

Denmark's investment in the Global Rust Reference Center (GRRC)

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The Global Rust Reference Centre (GRRC, www.wheatrust.org) was established in 2008 upon the request of [CIMMYT](#) and ([ICARDA](#)) and extended in 2011 by the support of the Borlaug Global Rust Initiative. GRRC serve as a global hub for investigating wheat rust fungi and can receive alive samples from all countries year round. The activities of GRRC comprise pathotyping of wheat yellow rust and wheat stem rust, as well as training of students and scientists, data handling and storage (databases) and reporting. The current research activities have a focus on evolutionary population biology, as well as basic genetic and genomic studies in yellow rust. The “Wheat Rust Toolbox” and the team behind has become part of the GRRC and all data generated by GRRC will be stored in this system. Data management, research activities and dissemination will be coordinated and integrated with partner information platforms at CIMMYT, ICARDA, Cornell University and other global partners. The quarantine greenhouse space has in recent years been enlarged by more than 50% allowing GRRC to take in more rust samples and students. The GRRC activities expanded significantly in 2011 and 2013 via grants from the Danish Strategic Research Council and the Ministry of Food, Agriculture and Fisheries. One of these initiatives, RUSTFIGHT, has a focus on understanding “aggressiveness” and involves a number of Danish and international partners, including ICARDA and CIMMYT, INRA and the John Innes Centre (UK), and private Danish plant breeding Industry.

A comparison of stem rust in oats and stripe rust in wheat: A Swedish example

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A number of rusts affect grain crops in Sweden, but stem rust on oats and stripe (yellow) rust on wheat appear to create the greatest problems in production. The epidemiology of these diseases is intimately connected to the overall cropping patterns of these two crops. In Sweden, oats are only sown in the spring, thus forcing any overwintering pathogen to survive a Swedish winter. This is easiest for *Puccinia graminis* f. sp. *avenae*, which apparently completes its full, sexual life cycle on the abundant barberry plants. The presence of barberry and clear indications of sexual reproduction by *P. graminis* suggests that *Pgt* could be a problem on wheat, but there are only sporadic reports of stem rust on wheat. Wheat cultivars grown in Sweden possess few effective genes for resistance to stem rust, and the lack of rust is probably due to a lack of *Pgt* in the region. Given the resurgence of barberry in the landscape this implies that stem rust on wheat could be a major problem if (or when) the pathogen returns. *P. striiformis*, in contrast, can survive the Swedish winters on fall sown cereal crops, and thus it is the fittest clones that survive and dominate in the population. A large number of factors can affect this fitness, most markedly resistance genes in the cultivated wheat, but it is also possible that extended asexual reproduction can reduce the fitness of these persistent clones (Muller's ratchet) so that they can be displaced by fitter clones. Despite the widespread occurrence of barberry plants, we have not found any aecia of *P. striiformis*, although there does seem to be some genetic variation in the alternate host. Simple models that simulate the appearance and competition between different clonal lineages of the pathogen indicate that fitter individuals will eventually dominate the population, but their initial appearance will be difficult, since they are only detectable after enough generations have passed to increase the population size above a detectable level.

A consensus map for Ug99 stem rust resistance loci in wheat

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The global effort to identify new sources of resistance to wheat stem rust, caused by *Puccinia graminis* f. sp. *tritici* race group Ug99 has resulted in numerous studies reporting both qualitative genes and quantitative trait loci (QTL). The purpose of our study was to assemble all available information on loci associated with stem rust resistance from 21 recent studies on *Triticum aestivum* (bread wheat) and *Triticum turgidum* subsp. *durum* (durum wheat). The software LPmerge was used to construct a stem rust resistance loci consensus wheat map with 1433 markers incorporating Single Nucleotide Polymorphism (SNP), Diversity Arrays Technology (DArT), Genotyping-by-Sequencing (GBS) as well as Simple Sequence Repeat (SSR) marker information. Most of the markers associated with stem rust resistance have been identified in more than one population. Several loci identified in these populations map to the same regions with known *Sr* genes including *Sr2*, *SrND643*, *Sr25* and *Sr57* (*Lr34/Yr18/Pm38*), while other significant markers were located in chromosome regions where no *Sr* genes have been previously reported. This consensus map provides a comprehensive source of information on 141 stem rust resistance loci conferring resistance to stem rust Ug99 as well as linked markers for use in marker-assisted selection.

