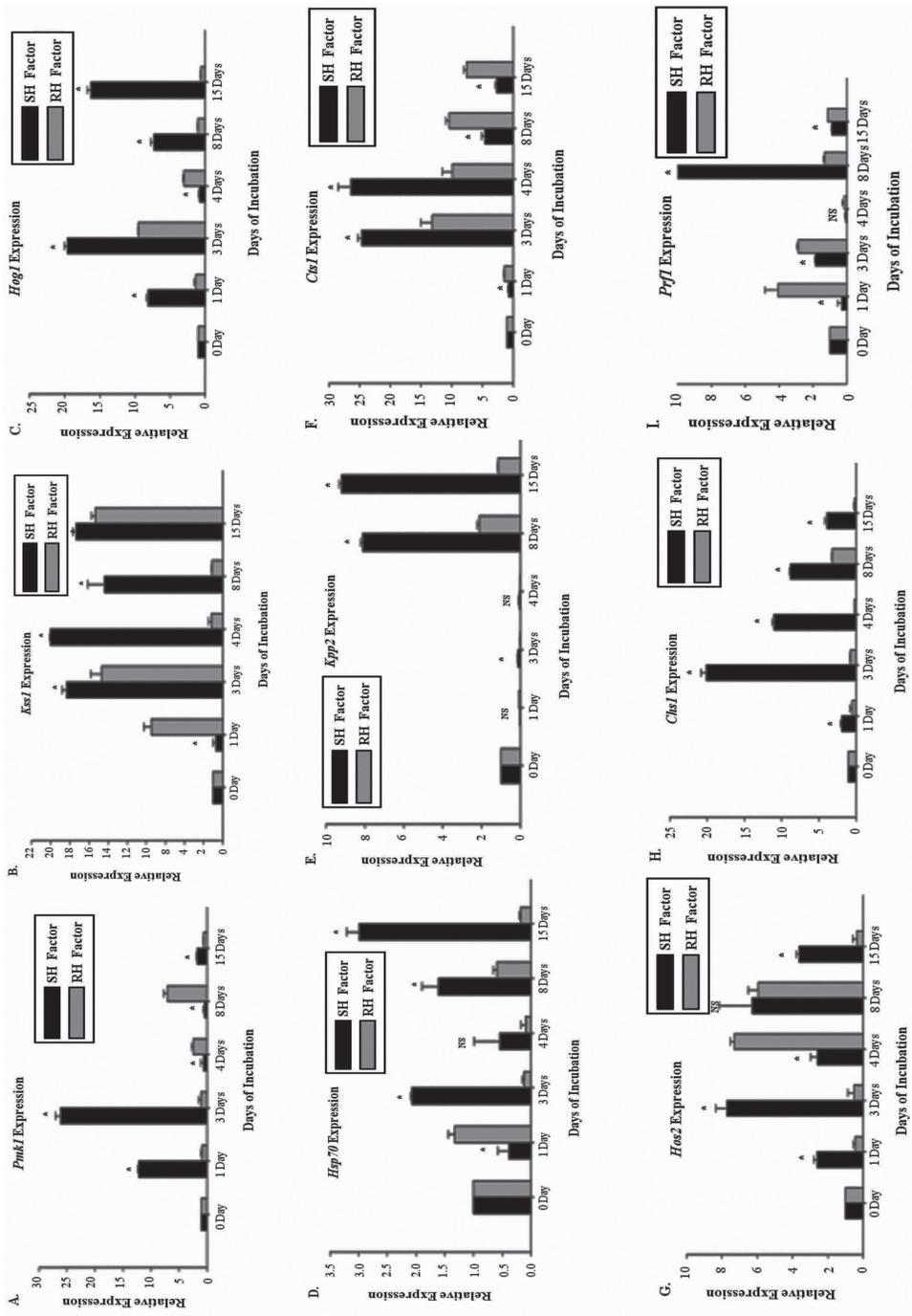
Figure 3A. Expression of putative pathogenicity-related genes, viz. *Pmk, Kss1, Hog1, Hsp70, Kpp2, Cts1, Hos2, Chs1, Prf1.*
NS – non-significant; * – significant in susceptible host factor
