A real-time, mobile diagnostics platform for wheat yellow rust in Ethiopia

Wheat yellow rust, caused by *Puccinia striiformis* f. sp. *tritici* (PST), is currently considered the most damaging disease of wheat globally (yield losses >60%). Furthermore, in the last decade new PST races have emerged that are adapted to warmer temperatures, have expanded virulence profiles, and are more aggressive than previously characterized races leading to wide scale epidemics. Understanding the rapidly shifting nature of pathogen populations and dispersal in near real-time is critical if effective early warning and control is to be obtained. This requires new, innovative, data-driven, diagnostic tools.

Considerable progress has been made in establishing global monitoring systems and surveillance networks for wheat rusts (www.rusttracker.cimmyt.org), but pathogen diagnostics still rely on lengthy and costly controlled bioassays undertaken at a very limited number of specialized laboratories. Advances in sequencing technologies and bioinformatics pipelines have permitted the development of new in-field pathogenomics methods which have been tested and validated for PST by JIC and partners in the UK (*Genome Biology* 2015 **16**:23). If these new pathogen diagnostic methods, plus open access software and data management tools, could be made quicker, cheaper and readily deployed in developing countries it would revolutionize pathogen surveillance and reduce the risk of small-holders.

The idea:

Develop and pilot an affordable, mobile in-field pathogenomics platform to revolutionize crop pathogen surveillance and diagnostics in real time. First use of the MinION mobile genome sequencer platform for crop pathogen diagnostics using wheat yellow rust as a test case and deployment assessed *in situ* in Ethiopia.

Why is the idea an unconventional or creative approach to the problem outlined in the topic?

Currently, no developing country has the capacity to undertake real-time pathogen diagnostics on important crop diseases like wheat yellow rust. Yet developing countries bear the brunt of epidemics. Human health outbreaks such as Ebola and Zika have seen the pioneering use of new, real time genomic surveillance approaches based on mobile sequencing platforms in resource-limited environments (Nature 2016, 530, 228-232). This study would be the first to evaluate a mobile genomic sequencing platform (and associated open informatics) for an important crop pathogen. In addition, in-country capacity in advanced pathogenomics will be developed through training and knowledge exchange with a world leading plant science institute, the John Innes Centre, with recognized excellence in crop disease and computational biology.

Describe the hypothesis for the proposal and why it is expected to succeed.

Current diagnostics for PST are slow (typically months between sample collection and final race identification), costly and limited to a small number of samples analyzed by specialized labs. This contrasts with the rapid evolution and migration of new PST races. Rapid, informed decision making on optimal control strategies within a growing season are therefore limited. However, recent advances in genomic technologies, bioinformatics and genomic-driven crop pathogen surveillance strategies that have been developed by Dr Saunders's lab provide a new opportunity to generate high-resolution in-field diagnostics capability. Active collaboration with Dr Loman (Birmingham University) will leverage his expertise of deploying the MinION in developing countries in response to the Ebola and Zika crises.

Publication of the draft reference genome and annotation of PST isolates in open-access data repositories (PLoS One. 2011;6:e24230, Nature Commun. 2013;4:2673) enabled the development of genomic-based PST diagnostic tools. The field pathogenomics approach for PST, successfully developed by Dr Diane Saunders at the JIC, UK uses an RNA-seq based genotyping approach running on an Illumina HiSeq platform. A new, more cost-effective method has been developed at JIC, based on targeted amplicon re-sequencing of single nucleotide polymorphism (SNP) regions that can be used to differentiate the dominant genetic groups of PST identified worldwide. This foundation provides a new opportunity to test and transfer the amplicon re-sequencing technique to the MinION mobile genome sequencer (Oxford Nanopore) and establish the first mobile, real-time crop pathogen diagnostics platform. Established networks for rust sample collection exist in Ethiopia and a rust early warning unit has been developed by EIAR and CIMMYT. Diagnostic results will be integrated into existing rust early warning systems that use state of the art dispersal and epidemiological models; this will assist validation and enhance the predictive power of the models. The EIAR National Biotechnology Centre at Holeta, Ethiopia is well-equipped and recognized as a centre of excellence for molecular biology.

This proposal builds upon existing platforms, and brings together a strong group of partners, with extensive experience, proven track-records and complementary expertise. It addresses a clear and urgent problem, with application to other crop pathogens and other geographies if successful.

Implementation Plan

To ensure success, rigorous validation of the MinION approach is needed plus the development of in-country capacity. The implementation plan includes the placement of an Ethiopian scientist (EIAR) in the JIC, UK lab for a period of 6-8 months, followed by full deployment and testing of the methodology in Ethiopia. A pilot MinION mobile genome sequencer platform with an open source analytical toolkit will be tested in Ethiopia based on the following timeline:

-Collection of representative Ethiopian PST samples (months 1-2) [EIAR+CIMMYT]

-Training of EIAR scientist at JIC, DNA extraction + creation of sequencing libraries (months 3-4) [JIC,EIAR]

-Test sequencing on MinION – comparison and validation, including known control samples that have previously been subjected to Illumina sequencing (months 4-5) [JIC, EIAR]

-Data analysis + analytical toolkit training at JIC (months 6-8) [JIC, EIAR]

-Deploy and test MinION platform and analytics in Ethiopia on PST field samples, if successful, integrate into existing early warning systems (months 9-12) [EIAR, JIC, CIMMYT]

Data Generation and Integration:

All PST isolate sequences generated will be stored in an open access data repository. Validated MinION sequence reads on control PST isolates, compared and matched against existing Illumina data to calibrate and evaluate the accuracy of the mobile platform. PST field samples from Ethiopia assigned to genetic groups and cross checked against Illumina results at JIC. In-country PST genetic group identification based on alignment with the open access database. Confirmed PST isolate genetic group data uploaded and displayed on RustTracker. Spatial and temporal distributions of validated PST genetic groups integrated into dispersal models to improve understanding of disease outbreaks.

Next Steps:

Further validation on a larger sample of field PST isolates in Ethiopia. Future: Field pathogenomics, using an Illumina platform, has already been applied to other crop pathogens – wheat blast (Bangladesh) and wheat stem rust. If the MinION platform can be validated and optimized then expansion to other pathogens, other crops and deployment in more countries is feasible. This diagnostic tool could be used for an array of pathogens - a platform for new disease outbreaks as well as tracking existing diseases.

Budget: Total US$100,000; 1 year