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Poster Abstracts

Understanding Rust Pathogens

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Molecular characterization of *Pgt* races in Kenya

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Regular rust surveys, sampling, and race identification provide knowledge on the pathogen population dynamics and evolution. The samples provide resources to underpin genetic studies and breeding. The genetic diversity of *Pgt* isolates collected in 2011 from the four major wheat-growing areas of Kenya are being investigated at the BecA-ILRI Hub using 11 simple sequence repeat (SSR) markers. The broad objective is to molecularly characterize Kenyan *Pgt* races, including isolates of the Ug99 lineage in order to determine whether there is a single population or discrete sub-populations in the different regions. By providing more detailed information about the pathogen population in Kenya, this study will complement work conducted at the USDA-ARS CDL, St Paul, as part of the global survey undertaken by DRRW. The information generated will be useful to both DRRW and the KARI wheat breeding effort.

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Identification of strategies for wheat stripe rust pathogenicity by deep transcriptome sequencing

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Stripe rust is a major constraint to wheat production worldwide. The molecular events that underlie host colonisation by *Pst* are largely unknown. The fungus creates a specialized cellular structure within host cells called the haustorium, which allows it to obtain nutrients from wheat, and to secrete pathogenicity factors called effector proteins. No effector protein has been identified to date in *Pst*. We are using genomic, transcriptomic and proteomic approaches to: 1. Understand how the pathogen establishes a compatible interaction with its host, and 2. Uncover the effector proteins that are synthesised and secreted by *Pst* during infection. We used next-generation sequencing platforms to characterise the *Pst* genome, and to generate two contrasting transcriptomes (germinating spores and haustoria). So far we have found ~900 potential secreted protein genes in haustoria and these are being tested *in vivo* to identify those with effector activity. Digital gene expression analysis comparing spores and haustoria revealed that spores mainly deploy their energy reserves for growth and development, whereas haustoria extract host nutrients for further growth and use them in biosynthetic pathways for the ultimate production of urediniospores. Finally, we developed a method to isolate highly purified haustoria, and it is being tested for proteomics analysis. This technique will be a useful aid to further effector discovery and *Pst* genome annotation. Together, these studies will substantially increase our knowledge of stripe rust effectors and provide new insights into pathogenic strategies of this important organism.

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Transcriptome analysis of six *Puccinia triticina* races

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Wheat leaf rust can cause yield losses of up to 20% in our wheat producing regions. During infection, the fungus forms an extracellular feeding structure called the haustorium. Proteins secreted from the haustorim enter the plant cell and effect changes in plant transcription, metabolism and defense. Race structure in *P. triticina* is defined by infection type on wheat lines containing different resistance genes. In this experiment, RNA was extracted from sets of wheat leaves infected with six different rust races at six days post inoculation. Illumina Solexa sequencing reads were assembled using Inchworm. To separate sequences by species of origin, contigs were BLAST aligned to either a wheat EST database or a *P. triticina* reference genome sequence. A total of 222,571 rust contigs were assembled from 165 million reads, with an average contig length of 744 bases. Translated secreted protein sequences were examined for the presence of SNPs resulting in amino acid changes and temporal expression profiles were developed for the corresponding genes.

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Genotyping field isolates of *Puccinia graminis* from Kenya, Ethiopia and Eritrea

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The diversity of SSR (Simple Sequence Repeat) genotypes in *Pgt* populations from East Africa was determined for stem rust samples collected by collaborators in 2009 and 2010, and received under Canadian Food Inspection Agency import permits. Living rust samples were purified and characterized for avirulence/virulence spectra on sets of host differentials. Processing was conducted in a PPC3 laboratory to ensure containment. DNA extracts from selected purified spore samples (2009: 14 Kenya; 2010: 15 Kenya, 10 Ethiopia, 23 Eritrea), and from unprocessed field samples imported in 2007 under permit (12 Kenya, 4 Ethiopia), were characterized for 11 SSR loci. Results were compared to reference data for 8 North American (NA) races and an early Ug99 race TTKSK accession (04KEN156/4). Of 78 samples, 35 were identified to race with all except 3 identified as members of the Ug99 race group: 18 TTKST, 2 TTKSK, and 11 PTKST. Genotypic patterns for all foreign samples were distinct from NA isolates. Diversity was low, with most collections, including all Ug99 race group isolates, sharing the same genotype across all loci and matching the reference TTKSK accession. There were two other genotypes, corresponding to races RTRJP and TRTTF. These results confirm that SSR genotyping is a useful tool for initial screening of field samples from routine surveys and confirming identifications of stored reference collections. Isolates can then be further characterized on differential lines as needed, or used in diagnostic SNP-based molecular assays when available.