MAGIC: An innovative approach to dissecting the genetic control of complex traits in bread wheat

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The multiparent advanced generation intercross (MAGIC) is a mapping population created by several generations of intercrossing among multiple founder lines (1-3). The NIAB MAGIC population is based on eight commercially available U.K. winter wheat varieties chosen in consultation with U.K. wheat breeders to capture traits of importance among lines of use in contemporary breeding programs. The use of multiple founders allows generation of diversity above that available in bi-parental mapping populations. To increase opportunities for recombination (and precision in QTL location) and generation of novel haplotypes, 210 independent funnel crossing schemes were followed over three generations to establish the population. In 2012 a glasshouse seedling test was conducted on the NIAB MAGIC population to test response to a new *Pst* race that had overcome the resistance of U.K. commercial variety 'Warrior'. All of the MAGIC founder lines are susceptible to this race. Transgressive segregation for resistance occurred in the MAGIC lines, with no sporulation being observed on several lines. Reactions to natural infection by powdery mildew, Fusarium head blight and stripe (yellow) rust were also scored in 2012 field multiplication plots and heritable variation and transgressive segregation were seen for each disease. The detection of potentially useful levels of resistance illustrates the strength of MAGIC in capturing diversity. The population is available immediately for mapping resistance to a new race or isolate of any pathogen in elite germplasm whereas classical mapping approaches commonly only begin by way of bi-parental crosses after a new race has emerged. In this presentation, segregation for additional traits of interest will also be discussed, as well as the opportunities available for using the NIAB MAGIC population to test methods for genomic prediction.

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Antagonistic interactions among stripe rust and stem rust resistance QTLs in wheat

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Stripe (yellow) rust (YR) and stem rust (SR) in wheat are among the most destructive wheat diseases worldwide and they continue to cause significant yield losses in many areas. In this study, we carried out a genome-wide association study (GWAS) using 200 elite ICARDA wheat germplasm lines genotyped with Diversity Arrays Technology (DArT®) and the 9,000 SNPs Illumina iSelect SNP assay. QTL analysis revealed nine genomic regions located on chromosomes 1DS, 2AL, 2BS, 3AS, 3BS, 5BL, 6AL, 6DL, and 7DS significantly associated with YR resistance. Eight QTLs on chromosomes 2BS, 3BS, 3DL, 4BS, 5AL, 5BS, 7AL, and 7BL were associated with SR resistance. Epistatic interactions at $P \leq 10^{-5}$ were investigated using linear regression including Q + K matrices as covariates. Twenty nine pairs of interacting markers, representing six different interactions, were detected when considering the YR phenotype against 524 pairs for SR phenotype, representing 24 different interactions. A total of 443 (84.5%) of the SR interactions were from seven clustered DArT and SNP markers on chromosome 3DL with 259 markers distributed on eight different chromosomes, viz. 1A, 1B, 2B, 2D, 4A, 5B, 6A and 7B. Only the YR QTLs on 3AS, 2BS and 6AL exhibited interactions with other loci whereas all SR QTLs showed interactions except for the 3BS and 5AL QTLs. Two SR QTLs interacted with two YR QTLs and the SR/YR interacting pairs were 2BS/2BS, 3DL/2BS and 3DL/6AL. Of significance is that the presence of YR resistance alleles in these interacting QTL pairs reduced SR resistance by 12.7, 20.3 and 22.8%, respectively, relative to the corresponding SR susceptibility alleles. An understanding of these interactions will facilitate the design of effective breeding strategies that maximize the potential for achieving durable resistance to both diseases.

Characterization of two pleiotropic loci conferring adult plant resistance to stripe rust and leaf rust in Indian cv. Sujata

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Stripe (yellow) rust and leaf (brown) rust are major biotic production constraints worldwide. Stripe rust is currently receiving increased interest due to the detection of highly aggressive and more widely virulent races in areas where the disease was previously rarely detected. Resistant wheat varieties are a key method to control these rusts. The tall Indian bread wheat cv. Sujata is highly resistant to stripe rust in the field and displays intermediate infection types in seedling greenhouse tests. It also possesses adult plant resistance (APR) to leaf rust. The genetic basis of the resistance was investigated in 133 F₅ RILs derived from a cross with the susceptible line Avocet S. The population was phenotyped for reaction to stripe rust and leaf rust over two and three years, respectively, at three Mexican field sites. Pleiotropic slow-rusting APR loci were detected on 1AS and 7BL, and were designated as *QYL.cim-1AS* and *QYL.cim-7BL*, respectively. The phenotypic variances explained (PVEs) by *QYL.cim-1AS* were 10.5 - 13.8% and 7.9 - 8.2%, respectively, whereas they ranged from 16.6 - 20.4% and 5.7 - 13.0% for *QYL.cim-7BL* for stripe rust and leaf rust, respectively. *QYL.cim-7BL* is a seedling resistance gene designated as *YrSuj* and confers intermediate reactions to stripe rust at the seedling stage, whereas it confers moderate resistance to both rusts at the adult plant stage. *QYL.cim-1AS* and *QYL.cim-7BL*, in combination with APR genes *Lr46/Yr29* and *Lr67/Yr46*, also mapped in the population, conferred high levels of resistance to stripe rust and leaf rust at three test locations.