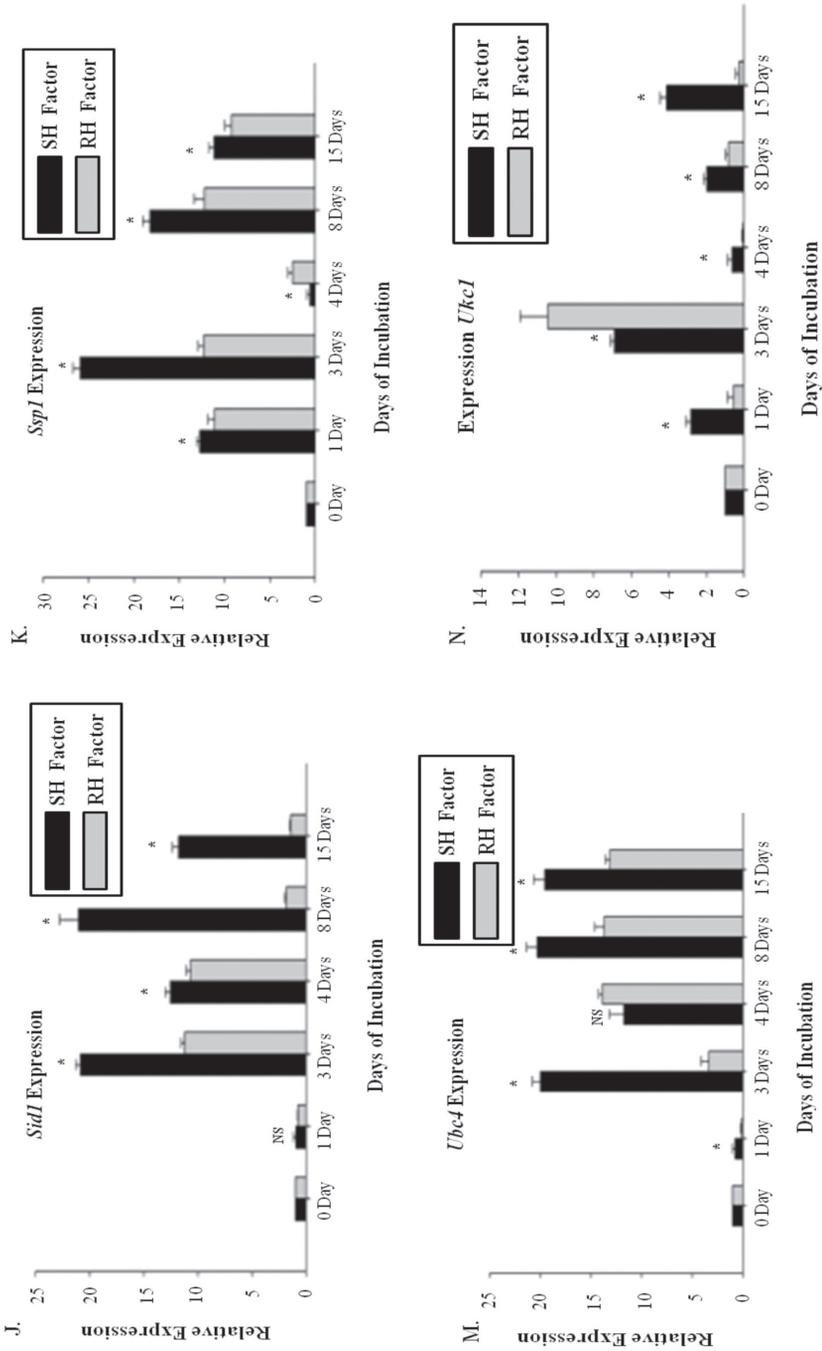
Figure 3B. Expression of putative pathogenicity-related genes, viz. *Ssp1, Sid1, Ube4, Ukc1*
NS – non-significant; * – significant in susceptible host factor

