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EMS mutagenesis of avirulent *Puccinia graminis* f. sp. *tritici* urediniospores

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*Pgt* race TTKSK has been recognized as a major threat to the world’s wheat crop. The emergence of virulent races such as TTKSK warrants further understanding *Pgt* avirulence genes and their ability to mutate and overcome resistance in wheat varieties. *Pgt* mutants defective in avirulence activity will serve as valuable resources for cloning avirulence genes and understanding wheat resistance. In this study, we are attempting to isolate virulent mutants of North American *Pgt* races MCCFC and RKQQC that are normally avirulent to the wheat stem rust resistance genes *Sr21* and *Sr35*, respectively. To this end, we have screened different concentrations of EMS (Ethyl Methane Sulfonate) (0.005 M, 0.008 M, 0.010 M, 0.040 M and 0.080 M) to determine the effects on germinating urediniospore viability. Preliminary results indicate that EMS concentrations of 0.010 - 0.015 M confer 50% urediniospore viability. This concentration is being used to mutagenize race MCCFC on monogenic wheat line ‘T.monocderiv./8*LMPG (Sr21) and race RKQQC on line DV92 (Sr35). Virulent pustules from the EMS treatments are being isolated and inoculated to the stem rust differential set to distinguish between mutants and contaminants.
Analysis of simple sequence repeats dynamics in the genic regions of wheat rust fungi (Puccinia sp.)

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Simple sequence repeats (SSRs) or microsatellites are one of the valuable sources for genetic markers because of their abundance and inherent potential for determining extensive allelic variation in the genomes. In this study, we analyzed and compared the abundance and organization of SSRs in the genic region of three important fungal pathogens of wheat, brown or leaf rust (Puccinia triticina), black or stem rust (Puccinia graminis f. sp. tritici), yellow or stripe rust (Puccinia striiformis). The total number of SSR ranged from 4026 to 5844 representing 0.3% of genic region. The relative abundance and SSR density was highest in stem rust followed by stripe rust and leaf rust. The distribution pattern of different SSR motifs provides the evidence of greater accumulation of dinucleotide followed by trinucleotide in leaf and stripe rust, but the frequency of dinucleotide and trinucleotide repeats was same in stem rust. AG dinucleotide repeats are more frequent. Among trinucleotide repeats, ACC repeats are more frequent in leaf rust with a frequency of 12.51 SSR/Mb. AAC and ATG repeats are more frequent in stem rust and stripe rust, respectively with a frequency of 27.12 SSR/Mb and 17.99 SSR/Mb. The information about the frequency, relative abundance, relative density and variation in length of different SSR motifs in Puccinia sp. will be useful for developing markers that can be used for analysis of genetic diversity, population genetics, race identification and acquisition of new virulence.
Analysis of effector proteins from the flax rust and wheat stem rust pathogens

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Stem rust caused by Puccinia graminis tritici (Pgt) is one of the most serious diseases in wheat and is combated mainly through the use of resistant varieties. Because the fungus evolves virulence towards previously resistant varieties, continuous breeding and identification of new sources of resistance is necessary to combat the threat of epidemics. Our work on the flax rust model system has provided insights into how the plant immune system recognises and responds to rust pathogens. These obligate parasites produce specialised infection structures called haustoria that penetrate infected cells and are the main sites of nutrient extraction. A suite of disease effector proteins are secreted from haustoria into the host cells where they promote infection. However these effectors can also be recognised by host immune receptors, known as resistance (R) proteins. To find effectors from Pgt that are recognised by wheat R genes, we used genome and transcriptome sequencing to predict ~ 400 candidate effector genes from Australian Pgt race 21-0. To screen for R gene recognition, we developed a bacterial Type III Secretion System delivery assay using Pseudomonas fluorescens. We screened candidate effectors on a set of 18 wheat cultivars carrying 22 different R genes and identified one effector that induces a cell death response specifically on a wheat genotype carrying Sr22. We are also analyzing sequence variation in effector candidates between clonal field isolates that have mutated to overcome resistance genes deployed in agriculture.
Genome analyses of the wheat stripe (yellow) rust pathogen *Puccinia striiformis* f. sp. *tritici* reveal polymorphic and haustorial expressed secreted proteins as candidate effectors