Evidence for recombination of *Sr2* and *Fhb1*

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Resistance from *Sr2* and *Fhb1* are considered important in the control of stem rust and Fusarium head blight but these genes on chromosome arm 3BS are known only to occur in repulsion. The objective of this study was to use a doubled haploid (DH) population of Carberry/AC Cadillac to seek a recombinant with the *Sr2* and *Fhb1* resistance alleles in coupling. Cultivar AC Cadillac is resistant to *Pgt* races TTKSK and TTKST. Carberry expresses a moderate level of resistance to FHB caused by *Fusarium* species. AC Cadillac has marker variants typically linked to the *Sr2* resistance allele, but does not have the marker variants that indicate the presence of the *Fhb1* resistance allele. Carberry does not have the marker variants associated with *Sr2*, but does have those associated with *Fhb1*. Both cultivars have other *Sr* and *Fhb* resistance genes. AC Cadillac expresses symptoms of pseudo black chaff (PBC) under some conditions but Carberry does not. The DH population was genotyped with 578 DArT®, 55 SSR, 2 BAC-derived, 2 CAPS, and 1 STS marker. The parents and 261 DH lines were evaluated for adult plant stem rust response (Ug99) at Njoro, Kenya, and at Swift Current, Canada (Canadian *Pgt* races). Response to FHB was evaluated in nurseries near Portage la Prairie, MB, Canada. PBC was scored in nurseries when symptoms were expressed. Using phenotypic and molecular marker data, and the very tight linkage or pleiotropic relationship of *Sr2* with PBC, DHs were classified into groupings of PBC and FHB. Several DH lines expressed FHB resistance superior to AC Cadillac, and expressed some PBC along with stem rust resistance. These DH lines had marker haplotypes that were consistent with the presence of *Sr2* and *Fhb1* resistance alleles in coupling.

Population divergence in the wheat leaf rust fungus *Puccinia triticina* is correlated with wheat evolution

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Co-evolution of fungal pathogens with their host species during the domestication of modern crop varieties has likely affected the current genetic divergence of pathogen populations. The objective of this study was to determine if the evolutionary history of the obligate rust pathogen on wheat, *Puccinia triticina*, is correlated with adaptation to hosts with different ploidy levels. Sequence data from 15 loci with different levels of polymorphism were generated. Phylogenetic analyses (parsimony, Bayesian, maximum likelihood) showed the clear initial divergence of *P. triticina* isolates collected from *Aegilops speltoides* (the likely B genome donor of modern wheat) in Israel from the other isolates that were collected from tetraploid (AB genomes) durum wheat and hexaploid (ABD genomes) common wheat. Coalescence based genealogy samplers also indicated that *P. triticina* on *A. speltoides*, diverged initially, followed by *P. triticina* isolates from durum wheat in Ethiopia and then by isolates from common wheat. Isolates of *P. triticina* found worldwide on cultivated durum wheat were the most recently coalesced and formed a clade nested within the isolates from common wheat. By a relative time scale, the divergence of *P. triticina* as delimited by host specificity appears very recent. Significant reciprocal gene flow between isolates from common wheat and isolates from durum wheat that are found worldwide was detected, in addition to gene flow from isolates on common wheat to isolates on durum wheat in Ethiopia.

Achieving sustainable leaf rust control in durum wheat: What have we learnt and how to move forward

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Durum wheat (*Triticum turgidum*) is an important crop in several developing countries, where varieties mostly derive from CIMMYT materials. Leaf rust poses a major threat to durum production. Within the last decade, new durum-specific virulent races rendered several cultivars susceptible in different parts of the world. To guarantee food security in these countries, incorporation of resistance to leaf rust in this crop is a high priority at CIMMYT. Previously, not much was known about the occurrence and nature of leaf rust resistance in durum, and extensive efforts were made in the last decade to increase knowledge and develop germplasm with enhanced resistance. Although new sources and diversity for resistance were identified in CIMMYT germplasm, three sources of race-specific resistance in Mexico lost their effectiveness in one decade due to new virulent races. To provide a more sustainable solution for leaf rust control, highly resistant durums should be developed based on pyramided slow rusting genes with minor, additive effects; an approach adopted for over 30 years in bread wheat. Characterization of diverse slow rusting sources and development of associated molecular markers will enhance genetic diversity for rust resistance and aid future breeding efforts.

Sources of stem rust resistance in cultivated and wild tetraploids

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Our research objective is to identify resistance genes in cultivated and wild tetraploids that are effective against Ug99 and other stem rust races that could be utilized in breeding. We characterized 4,500 durum and 360 emmer accessions for field resistance in Debre Zeit, Ethiopia, and Saint Paul, Minnesota. Accessions with resistant to moderately resistant responses in multiple field evaluations were characterized at the seedling stage for resistance to races TTKSK (Kenya), TRTTF (Yemen), TTTTF (USA), JRCQC (Ethiopia), and six representative U.S. races. We identified 168 durum and emmer entries resistant to moderately resistant in all field and seedling evaluations. These accessions likely possess useful genes for stem rust resistance in wheat improvement. Accessions susceptible at the seedling stage are evaluated for the presence of adult plant resistance genes. A search for resistance through seedling evaluations was also conducted on wild emmer (840 accessions) and four cultivated tetraploids (Persian, Polish, Oriental, and Pollard wheats, 560 accessions). About 20% of the accessions were resistant to race TTKSK. Wild emmer had the highest frequency of resistant responses and may have potentially new and diverse resistance genes. Inheritance studies of TTKSK resistance conducted on 40 resistant accessions of cultivated and wild tetraploids revealed that resistance to race TTKSK was conferred mostly by one or two genes. A bulked segregant analysis approach is being used to map resistance in selected lines using the 90K SNP platform. In addition to the AABB genome, we evaluated 1,220 accessions of six tetraploid *Aegilops* species for reaction to stem rust based on seedling tests. A high frequency of resistance was observed in these accessions. Populations are being developed in an attempt to determine the genetic control and mapping of stem rust resistance in these *Aegilops* spp.