using qPCR assay. The *TiUbc4* and *TiUkc1* were found significantly upregulated up to 20-fold (8 days) and 7-fold (3 days) (Fig. 3B) in susceptible. Moreover, *TiUkc1* was found to be upregulated up to 10-fold (3 days) in RH factors in correspond to 7-fold (3 days) in SH factors. The genes with diverse functions related to pathogenicity such as virulence (*Prf1*, *Hos2*), siderophore biosynthesis and pathogenicity (*Sid1*), the mobilization of storage lipids in spores (*Ssp1*) and protein folding and heat stress tolerance (*Hsp70*) assessed for transcripts pattern in response to host factors. *TiHsp70*, *TiHos2*, *TiPrf1*, *TiSid1* and *Tissp1* were found upregulated up to 3-fold (15 days), 8-fold (3 days), 15-fold (8 days), 21-fold (8 days), 26-fold (3 days), respectively (Fig. 3A, B), in response to susceptible factor.

Discussion

Karnal bunt (*Tilletia indica*) is one of the major biotic stresses affecting wheat crop as it is re-emerging disease in the North-western plains zone of India. *T. indica* is a heterothallic fungus in which primary or secondary sporidia or hyphae of compatible mating types must fuse to form dikaryon, at the time of causing infection but the pathogenesis mechanism is poorly understood. Resistance genes have not been identified in wheat against Karnal bunt. Earlier, many studies have been done on pathogenic behaviour and molecular/genetic diversity in the pathogen but the molecular basis of host pathogen interaction (Aggarwal et al. 2010; Thirumalaisamy and Singh, 2012) and of pathogenesis has not been worked out. Host factors may play important role in enhancing the disease potential of *T. indica* which provides signal (s) during morphogenetic development of fungus (Gupta et al. 2012). Therefore, the expression of different pathogenesis-related genes in response to host factors at different time points *in vitro* using real time PCR assay was investigated.

In the present investigation, host factors extracted from susceptible and resistant wheat cultivars at boot stage (Zadok's 49, S2) were utilized to study their effect on mycelial growth of *T. indica*. The radial growth of *T. indica* recorded in host-factor amended medium showed significantly clear differentiation at 8 days after incubation (DAI). After 30 DAI, The highest radial growth (3.4 cm) was observed in the presence of susceptible host factor after. It indicated that host factors may influence Karnal bunt disease development at post-anthesis stage in susceptible genotypes of wheat. Relative expression analysis of eleven genes, viz. *TiPmk1* (mating and pathogenic development)*, TiKss1* (mating and pathogenic development), *TiHog1* (appressorium, pathogenicity and stress tolerance)*, TiHsp70* (protein folding and heat stress tolerence)*, TiCts1* (chitinase)*, TiHos2* (virulence through direct regulation of mating type gene)*, TiChs1* (mating, virulence and dimorphic behaviour)*, TiSid1* (siderophore biosynthesis and pathogenicity)*, TiSsp1* (mobilization of storage lipids in spores)*, TiSte20* (role in mating and pathogenicity) and *TiUbc4* (filamentous growth) were observed to be upregulated after 3 DAI whereas three genes, viz. *TiPrf1* (sexual development)*, TiKpp2* (mating and pathogenic development) and *TiUkc1* (morphogenesis, pathogenecity and pigment formation) were significantly differentially expressed between 1 to 8 days after inoculation in the presence of susceptible factor.

Further, these genes can be used for functional characterization and to identify exact role in virulence of pathogen.

Moreover, MAP kinase genes related to mating and pathogenic development, viz. *TiPmk1, TiKss1*, *TiHog1*, *TiKpp2* and *TiSte20* genes were upregulated in response to susceptible at different time points as compared to resistant factor. Previously, few pathogenicity genes have been identified and characterized in other fungi like *M. grisea Hog1* homolog *OSM1* which is essential for survival because it is required for tolerating desiccation, UV-light, and osmotic stresses (Dixon et al. 1999). *MlpHog1* gene may play a role in the infectious growth of *Melampsora larici-populina* and the response of the fungus to various environmental stresses (Yu et al. 2016).

