14th International Cereal Rusts and Powdery Mildews Conference is organised by Aarhus University on behalf of the European and Mediterranean Cereal Rust Foundation (EMCRF). The conference takes place 5-8 July 2015 at Hotel Marienlyst, close to Copenhagen and neighboring the famous Kronborg Castle in Helsingør.

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Sponsors

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Abstracts from oral presentation

Session 1

Global landscapes of cereal rust and powdery mildew fungi

Worldwide spread of wheat yellow rust from the centre of diversity in Himalayas

Sajid Ali[1], Jérôme Enjalbert[2], Pierre Gladieux[3], Annemarie F. Justesen[4], Mogens S. Hovmoller[4] & Claude de Vallavieille-Pope[5]

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The worldwide population structure, migration patterns and the centre of diversity of wheat yellow rust pathogen *P. striiformis* was inferred based on the microsatellite genotyping of worldwide representative isolates. The population genetic analyses revealed the presence of at least six genetic clusters associated with their likely geographical origin. A high genetic and genotypic diversity and recombinant population structure was identified in the Himalayan and near-Himalayan regions (Nepal, Pakistan and China), while a predominant clonality and low diversity in other parts of the world. Together, the high diversity, recombinant population structure, high sexual reproduction capacity and the abundance of alternate hosts (*Berberis* spp.) suggested the Himalayan and near Himalayan region as the plausible centre of origin of *P. striiformis*. The clustering methods and approximate Bayesian computation (ABC) analyses were used to infer on the worldwide spread of *P. striiformis* from this plausible centre of origin and identify the sources of recent invasive strains/populations. The Himalayan and near Himalayan populations were identified as the most ancestral populations, while analyzing the ancestral relationship among worldwide populations. Among the recent invasive strains/populations, the Middle East-East Africa was identified the source of *PstS1*/*PstS2*; Europe as the source of South American, North American and Australian populations; and Mediterranean-Central Asian populations as the origin of South African populations. The most recent invasive race groups of Europe, "Warrior" and "Kranich", were identified to be originated in the pathogen's centre of diversity in the Himalayan and near Himalayan region. The worldwide spread of *P. striiformis* from its plausible centre of origin reveals the role of both human activities and air dispersal to its long distance dispersal.

PgtSNP chip: A high-throughput SNP genotyping array

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Understanding the genetic diversity and population structure of *Puccinia graminis* f. sp. *tritici* (*Pgt*) has been hampered by the obligate, dikaryotic nature of this pathogen and the lack of robust high-throughput genotyping tools. A custom 1536-SNP Illumina GoldenGate array (PgtSNP 1.5k chip) was developed based on *Pgt* reference genome (U.S. isolate 75-36-700) and NGS sequence data from four selected isolates (78-21-BB463, U.S.; 04KEN156-4, Kenya; 06YEM34-1, Yemen; ANZ21-0, Australia). A balanced selection scheme was use to identify candidate SNP loci evenly distributed along the largest supercontigs, covering 50% of the *Pgt* genome. A final set of 1524 SNPs was selected with loci that are evenly spaced (average spacing of 27 kb) and spanning supercontigs 1-26. These loci are partitioned between genic (82.9%) and intergenic (17.1%). The genic loci are further partitioned between coding (66.5%), intronic (14.5%) and untranslated (2.9%) regions. Twenty-two additional loci previously identified for genotyping members of the "Ug99" race group were added. To test the performance of PgtSNP 1.5k chip a representative sample set of 50 *Pgt* isolates, spanning both geographical (15 countries) and temporal (56 years) diversity, was used. Replicates of each sample were included. Data was filtered using the following criteria: GenTrain score > 0.6; 10% GC score > 0.6; call frequency > 95%; and no replicate errors. The resulting SNP set containing 1236 loci was analyzed by Principal Component analysis with the software package 'Poppr'. The 50 *Pgt* isolates were divided into six genetic clusters. In general, the isolates divided geographically, with the North American samples distributed between two of the clusters and the remaining four clusters containing samples from Africa, Asia and Europe. In a second study, 41 *Pgt* isolates collected in Ethiopia during the 2013/14 main wheat-growing season was analyzed. Phylogenetic analysis divided these isolates into four well-supported clades, based on 918 SNP loci. Each of these clades represented a distinct *Pgt* race phenotype or race group: clade I, Ug99 race group; clade II, JRCQC; clade III, TRTTF/RRTTF; clade IV, TKTTF. Clade IV, was further subdivided into two distinct sub-clades and represents the *Pgt* race that was responsible for the 2013 wheat stem rust epidemic in Ethiopia. Analysis of Ethiopian *Pgt* collections made during 2014 wheat season will be discussed.