New germplasm development using synthetic and other approaches to transfer stem rust resistance from tetraploids to hexaploids

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In the *Triticum* genus, tetraploid *T. turgidum* is a useful resource for germplasm improvement of hexaploid common wheat (*T. aestivum*). Several recent studies demonstrated that *Pgt* race TTKSK resistant genotypes were abundantly present among seven tetraploid subspecies (*T. turgidum* subsp. *carthlicum*, *dicoccum*, *dicoccoides*, *durum*, *polonicum*, *turgidum*, and *turanicum*). In an effort to improve common wheat for TTKSK resistance, we have been transferring stem rust resistance from tetraploid to hexaploid wheat through production of synthetic hexaploid wheat (SHW) or direct hexaploid × tetraploid hybridization followed by backcrossing. For production of SHW lines, we selected 181 unique tetraploid genotypes from the seven tetraploid subspecies for crosses with 14 accessions of *Aegilops tauschii* ($2n = 2x = 14$, DD) and developed 200 new SHW lines from these crosses. We are currently characterizing these lines for reaction to stem rust. So far, 80 SHW lines and their parents have been evaluated for reaction to races TTKSK, TRTTF, TTTTF and six other U.S. races and genotyped using molecular markers linked to known resistance genes previously identified in *T. turgidum* subsp. *dicoccum* and *Ae. tauschii*. The evaluation data showed that 42, 40, and 52 SHW were resistant to races TTKSK, TRTTF, and TTTTF respectively, with 21 lines being resistant to all three races. Based on marker analysis and race specificity, we postulated that a number of SHW lines have novel genes conferring resistance to TTKSK and other races. For gene introgression through direct hybridization, we have transferred *Sr47*, which was recently transferred from *Ae. speltoides* into durum through marker-assisted chromosome engineering, from durum into adapted hard red spring wheat germplasm. The new SHW lines and adapted germplasm carrying unique stem rust resistance genes from the tetraploids represent new sources of stem rust resistance for hexaploid wheat improvement.

Advances in breeding for resistance to stem rust caused by Ug99 and Ethiopian *Pgt* races in durum wheat

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Stem rust (SR) resistance is required for CIMMYT durum germplasm to keep relevance in Ethiopia, where Ug99 and other *Pgt* races are a major yield-limiting constraint, and in countries along the possible dissemination paths of these races. Resistance to Ug99 is widespread in most durum germplasm groups when tested in Kenya, but resistance is lost when exposed to Ethiopian races; hence selection at the Debre Zeit site in Ethiopia is essential for durum wheat. Due to difficulties with shuttling segregating populations between Mexico and Ethiopia, we have adopted a strategy involving the identification of resistant/moderately resistant lines at Debre-Zeit, and inter-crossing in Mexico followed by selection for resistance to leaf rust and agronomic type and finally screening for SR reaction in the resulting F₆ lines at Debre-Zeit at the same time as they are tested for yield and quality in preliminary yield trials in Mexico. This has generated a significant increase in the proportion of resistant and moderately resistant genotypes within outgoing CIMMYT germplasm, from less than 3% at the onset of the initiative in 2008 to 16% in 2011, and 38% in 2013. SR-resistant germplasm was characterized by similar frequency distributions to other traits in the overall germplasm such as yield potential, drought tolerance and industrial quality parameters. Advances have also been realized using marker-assisted selection (MAS) to introgress *Sr22* from bread wheat and to combine it with *Sr25*, producing advanced lines with 2-gene stacks with confirmed outstanding resistance and superior quality attributes. Since the two genes are closely linked but from different sources bringing them together required a very rare recombination event finally detected via MAS among thousands of plants. They are now essentially inherited together with a very low likelihood of generating recombinant individuals with either gene. The yield potential and stability of these lines are under evaluation in Ethiopia and the best lines are being used in a second round of breeding.

The global occurrence and economic consequences of stripe rust in wheat

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There is emerging evidence that the geographical footprint of stripe rust is expanding, opening up prospects for an increase in economic losses attributable to this disease worldwide. Drawing on newly compiled data, along with insights obtained from a survey initiated at the BGRI meeting in New Delhi in August 2013, this talk will report on efforts to model the global occurrence and persistence of stripe rust in a geo-spatially sensitive fashion. Using the available data in conjunction with these newly developed climate suitability maps, I will present probabilistic crop production losses associated with the disease and place an economic value on the prospective losses. Given changes in the geographical spread of this disease, and the associated uncertainties about its likely wheat yield and economic effects, various scenarios will be assessed to inform and thereby help shape the research investment decisions regarding crop breeding and other options for ameliorating these prospective losses over the longer term.