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Analysis of genetic diversity within the *Pgt* Ug99 race group using SNP based markers

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Since the discovery of Ug99 (race TTKSK), six additional variants have been found. Standard race typing of *Pgt* using wheat differential lines has provided essential phenotypic information, but little is known about the underlying genetic variation within this race group. DNA sequence data from nine isolates of *Pgt* was mined to identify SNPs that are polymorphic between members of the Ug99 race group. Thirty-four SNP markers were developed and used to analyze the genetic diversity of 106 *Pgt* isolates from the group. This collection of isolates represented six of the seven known members of the Ug99 race group: TTKSK, 42 isolates; TTKST, 19 isolates; TTTSK, 15 isolates; TTKSF, 19 isolates; TTKSP, 5 isolates; and PTKSP, 6 isolates. Nineteen different genotypes were observed and the number of genotypes per race phenotype ranged from 1 to 6 with, TTKSK containing the most genotypes. Principle coordinate analysis showed that three of the race phenotypes (TTKSF, TTKSK and TTTSK) each contained two distinctive genetic groups. In addition, genetic clusters contained multiple race phenotypes. This data suggests that races within the Ug99 race group have likely evolved multiple times from different genetic backgrounds. These results demonstrate the importance of genotypic data for monitoring long distance movement of Ug99 and related races.

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Development of a molecular assay system for rapid detection and identification of Ug99 and related *Pgt* races

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In the last decade the complexity and distribution of the *Pgt* Ug99 race group has dramatically expanded. In order to provide rapid tools, a two-stage molecular assay system was developed using real-time polymerase chain reaction methodologies and TaqMan probes. Stage-1 uses a suite of four assays to identify samples belonging to the Ug99 race group. An internal control, based on the actin gene, is included to control for false negatives. Only samples from the Ug99 race group are positive for all four assays. The stage-2 assay uses a suite of ten allele-specific SNP markers to predict the specific race phenotype of a sample within the Ug99 race group. A worldwide collection of over 400 samples was used to validate the assay system. The assay performed reliably with a range of samples including pure fungal tissue, infected plant tissue and ethanol-killed infected wheat stem tissue. This assay system provides a rapid and accurate method for diagnosis of the Ug99 race group and provides new tools for monitoring the movement Ug99 and related races.

Evaluation of a SNP-based qPCR identification system for the *Pgt* Ug99 race group using field stem rust samples collected in South Africa

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Annual surveys aim to monitor the distribution and composition of the *Pgt* population within South Africa. The number of isolates that are processed is limited by physical and financial constraints and the problem of non-viable spores. An accurate DNA-based identification method would increase and expedite the number of isolates that are screened. A two-stage SNP based qPCR identification system for individual members of the Ug99 race group was recently developed by the USDA-ARS Cereal Disease Laboratory. The first stage distinguishes between Ug99 lineage and non-Ug99 lineage isolates, whereas the second allows for the accurate identification of individual Ug99 races. Since four of the eight described Ug99 variants have been detected in Southern Africa, the efficiency of this identification system was evaluated using field samples of stem rust collected during recent surveys in South Africa. Using infected wheat stem tissue as DNA sources, randomly selected isolates were identified with qPCR and their identities compared to those determined by traditional race analysis. Preliminary results have indicated an excellent correlation between the two methods.

Identification of effectors in *Puccinia graminis* tritici (*Pst*)

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Obligate biotrophic fungi such as *Pst* secrete small effector molecules into host cells to suppress defense responses. To understand the basic features of effectors, we predicted potentially secreted proteins (SPs) without TM domains in the *Pst* genome and obtained 2100 candidate SPs. The sequences showed high diversity with a few members grouping into small families. There were no clearly conserved motifs that might function as uptake signals. Thus, it is not known whether fungal effectors use similar or different uptake mechanisms. SP genes exhibited different expression patterns in germ tubes, and in infected wheat and barberries. Most of them were significantly induced during *Pst*-wheat interaction, suggesting diverse roles, e.g. suppression of the host immune system, or the reprogramming of infected host cells. Three SP genes were specifically expressed in *Pst*-infected barberries suggesting they might have critical roles as specialized effectors in *Pst*-*Berberis* interaction. We also assayed their virulence or avirulence functions in tobacco leaves. Twelve of 198 SPs inhibited cell death triggered by BAX and permitted virulence functions in *Nicotiana benthamiana*, implying that these candidate effectors could suppress plant immunity responses. These potential effector proteins will be used to further decipher their functions in triggering or suppressing host resistance mechanisms.

