Identification and Expression Analysis of Pathogenicity-related Genes in *Tilletia Indica* Inciting Karnal Bunt of Wheat

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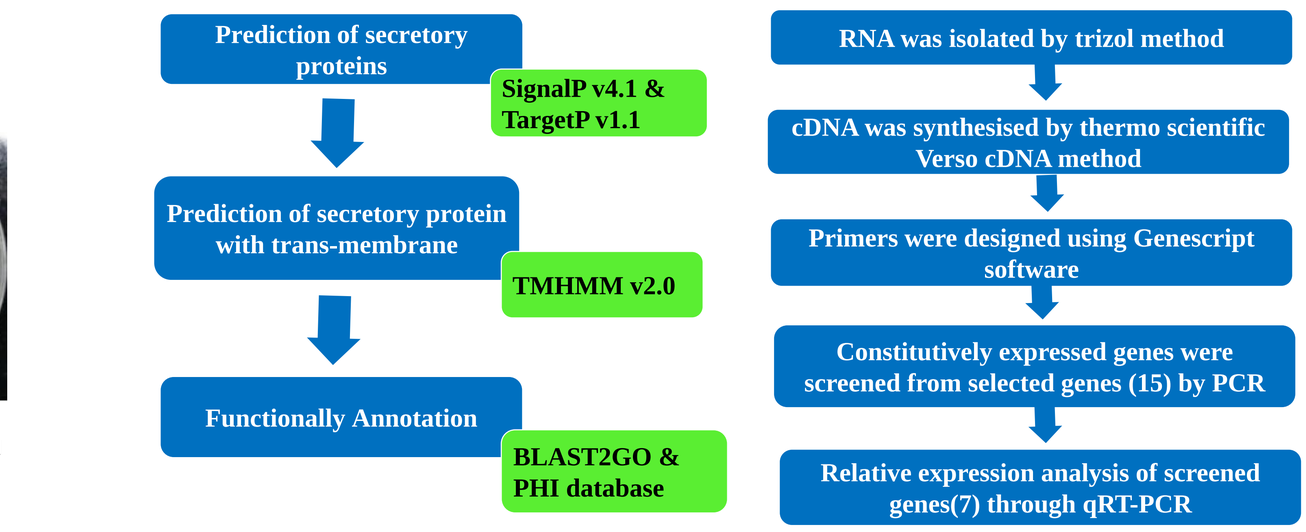
Introduction

- Wheat (*Triticum aestivum*) is the most prominently cereal crop grown worldwide. India is second largest producer in the world with the production of 98.38 million tons (Economic Survey of India, 2017).
- The prevalence of the Karnal bunt (KB) disease in Northern-western plain zone of India, is barrier on wheat export due to the Sanitary and Phytosanitary Agreement, such as restricted movement of consignment to other countries (FAO, 1996).
- Karnal bunt or partial bunt of wheat caused by the heterotallic fungus *Tilletia indica,* was first reported from Karnal (Haryana) by Mitra
- In modern biotic resistance breeding, effectors are efficiently used to identify, functionally characterize and deploy resistance genes.
- The present study was undertaken to understand mechanism(s) of pathogenesis required to manage this disease by analyzing whole genome sequence data generated earlier in Fungal Molecular Biology Lab (ID: RAKB_UP_1; Accession No. MBS00000000) IARI.
- Keeping this in view the studies were conducted to identify secretory genes related to pathogenicity by *in silico* tools and expression analysis of selected genes.

Karnal Bunt disease showed infected (a) wheat spike, (b) infected wheat grains, (c) mass of teliospores and (d) mycelial culture of *Tilletia indica*

Methodology

- Putative proteins from WGS of *Tilletia indica* were analyzed by SignalP v4.1 as well as TargetP v1.1 for prediction of secretory signal peptides and for the presence of trans-membrane domain using TMHMMv2.0.
- Predicted secretome was functionally annotated by using BLAST2GO and analysed by using PHI database.
- Primers was designed for selected (15) putative genes of *T. indica* using GeneScript software.
- Under *in vitro* conditions, KB1 isolates was cultured on minimal media amended with susceptible / resistant host factor.
- Under *in planta* conditions, susceptible and resistant genotype plants were grown and inoculated at pre-booting stage.
- RNA was isolated, cDNA was synthesized and relative gene expression analysis was done through qPCR under *in vitro* and *in planta* conditions.