The worldwide *Pst* population structure and its temporal maintenance in Pakistan

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Inferences on worldwide population structure of *Puccinia striiformis* f. sp. *tritici* (*Pst*) were made through analyses of a set of 409 worldwide representative isolates from the core collections of INRA, France, and GRRC, Denmark. Bayesian and multivariate clustering methods partitioned the set of multilocus genotypes into six distinct genetic groups associated with their likely geographical origin. Analyses of linkage disequilibrium and genotypic diversity indicated a strong regional heterogeneity in levels of recombination, with clear signatures of recombination in the Himalayan (Nepal and Pakistan) and near-Himalayan (China) regions, and predominant clonal population structures in other regions. The ancestral relationship among geographically spaced populations assessed through the use of approximate Bayesian computation (ABC) analyses confirmed the Himalayan and near Himalayan populations to be the most ancestral to world populations. The existence of high genotypic diversity, recombinant population structure, high sexual reproduction ability, and abundance of alternate hosts (*Berberis* spp.) in the Himalayan and neighboring regions suggest the region as a plausible center of origin for *Pst*. A detailed analysis was carried out on a set of 684 isolates collected from 14 locations in Pakistan over three seasons to assess temporal maintenance of *Pst* in this presumed centre of origin. A highly genotypic diverse, recombinant population structure, and lack of differentiation between samples across two sampling years, and re-sampling of multilocus genotypes over-years demonstrated the contribution of both sexual recombination and off-season over-summer survival to the temporal maintenance of the pathogen. Fifty years ago, Norman E. Borlaug initiated the “green revolution”, putting strong emphasis on Pakistan wheat production. Our work on *Pst* population biology brings another focus to the Himalayan region, and particularly Pakistan, on the wheat stripe (yellow) rust pathogen and its worldwide population structure. Further studies on the role of alternate hosts in the epidemiology of stripe rust in the region should help better disease management.

Molecular characterization of *Pst* isolates from Western Canada

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The stripe (yellow) rust pathogen of wheat is evolving rapidly and generating new races that overcome host resistance genes. The genome size of this pathogen is ≈ 110 Mb. In contrast to isolates that predominated during 1960-2000 that optimally germinate at temperatures of 12-14°C, the recent post-2000 *Pst* isolates germinate optimally at higher temperatures of 16-18°C and have now become widespread. The main objective of this project is to identify genetic elements in *Pst* related to differences between the pre- and post-year 2000 isolates. The genomes of eleven isolates, that included both pre-2000 and post-2000 isolates, were sequenced using next-generation sequencing (MiSeq & HiSeq, Illumina) technology. The sequence reads were assembled both *de novo* and against the reference strain Pst78 isolated in year 2000 (Dr. Cuomo, Broad Institute, Cambridge, MA, USA). Assembly of three isolates sampled in the 1990s and three post-2010 isolates, yielded similar numbers of contigs ($\approx 16,700$) although more reads (61%) were assembled in the older isolates than in the new ones (48%). To distinguish among the *Pst* isolates, phylogenetic trees based on rRNA IGS and randomly selected sequences were obtained. Unique and enriched sequences to old (234 + 131) and new (88 + 211) isolates were identified using BLAST2GO. The unique and enriched genes from the pre-2000 isolates included numerous genes related to phosphorylation, cell signaling, transport, and DNA modification and those from the post-2000 isolates included numerous genes related to transport, response to exogenous molecules, RNA metabolism and modification of cell wall. Results will be presented in terms of evolutionary changes by focussing on genomic differences between the collected pre- and post-2000 isolates.

Field pathogenomics of wheat stripe (yellow) rust

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| Traditionally, the surveillance of emerging and re-emerging plant pathogens has hinged on field biology and virulence tests to provide phenotypic knowledge on pathogen diversity. However, with the cost of gene sequencing rapidly decreasing, now is the time to integrate genotypic data into pathogen surveillance activities. We call this method “field pathogenomics”. Focusing initially on stripe (yellow) rust, we collected nearly 200 infected wheat samples directly from the field in 2013, with 40 subjected to transcriptome sequencing. With up to 80% of the data of pathogen origin, comprehensive analysis of population dynamics at the field level is underway. The genotypic data have also provided information for selection of isolates for subsequent complementary labor-intensive phenotypic characterization. Integrating such high-throughput sequencing technology into the U.K. Cereal Pathogen Virulence Survey will ultimately provide a broader understanding of pathogen population dynamics thereby assisting informed decision-making regarding the best crop varieties to deploy in the field.

