Development of a real-time field pathogen monitoring system for devastating rice and wheat blast diseases

Rationale:

The intimate co-evolution between host and pathogen often results in the emergence and dominancy of virulent pathotypes in a short duration after the deployment of resistant varieties containing a single dominant resistance gene. The most recent appearance and spreading of wheat blast in Bangladesh and India has raised a huge socio-economical concern of this devastating fungal disease, caused by *Magnaporthe oryzae*, to the stable food production in Asia and beyond (News in focus, Nature, 2016). Most recently, Inoue et al. (Science, 2017) demonstrated that the evolution of the wheat blast fungus from *Lolium* isolates triggered by the functional losses in a host specificity determinant was mainly attributable to the emergence and epidemic of wheat blast in South America in 1980s and onwards. It was also found that wheat and rice blast pathotypes shared extremely high genome identity although they did not cross infect each other till now (Wang et al., unpublished). Distinct from South America, South Asia employs an intensive rice/wheat cropping system, which could make the blast disease management more complicated. **Thus, monitoring of rice and wheat blast pathotypes becomes urgent and vital for 1) the containment of wheat blast from further spreading, 2) the proper management of rice and wheat blast, and 3) the investigation of their potential host drift.**

Given the fact that fungal effectors are the main determinants of host specificities between different pathotypes and resistance spectrum of cognate resistance genes within same host species, we will focus on the effectome of both wheat and rice blast pathotpyes as targets for monitoring the virulence spectrum and dynamics of the pathogen population in the field. Due to the complexity of sequence diversity including single nucleotide polymorphisms (SNPs) and small insertion and deletion (InDel), we will employ the TruSeq Custom Amplicon (TSCA) platform recently developed in Illumina (https://www.illumina.com/content/dam/illumina-marketing/documents/products/datasheets/datasheet_truseq_custom_amplicon.pdf) for de novo sequencing the key effectome of both rice blast and wheat blast isolates in the field. Three groups of effectomes: 1) known avirulence (Avr) genes of rice pathotype, 2) highly polymorphic effectome of rice pathotype, 3) highly polymorphic effectome of wheat pathotype, will be selected for the sequencing. Given that most effector-coding genes are small in size, we will select the module of 2X300bp capable for covering both promoter and coding region of a single effector gene in the probe design. The sequences of effector genes in different samples will be analyzed and compared to determine the virulence spectrum and dynamics of the pathogen population in given areas, which can be used as guidance for the smart deployment of host resistance genes as well as proper disease management. Starting from as little as 50 ng of genomic DNA (3-4 lesions from a single leaf), this assay is amendable to quickly capture the virulence spectrum and dynamics of blast pathogen population derived from up to 96 field samples. Compared to the conventional pathotyping, which requires tedious single spore isolation and differential variety-based pathotyping, and field pathogenomics, which is relatively costly and less specific to interested effectome, this platform provides a real-time and specific effectome targeted diagnosis at a relatively high-throughput scale. The in-house facility of TSCA assay established at IRRI allows us to conduct the assay using the fresh samples from the field in Philippines. A rice blast hotspot developed in Bohol with the understanding of the structure of rice blast pathogen population

(Selisana et al., 2017) provides an ideal experimental site and dataset for validating the concept on the pathogen monitoring using the TSCA assay.

Implementation of the research plan in the pilot phase:

The pilot phase is consisted of the following activities with the estimated budget and timeline:

1) Identification of key effectome of both rice and wheat blast pathotypes

Effector genes including 1) 9 rice blast AVR genes including *AvrPik*, *AvrPii*, *AvrPia*, *AvrPiz-t*, *AvrPi9*, *AvrPib*, *Avr1-CO39*, *AvrPita*, and *AvrPi54*; 2) 200 rice blast non-Avr effector genes showing high polymorphism in different rice blast isolates; 3) 200 wheat blast non-Avr effector genes showing high polymorphism in different wheat blast isolates will be identified by bioinformatics analyses. Effector genes showing sequence variations including single nucleotide polymorphisms, InDels, and presence/absence among different isolates are considered as target genes for the probe synthesis. Rice and wheat blast housekeeping genes and non-blast genes used as controls are to be included in the probe design. This work will be done at Guo-Liang Wang's lab at The Ohio State University (OSU). 0.2 FTE for 2 months for a postdoc is needed.

2) Probe design and synthesis using TSCA platform

A total of 409 effector genes and controls will be design and synthesized using DesignStudio (Illumina). This work will be outsourced in Illumina and the cost will be determined as early as possible.

3) TSCA assay

In-house assay will be conducted using two sets of 96 samples. One set for rice blast will include 80 field samples collected in the rice blast hotspot in Bohol, Philippines by pooling 3-4 lesions in a same leaf and 16 standard genomic DNA samples of wheat and rice blast isolates. Another set for wheat blast will include 80 field samples of wheat blast infected panicles with the same 16 standard DNA samples. This assay will be conducted at IRRI. The disrupted wheat blast samples used for the wheat blast set will be sent through CIMMYT to IRRI. Standard DNAs of wheat and rice blast isolates will be received through MTA from Bangladesh and other countries facilitated through CIMMYT. 0.6 FTE for 6 months for an Assistant Scientist (AS) at IRRI is needed. 0.5 FTE for 1 month for an AS at CIMMYT is needed.

4) Data analysis and release

After base calling from TSCA assay, the sequences of 409 target effector genes from each sample will be analyzed. The haplotypes of individual effector genes in different samples and their frequency in the population will be calculated. The virulence spectrum and dynamics of population structure of the rice blast pathogen population in Bohol will be compared with the one determined previously. The data will be disclosed through open-accessible website operated by IRRI. This work will be conducted at IRRI and OSU. 0.1 FTE for one month for a postdoc at OSU and 0.5 FTE for 2 months for an AS at IRRI are needed.

Scale up after the pilot phase:

If the effector-based pathogen monitoring system is working for determining the virulence spectrum and diagnosis of different host pathotypes, we will scale up the assay system in different rice and wheat growing regions vulnerable to blast diseases mainly in South Asia, Southeast Asia, Africa, and South America through different networks, e.g., RICE and WHEAT CGIAR Research Programs, Africa rice blast networks, JIRCAS rice blast networks, TRRC rice blast networks, and Stress tolerant rice in South Asia and Africa (STRASA).