Mitogen activated signal transduction pathway play a crucial role in development of virulence in pathogens. Through mitogen activated protein kinase (MAPK) pathways, pathogens respond to external stimuli and alter their own features such as cell wall integrity, mating, morphological transition, adaptation to stress factors and generate different virulence levels. In pathogens, the *Hog1*-type mitogen-activated protein kinase (MAPK) gene is involved in both pathogenesis and the response to various stresses, including osmotic stress. The most conserved function of the *Hog1* MAP kinase pathway in yeast and filamentous fungi described regulating to hyperosmotic stress (Turra et al. 2014). Teliospores of *T. indica* survive in soil up to 5 years which are exposed to stress conditions in terms of temperature and moisture. The survival of teliospores under stress conditions may be associated with presence of certain genes like *TiHog1* which have role in pathogenesis and withstanding osmotic stress. In present study also expression of *TiHog1* along with other genes were analysed at different time points induced by host factor. Presence of wheat host factors showed the high expression of MAP kinases, viz. *TiPmk1* and *TiKss1* in *T. indica* after 7 and 14 days (Gupta et al. 2012). *Pmk1* was found to be essential for appressorium formation and pathogenicity in various fungal pathogens, viz. *Ustilago maydis*, *M. Grisea* (Mey et al. 2002).

In present investigation, chitin metabolism genes like *TiChs1* (chitin synthase) and *TiCts1* (chitinase 1) were upregulated in presence of susceptible factor after 3 and 4 days of incubation. In the presence of susceptible host factor, the *TiCts1* and *TiChs1* were also highly expressed showing up to 26.46-fold and 20.14-fold at 4 ad 3 days, respectively. Chitin synthesis as well as breakdown is important for fungal growth and development. Similarly, *Chs1* and *Cts1* genes were found to play important role in pathogenicity of *Ustilago maydis* (Langner et al. 2015). In this study, *TiUbc4* and *TiUkc1* in *T. indica* were differentially expressed in response to host factor. The *TiUbc4* gene in *T. indica* was found to be upregulated in the presence of susceptible factor. It may play role in filamentation and high pigment formation during pathogenesis of *T. indica*. Earlier researchers reported that the *Ubc4* gene encodes a putative MAPKKK which functions upstream of *Kpp2* in the same MAP kinase pathway (Andrews et al. 2000). In present study, the genes with diverse functions like virulence (*Prf1*, *Hos2*), siderophore biosynthesis and pathogenicity (*Sid1*), the mobilization of storage lipids in spores (*Ssp1*) and protein folding and heat stress tolerance (*Hsp70*) were also studied. *TiHsp70* (heat shock protein) was down regulated in both host factors at 3 and 4 days of incubation. Moreover, the *TiSsp1*, *TiHos2*,

TiSid1 genes found to be highly upregulated except *TiPrf1* in susceptible host (WH 542) at 3 days. In our study in response to susceptible host factors, *TiPrf1* was found upregulated on 8th day of incubation which suggests that it may play role in pathogenic and mating development in *T. indica*. Previously, Muller et al. (1999) reported that the reduction in expression of the HMG box transcription factor *Prf1* in Δ*kpp2* mutants of *U. Maydis*.

The process of population shifts during developmental stage of *T. indica* accompanied the change in morphology and in turn appearance disappearance of virulence genes on fungal populations. In conclusion, most of the putative pathogenesis-related genes in *Tilletia indica* were highly expressed showing higher transcripts in susceptible host factor. Further, some genes, viz. *TiHog1*, *TiPmk1*, *TiKss1*, *TiCts1* and *TiUkc1* can be utilized to identify function in relation to pathogenesis and to understand infection mechanism of *T. indica*. In addition, it will provide basis for further functional genomics to unravelling infection mechanism of *Tilletia indica* fungus in context to host resistance of Karnal bunt disease.

Acknowledgements

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Electronic Supplementary Material (ESM)

Electronic Supplementary Material (ESM) associated with this article can be found at the website of CRC at http://www.akademiai.com/content/120427/

Electronic Supplementary *Table S1*. Fold changes of pathogenicity-related genes in *Tilletia indica*