New evidence supporting the occurrence of sexual reproduction in the wheat stripe rust fungus on barberry spp. in China

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Wheat stripe rust is a destructive disease of wheat in China. Resistant cultivars are an effective and low-cost approach to control this disease. However, the resistance in wheat cultivars can often be overcome by new races, that later cause epidemics and consequent yield losses. The Chinese *Pst* population is reported to have higher genetic diversity than populations in other regions. This may be due to sexual reproduction, especially in northwestern regions. More than 20 barberry species (*Berberis* spp.) have been identified as possible alternate hosts of *Pst* in China. Field investigations demonstrated that aecial infections of *Berberis* spp. are quite common in the region. In 2011, we identified 4 *Pst* isolates from naturally rust-infected barberries that were different from known *Pst* races. Of 9,265 single aecia (or aecial-cup clusters) collected from diseased leaves of *Berberis* spp. in Gansu, Shaanxi and Tibet in 2013 and inoculated to susceptible wheat cultivar Mingxian 169, 16 *Pst* isolates were obtained from three *Berberis* spp. (8 from *B. aggregata*, 4 from *B. shensiana*, and 4 from *B. spp.*). On the 19 genotype Chinese host differential set we identified various groups of isolates: 4 isolates matched the current predominant race CYR32, 9 isolates were in a race group virulent on Suwon 11, and 3 were in a race group virulent on Hybrid 46. These results provide further evidence for sexual recombination in *Pst* on barberry in northwestern China. Winter survival of teliospores, production of basidiospores (following meiosis) and infection of barberry in spring, followed by the release of aeciospores that infect nearby wheat in spring/early summer completes a full macrocyclic cycle that is important in epidemiology and in the origin of new races.

An experimental genetic system using *Berberis vulgaris* confirms sexual recombination in *Puccinia striiformis*

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An effort to develop an experimental genetic system for the stripe (yellow) rust fungus using *Berberis vulgaris* as an alternate host has been made by INRA Grignon (F) and GRRC (DK). The first attempts to achieve infection using European isolates and *B. vulgaris* plants from France were unsuccessful in both laboratories despite high numbers of viable basidiospores and subsequent pycnial infections. Similar results occurred when plants of *B. chinensis* and *B. shensiensis* of North American origin were inoculated. Lack of host-pathogen compatibility was considered a possible explanation. In contrast, the use of *B. vulgaris* plants originating from nature reserves in Sweden and Denmark proved to be successful for infection and selfing a European *Pst* isolate in the Danish laboratory in 2013. The progeny isolates in the S₁ generation were genotyped with microsatellite markers to confirm parental origin and to study genotypic diversity. The markers confirmed the parental origin and markers that were heterozygous in the parent generally segregated in the S₁ progenies. A largest number of multilocus genotypes observed among the progeny isolates confirmed successful sexual recombination. Segregation for avirulence and virulence was investigated using 15 single R-gene wheat lines. The sexual structures and spore forms were documented by microscopic and macroscopic imaging at crucial time points during the life cycle of *Pst* on the alternate host.

Host induced gene silencing of wheat rust pathogen genes to identify processes essential to pathogenicity and targets for engineering resistance

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Expression of dsRNA fragments of rust pathogen genes in wheat seedlings through the barley stripe mosaic virus (BSMV) system can reduce the expression of the corresponding genes in the rust fungus. The highest levels of suppression have generally been observed in genes that are expressed mainly in haustorial cells. Comparative RNA sequencing was used to identify genes from *Puccinia graminis* f. sp. *tritici* (*Pgt*) with haustoria-specific expression. BSMV constructs with fragments of nearly 100 *Pgt* genes, or *Puccinia striiformis* f. sp. *tritici* (*Pst*) homologs, were tested for their ability to interfere with pathogenicity of these rust fungi. Partial suppression of most of the tested genes does not interfere with pathogenicity, but 10 genes appeared to show reduced pathogenicity in silencing assays. These included a gene encoding a predicted glycolytic enzyme, three proteins probably involved in carbohydrate or sugar metabolism, an ABC transporter-like protein, a protein involved in thiazol biosynthesis, and an amino acid permease. One of the most promising genes (designated *Pgt-IaaM*) encodes a putative tryptophan 2-monooxygenase, an enzyme that several plant pathogenic bacteria and a few fungi use to make the IAA precursor indole-3-acetamide (IAM). *Pgt* infection caused wheat to accumulate auxin in infected leaf tissues. In addition, expression of *Pgt-IaaM* in *Arabidopsis* caused a typical auxin overexpression phenotype and promoted susceptibility to the bacterial pathogen *Pseudomonas syringae* pv. *tomato* DC3000. The observation that transient silencing of this gene in infected wheat plants interfered with rust development indicates that stimulation of auxin biosynthesis is a process that is required for full pathogenicity. Transient silencing of some of the more conserved wheat rust pathogen genes reduced development of both *Pgt* and *Pst*, indicating that it may be possible to engineer resistance to multiple rusts with a single gene in transgenic wheat plants.