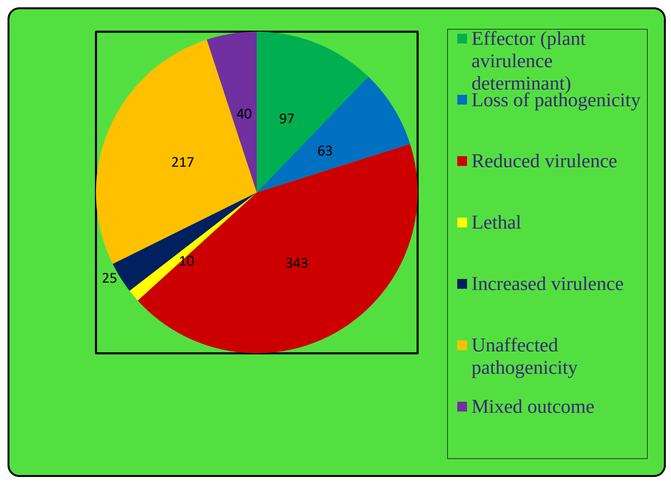
Results and Discussion

- In total, 1,337 unique proteins having secretory signatures were predicted using SignalP v4.1 as well as TargetP v1.1.
- PHI based analysis suggested that 97 genes were related to effector, 25 genes to increased virulence, 63 genes to loss of pathogenicity and 7 genes to resistance to chemicals.
- Expression studies conducted *in vitro* conditions using resistant/ susceptible host factor amendment showed not more than 3 folds increase in expression *of Ti 57, Ti 198, Ti 2035, Ti 2347, Ti 3774* genes but, 3 folds change was observed only in *Ti 12741* and *Ti 10340* at 24hrs post amendment with susceptible genotype host factor. However, the genes showing less expression under *in vitro* conditions at all the time points may be non-significant.
- Out of the 7 genes taken for the study under i*n planta* conditions, 3 genes (*Ti 2035, Ti 2347* and *Ti 3774*) showed maximum expression oat 3dpi in both the genotypes, further two genes (*Ti 57* and *Ti 198*) showed maximum expression at 3dpi followed by 10dpi and 15dpi respectively. These genes may have role in penetration, infection and in establishment of local systemic infection. Two genes (*Ti 10340* and *Ti 12741*) showed highest expression at later stages (i.e. 10dpi and 15dpi) only in susceptible genotype. So, these genes can have role in pathogen establishment and sporulation.

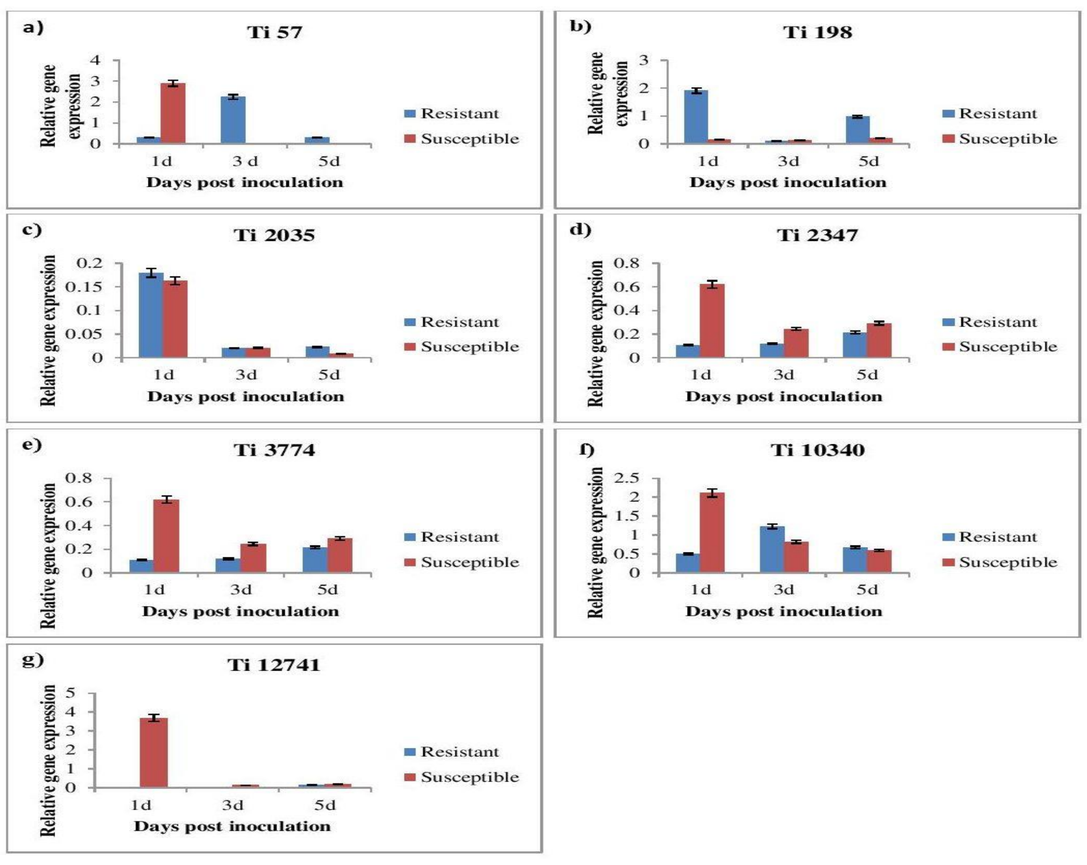
Secreted proteins identified in WGS of *Tilletia indica* using *in silico* tools