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To design effective breeding strategies that maximize the potential for durable disease resistance it is important to understand the molecular basis of pathogenicity. In particular, characterization of the structure, function and evolutionary dynamics of secreted effector proteins that are detected by host immune receptors can help guide and prioritize breeding efforts. However, to date, our knowledge of the effector repertoire of cereal rust pathogens is limited. We re-sequenced four *Pst* isolate genomes to identify effector candidates and relate them to their distinct virulence profiles. We implemented a bioinformatics pipeline to integrate genomics, transcriptomics, and effector-focused annotations to identify and classify effector candidates in *PST*. RNAseq analysis highlighted transcripts encoding secreted proteins that were significantly enriched in haustoria compared to infected tissue. The expression of 22 candidate effector genes was characterized using qRT-PCR, revealing distinct temporal expression patterns during infection in wheat. Lastly, we identified proteins that displayed non-synonymous substitutions specifically between UK isolates PST-87/7 and PST-08/21, which differ in virulence to two wheat varieties. Integration of genomics, transcriptomics, and effector-directed annotation of *Pst* isolates has enabled us to move beyond the single isolate-directed catalogs of effector proteins and develop a framework for mining effector proteins in closely related isolates and relate these back to their defined virulence profiles. This should ultimately lead to a more comprehensive understanding of the *Pst* pathogenesis system, an important first step towards developing more effective surveillance and management strategies.
Next-generation sequencing to characterize wheat stripe rust races from western Canada


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Wheat stripe (yellow) rust (*Puccinia striiformis* f. sp. *tritici*) is a highly aggressive pathogen of wheat quickly evolving new races that overcome existing resistance worldwide. The genome size of this pathogen is ≈110 Mb. New and highly virulent races of stripe rust already occur worldwide for which very limited genetic resistance exist and are a continuing threat to global food production thus posing a serious threat to wheat production around the world. The goals of the project are to identify stripe rust genetic elements related to strain aggressiveness and tolerance to high temperature and strain-specific sequences to further facilitate their recognition. A challenging issue has been to obtain enough single pustule isolated spores (300 mg) from this biotrophic organism to isolate sufficient DNA for sequencing (1 µg). So far, we have obtained Illumina sequencing information for 8 strains isolated from southern Alberta. Sequences were de novo assembled using Velvet and Geneious software as well as using data from PST-78 as a reference assembled genome (Dr. Cuomo, Broad Institute). Initial mapping of two contrasting strains against PST-78 suggested significant differences between these lines in both the mapped and unmapped sequence complements. Sequence representativeness between the output results of the HiRes and MiSeq instruments will be discuss as the later one requires only 50 ng of DNA. Results of assembled sequences will be discussed in function of their isolation dates (1990’s vs. 2010’s) and annotation of group specific genes’ will be presented and discussed in term of temperature tolerance and aggressiveness.
Identification and characterization of microRNAs and their putative target genes in *Puccinia* spp.

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MicroRNAs, small, single-stranded, non-coding RNAs with 18 - 22 nucleotides, regulate gene expression post-transcriptionally. Although the role and targets of miRNA are extensively identified in diverse plants and animals, they remain largely uncharacterized in filamentous fungi; no miRNAs have been reported in rust fungi. Wheat is host to three different rust fungi, each of which is capable of causing serious losses to wheat production. In this study we identified and characterized miRNAs in the genomes of all three fungal pathogens using a computational pipeline. Contigs of genomic sequences of *Puccinia striiformis* were downloaded from the Broad Institute website (http://www.broadinstitute.org/). Most of the genes targeted by the predicted miRNAs were highly conserved across the three species, and were genes involved in transcription regulation, DNA binding, and ATP binding, but many were also hypothetical proteins. Our results provide new insights into regulatory and pathological functions of small RNAs and provide potential initiatives for study of plant pathogenic fungi. The study lays a foundation for understanding miRNA function in rust fungi and provides opportunities for exploiting RNA silencing in various applications, such as engineering plants resistant to fungal pathogens.
Characterization of seedling yellow rust resistance in wheat commercial cultivars, landraces and elite genotypes from Syria and Lebanon

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Multi-pathotype test on 87 elite line, 17 commercial cultivars, and 35 wheat landraces was carried out with 11 Pst pathotypes at INRA. Yr1, 3, 4, 6, 7, 9, 17, 25, and 27 were postulated singly either in landraces, cultivars or in elite lines and gene combination of Yr6+9, 6+17, 7+1, 7+4, 9+1, 9+3, 9+4 were only found in elite genotypes and the Yr6+7 was only postulated in the landraces. Yr27, 7, 6, 3, and 17 were the most common postulated genes in the cultivars, whereas the frequency of these genes were slightly different in elite lines. In landraces, Yr25, Yr3, and Yr9 were the most frequent postulated genes. Yr1 was only postulated in elite lines and Yr4 was postulated in landraces and elite lines. Since the frequency of virulence Yr1, Yr3, and Yr4 is low in the present Pst population in CWANA, utilization of these genes alone in large scale in farmer cultivars could result in increasing frequency of virulences and consequently devastating epidemics.