Understanding resistance gene mediated recognition of stem rust in wheat

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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 are necessary to combat the threat of rust epidemics. Our work on the flax rust model system has provided insights into how the plant immune system recognises and responds to rust pathogens. We have been extending this work to wheat stem rust by targeted cloning of resistance (*R*) genes in wheat and corresponding Avr genes in *Pgt*. Plant *R* genes encode immune receptors that recognise and respond to pathogen effector proteins delivered into host cells from haustoria. We recently isolated the *Sr33* and *Sr50* resistance genes from wheat and have begun functional analyses to determine how they trigger defense responses. We are also targeting 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 recognition of these proteins by wheat *R* genes, we developed a bacterial Type III Secretion System delivery assay using *Pseudomonas fluorescens* to inject the effector candidates into wheat leaf cells. We are screening candidate effectors on a set of 18 wheat cultivars carrying 22 different *R* genes and have so far identified one effector that induces a cell death response specifically on a wheat genotype carrying *Sr22*. Understanding the nature of wheat *R* genes and the Avr proteins that they recognize will allow better prediction of *R* gene durability and enable the possibility of rational design of novel *R* genes. We are also developing techniques for stacking *R* genes in cassettes for deployment of multiple genes at a single locus in wheat.

Global transcriptional profiling of *Brachypodium distachyon* during early stages of stem rust infection

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Brachypodium distachyon has been developed as an experimental model to study temperate cereals and grasses. The natural genetic variation of *B. distachyon* and its genotype-dependent resistance against pathogenic rust fungi support the potential of this species to provide durable disease resistance to important crops. *B. distachyon* is considered a non-host to the causal agent of stem rust in wheat and barley, *P. graminis* f. sp. *tritici* (*Pg-tr*), and a pseudo-host to *P. graminis* f. sp. *lolii* (*Pg-lo*) and *P. graminis* f. sp. *phlei-pratensis* (*Pg-pp*), both pathogens of forage grasses. Comparison of disease severities in a collection of *B. distachyon* inbred lines inoculated with *Pg-tr*, *Pg-lo* and *Pg-pp* allowed us to select line Bd1-1 to investigate the mechanisms that mediate pathogen recognition and defense to these rust fungi. Histological studies suggest that Bd1-1 exhibits pre-haustorial resistance to *Pg-tr* and post-haustorial resistance to *Pg-lo* and *Pg-pp*. Thus, a comparative RNA-Seq analysis of the early responses of Bd1-1 to *Pg-lo*, *Pg-pp* and *Pg-tr* was conducted. Gene expression profiles were determined for two time points to report the plant responses to distinctive stages of infection, appressorium formation (12 hours post-inoculation (hpi)), and fungal penetration (18 hpi). Here, we describe some of the specific and common molecular and cellular activities that occurred during interactions between Bd1-1 and *Pg-tr*, *Pg-pp* or *Pg-lo*. In combination all three different fungal inoculations at both time points, induced differential expression in a total of 603 genes, including 47 non-annotated genes and 49 genes predicted to encode receptor-like proteins. In addition, the data suggest the common occurrence of cell wall reinforcement and production of reactive oxygen species.

Non-host resistance, near-host resistance, or just plain host resistance? Genetic analysis of resistance to different *formae speciales* of *Puccinia graminis* and *P. striiformis* in cereals

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Non-host resistance (NHR) that is regarded as being non-race-specific and hence durable has been proposed as an alternative approach for disease resistance breeding. Previous research has indicated that the distinction between host resistance and NHR is not clear-cut, and may form part of a continuum. The research outlined in this paper is aimed at understanding the genetic basis of resistance to the wheat stem rust pathogen *Puccinia graminis* f. sp. *tritici* (*Pgt*) in oats, the oat stem rust pathogen *Puccinia graminis* f. sp. *avenae* (*Pga*) in barley and wheat, and to the wheat stripe rust pathogen *Puccinia striiformis* f. sp. *tritici* (*Pst*) in barley. In a Doubled Haploid (DH) population derived from Australian barley cultivars Yerong and Franklin, the observed segregation of rust response (5:3, R:S) suggested the presence of two complementary genes for resistance in Yerong and a single independently assorting gene in Franklin. Overlapping QTLs were detected from independent tests with three diverse *Pga* pathotypes, and further comparative mapping demonstrated co-location to previously identified NHR genes conferring adult plant resistance to powdery mildew and to cloned genes such as *Mla* and *Ror1* located on chromosome 1H. Histological analysis at 2, 5, and 10 days post infection determined that the resistance contributed by Yerong was pre-haustorial. Patterns of fluorescence in mesophyll cells demonstrated increased callose deposition and possible papillae formation in resistant parental and DH lines. Other work presented will include the identification of resistance to *Pst* in barley, and to *Pgt* using association mapping in a diverse international oat collection. The results suggest that while resistance in these non-adapted rust pathogen: host interactions is not pathotype specific and often polygenic, it appears mainly to be under major gene control typical of adapted pathogen: host interactions. Both current and future work is focused on fine mapping and cloning resistance genes in barley effective against different *formae speciales* of *P. graminis*.