	Number of Protein
Total proteins	10113
SignalP Y	772
TargetP S	1307
Merged and Duplicate removed	1337
TmHmm 0	829
TmHmm 1* (TM overlapping with signal peptide <10 TM aa in mature peptide)	185
Highly probable GPI anchor containing sequences	34

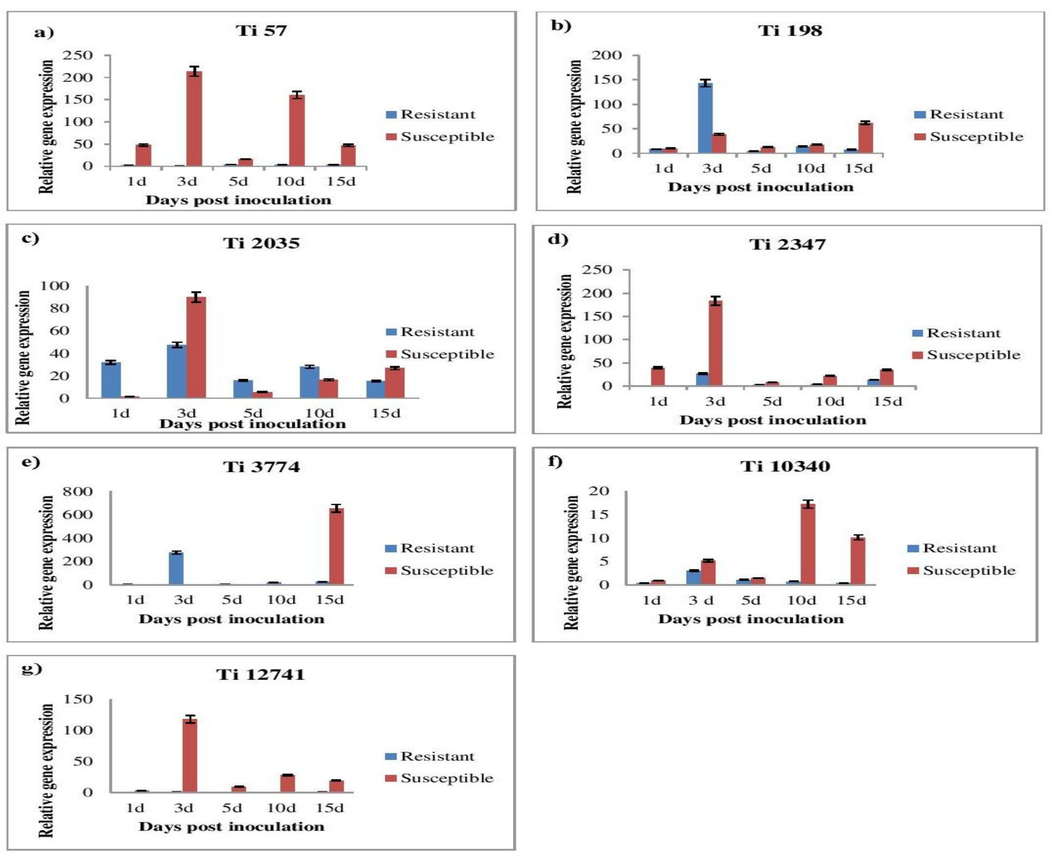
Functional annotation of predicted genes using PHI database



Expression pattern of gene in *T. indica* (KB-1) raised in PDA media amended with resistant (HD29) / susceptible (WH542) host factor using qPCR at different time periods



Expression patterns of genes in *T. indica* on inoculation of wheat resistant (HD29) and susceptible (WH542) genotypes with KB-1 isolate of *T. indica* by qPCR at different time periods



References

Gupta AK, Joshi GK, Seneviratne JM, Pandey D, Kumar A (2013) Cloning, *in silico* characterization and induction of TiKpp2 MAP kinase in *Tilletia indica* under the influence of host factor (s) from wheat spikes. Mol Biol Rep 40(8): 4967–4978.

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- All lab mates and supporting staff.

Conclusion

- The secretory proteins were identified in whole genome sequence of *Tilletia indica.*
- The genes related to infection, establishment and sporulation of *T. indica* were identified through relative expression analysis using qPCR.
- Functional analysis of these genes will help to better understand the pathogenesis mechanism of the *T. indica* and will help to develop novel strategies to manage Karnal bunt disease of wheat.