Ultrastructural studies of the sexual stage of *Pst* by inoculation of barberry with germinating teliospores and wheat with aeciospores

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We performed electron microscope studies on *Pst* teliospore germination and infection of barberry with basidiospores to produce pycnia and aecia, and of aeciospore infection of wheat. The following results were noted:
(1) Teliospores germinated and produced basidiospores, which germinated on the upper leaf surface of barberry to produce germ tubes that formed appressoria and directly penetrated epidermal cells to develop monokaryotic intercellular hyphae. These produced papillae to perforate cell walls and form monokaryotic haustoria in barberry cells.
(2) Flask-shaped pycnia below the upper leaf epidermis, formed pycniophores and sterile paraphyses inside the pycnia. Single pycniophores produced successions of mononucleate, elliptical pycniospores with smooth surfaces. An obvious annular scar remained on pyciniophore after mature pycniospores had dropped off.
(3) The aecia are cylindrical or trumpet-shaped and produce numerous binucleate aeciospore chains inside. The mature aeciospore enclosed by internal (secondary) and external (primary) walls has electron-dense cytoplasm containing numerous lipid drops and glycogen granules. The surfaces of mature aeciospores are verrucose and covered with remanent material.
(4) Germinating aeciospores produce germ tubes and appressoria that penetrate wheat leaves through stomatal openings and form intercellular hyphae that develop to form haustorial mother cells and penetration pegs to invade the host cells. Uredinospores are produced from the sporogenous cells and become exposed on the wheat leaf surface.

Genetic variability within and among *formae speciales* and putative somatic hybrids of the stem rust pathogen

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Barley in Australia is affected by three forms of the stem rust pathogen *Puccinia graminis*; viz. the form virulent on wheat and triticale (*P. graminis* f. sp. *tritici, Pgt*), the form virulent on cereal rye (*P. graminis* f. sp. *secalis, Pgs*) and a variant considered to be a somatic hybrid between the two forms, locally referred to as 'scabrum' rust. Scabrum variants are avirulent on most wheat stem rust differential genotypes, including those lacking known genes for resistance to *Pgt*, and when tested on cereal rye and barley, most are avirulent on rye, but are virulent on certain barley genotypes. Sixty-six SSR makers were used to study genetic variability among isolates of *P. graminis*, including 14 scabrum isolates, nine *Pgt* isolates and two *Pgs* isolates. On the basis of results generated at 25 polymorphic loci, 12 genotypes of scabrum rust were identified, indicating that potentially 12 independent hybridization events had occurred in nature. Three scabrum isolates that generated identical fingerprint patterns were pathogenically distinct, providing evidence for the first time that scabrum variants evolve by acquiring new virulences. Whereas the majority of scabrum isolates identified to date are avirulent on commercial wheat cultivars, the results show that this pathogen has the potential to adapt to wheat. This warrants further research to assess the full implications that somatic hybridization may have on breeding for stem rust resistance in wheat.

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A draft genome of *Puccinia striifomis* f. sp. *triciti*

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Molecular deciphering of pathogenic variation in *Pst* will depend on knowledge of the genome. We generated an improved genomic draft of *Pst* race CY32 comprising 130.7 Mb of total scaffolds; 27,964 protein coding genes were predicted. Comparative genomic analysis showed 4,310 gene clusters shared by all three wheat rust fungi, and 7,353, 8,286, and 2,443 genes are *Pst*-, *Pgt*- and *Pt*-specific, respectively. The specific genes are mainly secreted protein genes (SP), reflecting the important role of SPs. Phylogenic relationships based on the whole genome show that the *Pst* genome is quite different from the other two pathogens, although *Pst* shares the same alternate host species with *Pgt* and possesses a similar genome size to *P. triticina*. This is the first molecular evolutionary snapshot of the three wheat rust fungi. Comparative secretome analysis indicated that the SP gene sets are more complex than those of other fungi. Limited similarity was found among rust pathogen SPs and those in other fungi, highlighting the diversity and lack of sequence similarity or conservation among fungal avirulence gene products. Genome sequencing of *Pst* offers a way to understand its survival mechanisms and avirulence/virulence evolution.

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Genetic differentiation at mitochondrial locus among *Zymoseptoria tritici* populations infecting bread wheat and durum wheat

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Zymoseptoria tritici (synonym *Septoria tritici*) is considered the most important foliar disease of both durum (*Triticum durum*) and bread wheat (*Triticum aestivum*) collected from Tunisian and European fields. The objective of this work was to investigate the host species pattern of adaptation of the fungus, on populations isolated from bread and durum wheat fields using 11 highly polymorphic nuclear microsatellites and one mitochondrial DNA (mtRFLP4) nucleotide sequence. Populations' subdivision was based on both wheat species and the presence/absence of the specific mtRFLP4 sequence. Gene flow estimates showed high migration levels between all populations suggesting low differentiation with an exception of less than one migrant per generation (Nm<1) observed between populations from bread wheat not carrying the specific mtRFLP4 sequence and all the others populations. Genetic differentiation was subsequently assessed using pairwise F_{ST} and *Jost*'s *D* estimates. The analyses indicate significant difference between bread wheat adapted population not carrying mtRFLP4 sequence and all others *Z. tritici* populations. Results suggest that wheat species specificity in *Z. tritici* might be altered by the specific mitochondrial *mtRFLP4* locus.

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