GRADUATE AND POSTDOCTORAL STUDIES

MCGILL UNIVERSITY

FINAL ORAL EXAMINATION
FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY

OF

SHIVAPPA HUKKERI
DEPARTMENT OF PLANT SCIENCE
Dissection and functional characterization of wheat QTL-Fhb5 based on forward and reverse genetics approach

December 12, 2016
1:15 pm
Raymond Building, ROOM R3-038
McGill University, Macdonald Campus

COMMITTEE:
Dr. Lyle Whyte - (Pro-Dean) (Natural Resource Sciences)
Dr. Danielle Donnelly - (Chair) (Plant Science)
Dr. Ajjamada Kushalappa - (Supervisor) (Plant Science)
Dr. Valerie Gravel - (Internal Examiner) (Plant Science)
Dr. Alan Watson - (Internal Member) (Plant Science)
Dr. Robin Beech - (External Member) (Parasitology)

Dr. Josephine Nalbantoglu, Dean of Graduate and Postdoctoral Studies
Members of the Faculty and Graduate Students are invited to attend
ABSTRACT

Fusarium Head Blight (FHB) is a devastating and dreadful disease of wheat (*Triticum aestivum* L), which not only reduces the yield but also, affects the grain quality by contaminating with health hazardous mycotoxins. Resistance to FHB in wheat is quantitative in nature, and have led to the identification of several quantitative trait loci (QTL), indicating the additive effects of several genes in governing the resistance. Though, more than hundred QTL are identified in wheat conferring FHB resistance, genetic controls underlying them are still unknown. The QTL-Fhb5 is one of the major FHB resistant QTL conferring high spikelet resistance and has been consistently mapped using different mapping populations in various environments. However, the gene(s) underlying QTL-Fhb5 conferring resistance and the resistance mechanisms are not elucidated. In our study, we made an attempt to dissect QTL-Fhb5 and functionally characterize using integrated metabolo-genomics approach to identify the putative candidate gene(s) and the plausible mechanisms of resistance. To further explore the candidate genes from QTL-Fhb5 the wheat near-isogenic lines (NILs) carrying resistant (R-NIL) and susceptible (S-NIL) alleles of QTL-Fhb5 derived from Sumai3 genetic background were subjected to semi-comprehensive metabolomic profiling. The metabolomic profiling of NILs identified several resistance related (RR) metabolites belonging to phenylpropanoid pathway in R-NIL as compared to S-NIL. Mapping of RR metabolites in metabolic pathways identified phenylalanine ammonia-lyase (*PAL*), chalcone synthase (*CHS*) and
agmatine p-coumaroyl transferase (ACT) as key metabolic pathway enzymes encoding genes. Further, upon dissection of QTL-Fhb5 using flanking markers, we identified an R2R3 MYB transcription factor, designated here as TaMybFhb5, GenBank ID: AHZ33834.1 is localized within the QTL locus. The transcriptional regulation of RR metabolite biosynthetic genes by TaMybFhb5 TF was confirmed through electrophoretic mobility shift assay (EMSA). The functional characterization of TaMybFhb5 gene through virus induced gene silencing (VIGS), not only reduced the RR metabolite abundances through downregulation of metabolic pathway genes expressions but also increased the fungal biomass accumulation and disease severity in silenced R-NIL as compared to non-silenced R-NIL. Further, the resistance functions of TaMybFhb5 gene was also validated by silencing in Sumai-3 (resistance source of QTL-Fhb5) based on VIGS. To identify the spikelet resistance genes and metabolites induced during F. graminearum (Fg) invasion we inoculated two wheat cultivars Sumai-3 (resistant) and Roblin (susceptible) with trichothecene producing wild-isolate (FgT) and non-producing mutant-isolate (Fgt) of Fusarium graminearum and subjected them to metabolome profiling and disease severity analysis. Interestingly, both the cultivars showed spikelet infection symptoms within 48 hours post inoculation (hpi) with FgT and Fgt, indicating both the isolates can cause infection. This clearly suggested that the trichothecenes are not essential to infect spikelets. However, the disease severity was higher in susceptible than in resistant cultivar, especially following FgT infection. In addition,
we observed a high accumulation of phenylpropanoids, lipids, fatty acids and flavonoids in spikelets of resistant than in susceptible cultivar. A semi-quantitative and real-time quantitative PCR revealed the differential accumulation of transcripts for selected biotic stress resistance R genes, in Sumai3 and Roblin spikelets infected with \( FgT \) and \( Fgt \). The fungal biomass and deoxynivalenol (DON) trichothecene accumulation in spikelets of Sumai3 were significantly lower than in Roblin. This is the first report on decoding the genetic controls underlying QTL-Fhb5 in wheat for FHB spikelet resistance. The \( TaMybFhb5 \) gene can be used for replacement in commercial elite wheat cultivars, based on genome editing to improve resistance against FHB.
CURRICULUM VITAE

UNIVERSITY EDUCATION
Since 2011  Doctor of Philosophy, Plant Science
2004-2006  Master of Science – Crop Physiology (University of Agricultural Sciences, GKVK, Bengaluru, India)
2000-2004  Bachelor of Science - Agriculture (University of Agricultural Sciences, Dharwad, India)

EMPLOYMENT
2006-2009  Research Associate – Molecular genetic markers development and validation in different agricultural crops (Avesthagen Pvt Ltd, Biotechnology company, India).
2009-2011  Research Associate – Development of single nucleotide polymorphic (SNP) markers in rice (DuPont-Pioneer Hi-Bred Seeds, DKC, Hyderabad, India)

AWARDS

- Graduate excellence Fellowship (GEF), Department of Plant Science, McGill University, Canada (2011-2013).
- Shasthri Indo Canadian Institution (SICI) Fellowship from SICI Institute, India (2011-2013).
- Ph.D. Scholarship from Réseau Innovagrains for extensive research on grains, Quebec, Canada (2013).
- Research assistantship for pursuing PhD, McGill University, Canada (2012-2016).
- University tuition support bursary, McGill University, Canada (2013-2016).
- GREAT (Graduate research enhancement and travel) travel award for attending Plant & Animal Genomics conference, Department of Plant Science, McGill University (2012).
PUBLICATIONS

- **Hukkeri S**, Shailesh Karre, Jean-Benoit Charron, McCartney, C and Ajjamada Kushalappa* (2016). A transcription factor TaMYBFhb5 in QTL-Fhb5 regulates downstream resistance genes to biosynthesize hydroxycinnamic acid amides and flavonoids conferring spikelet resistance against *Fusarium graminearum* (To be submitted).

- **Hukkeri S**, Udaykumar, and Ajjamada Kushalappa* (2016). The TaMYBFhb5 gene from wheat QTL-Fhb5 regulates downstream resistance related metabolite biosynthetic enzymes encoding genes in Sumai3 during *Fusarium graminearum* infection (Publication under preparation).


- Udaykumar Kage, **Shivappa Hukkeri**, Dhananjay Dhokane, Arun Kumar, Shailesh Karre, Ajjamada C Kushalappa (2016). Advances in genome editing with CRISPR/Cas9 system and their application in crop improvement (Under review).


Conference/Symposia publications:

- **Hukkeri, S**, Ajjamada Kushalappa, Jean-Benoit Charron and McCartney, C. “Type-I fusarium head blight resistance mechanism unraveled through


- Impa. S.M, Nadaradjan. S, Sheshshayee. M.S, **Shivappa. H**, and