Alterations in host transcriptional activity to rust pathogens

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The presence of plant pathogens alters the host transcriptome throughout the infection process. Differential expression patterns can be the result of pathogen sensing and subsequent host defense responses or pathogen mediated changes in host transcription to aid the infection process. Rust pathogens are obligate biotrophs which must infect, obtain nutrients, and proliferate in host tissues while evading or tolerating host defenses. To gain an understanding of host-rust pathogen interactions, we examined host differential expression in barley and soybean with and without rust infection. The barley line Morex was inoculated with *Pgt* race TTKSK (Ug99) and soybean line Williams 82 was inoculated with a field collection of *Phakopsora pachyrhizi* collected from kudzu in northern Florida. For each system a mock inoculation of only the spore carrier was performed. Total RNA was extracted from 6-10 bulked plants at 6, 12, 24, and 72 hours post inoculation (hpi). Two biological replicates were used per time point. RNA-seq was performed using the Illumina HiSeq platform at 20 million 100 bp paired-end reads per sample. Transcriptomes were assembled through genome-guided assembly using the Galaxy program. Peak differential expression was observed in Morex at 12 and 72 hpi with a pronounced reduction in differential expression occurring at 24 hpi and no differential expression at 6 hpi. Peak differential expression occurred in Williams 82 at 6, 12, and 72 hpi; a pronounced reduction in differential expression was also observed at 24 hpi. Significant proportions of differentially expressed genes in both systems were non-annotated and are currently undergoing annotation. At present another host-pathogen system is being added to the analysis. The maize line B73 was infected with an isolate of *P. sorghi* collected from a field at St. Paul, MN. Results from this study not only illuminate our understanding of host-rust pathogen interactions, but also provide candidate host (susceptibility) genes that are necessary for successful rust colonization. Manipulation of these ‘susceptibility’ genes may lead to novel and broad-spectrum rust resistance.

Global *Pgt* Initiative: An international genetic resource to combat stem rust

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An important component of the management of wheat stem rust is an understanding of the population diversity of the pathogen, *Puccinia graminis* f. sp. *tritici* (*Pgt*). The discovery of “Ug99” resulted in renewed efforts on pathogen surveys, sample collections and pathotyping of *Pgt*, with a primary focus on Africa. In the last few years these efforts have been expanded to include other targeted regions, however a global effort is needed. The aims of the “Global *Pgt* Initiative” is: to capture and maintain living cultures that collectively reflect the entire global diversity of *Pgt* in the years 2014 - 2016; pathotype and genotype this collection; develop DNA-based diagnostic tools that will be able to rapidly detect shifts in *Pgt* populations, and provide an early warning system of the vulnerability of wheat to new virulent strains; and provide a genetic baseline for comparison of *Pgt* populations over time, both forward and backwards. This initiative will provide the wheat rust community with a geographically distributed, well characterized, living culture collection that represents the global diversity of *Pgt*; a global open access knowledge bank on *Pgt* pathotypes and genotypes; and advanced molecular diagnostic tools for rapid detection and tracking of *Pgt* populations. The Global *Pgt* Initiative represents the most comprehensive effort to capture and characterize the global diversity of *Pgt* and provide a unique resource to the global wheat rust community.

Cultivating Success in Ethiopia: The contrasting stripe rust situations in 2010 and 2013

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In 2010, Ethiopia experienced one of the largest stripe rust epidemics in recent history. Over 600,000 ha of wheat were affected, an estimated 60 million Ethiopian Birr (\$US3.2 million) were spent on fungicides and large production losses were observed. Factors associated with the 2010 epidemic were conducive climatic conditions (prolonged rain and apparently optimal temperatures), large areas planted to susceptible cultivars, early infection and rapid spread of a virulent pathogen, a low level of awareness, and ineffective control measures. In 2013, highly favourable climatic conditions and early appearance of stripe rust showed remarkable similarity to the conditions observed in 2010, prompting fears of a similar major rust epidemic. However, no stripe rust epidemic developed in 2013. In contrast, only limited and localized outbreaks of stripe rust were observed in 2013; wheat crops remained in good condition and a good harvest was achieved. It seems that a series of positive and timely actions in Ethiopia contributed to the markedly different stripe rust situation in 2013 compared to 2010. The principle factors associated with the positive outcomes in 2013 are (i) effective promotion, plus rapid and widespread adoption of rust resistant wheat cultivars since 2010 - this dramatically reduced the vulnerability of the Ethiopian wheat crop; and (ii) timely and coordinated surveillance efforts, coupled to good information exchange amongst different stakeholders - this resulted in effective control and awareness campaigns that targeted emerging stripe rust outbreaks. A comparative analysis is presented which highlights the similarities and disparities between the 2010 and 2013 stripe rust situations in Ethiopia. The roles and contributions of different organisations are examined and an in-depth analysis of the biophysical conditions in the different years is presented.