Genetic Differentiation and Migration in Worldwide Populations of the Wheat Leaf Rust Fungus, *Puccinia triticina*

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Collections of the wheat leaf rust fungus, *Puccinia triticina*, were obtained from North America, South America, Central Asia, the Middle East, Europe, Turkey, Russia, Ethiopia, and China from common wheat and durum wheat to examine the genetic diversity within each continental region and genetic relationships between regions. Approximately 100 single uredinial isolates from each region were tested for virulence to 20 near-isogenic Thatcher wheat lines and for molecular genotype with 23 SSR primers. There was relatively little variation in the average number of SSR alleles per locus between regions, varying from 2.2 (South America) to 3.1 (China). Populations in all regions had significantly higher than expected SSR allele heterozygosity and had very high (>3.0) index of association values, indicating clonal reproduction worldwide. All populations had significant correlation with virulence, varying from 0.43 (North America) to 0.56 (Europe). Genetic differentiation of SSR genotype groups based on principal coordinate plots and Baysian analysis varied from eight groups in Europe to three groups in China and two groups in Russia. In all regions overall differentiation based on R_{ST} was greater than differentiation based on F_{ST}. Mutation and genetic drift likely contribute to differentiation of SSR genotype groups in *P. triticina*. Within all populations F_{ST} and R_{ST} differentiation based on SSR genotype groups was higher than differentiation based on geographical regions, indicating the migration of SSR genotypes within continental regions. Collections from tetraploid durum wheat in North America, South America, Europe, the Middle East, and Ethiopia had distinct virulence pathotypes that were avirulent to most genes in the Thatcher differential lines and had distinct SSR genotypes compared to collections from common, hexaploid wheat. There was little or no significant differentiation of SSR genotypes of isolates collected from durum wheat from the various regions, indicating a likely recent migration of *P. triticina* types from a single source. Isolates from hexaploid wheat in North America, South America, Europe, the Middle East, Pakistan, and Ethiopia that have virulence to *Lr1, Lr3, Lr3bg, Lr17, Lr26*, and are avirulent to *Lr28* were first detected in Mexico in the mid 1990s and were subsequently detected in the US and Canada (1996), Uruguay (1999) France (2000) and Israel (2000). SSR genotypes of these isolates were nearly identical indicating a rapid migration between continental regions. In Ethiopia, tetraploid landrace emmer and durum wheats have historically been grown and common hexaploid wheats have increased in cultivated area in recent years. The *P. triticina* population in Ethiopia was highly distinct for virulence and SSR genotypes, likely due to the selection pressure exerted by the diverse host population. Isolates collected from the tetraploid wheats were either avirulent to the susceptible common wheat Thatcher, and had very distinct SSR genotypes, or had virulence and SSR genotypes identical to other isolates collected from cultivated durum wheats worldwide. Isolates from common wheat were almost exclusively identical for virulence and SSR genotype to the isolates that were first detected in Mexico and spread to the US, Canada, Europe, and the Middle East. The lack of virulence and SSR diversity suggests that these isolates were recently introduced to Ethiopia.

Population structure and diversity of *Puccinia striiformis* in the past

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The past worldwide population structure and diversity of the yellow rust causing fungus *Puccinia striiformis* have not been investigated previously at the molecular level. Knowledge on this would shed light on the evolution and temporal dynamics of the pathogen on a global scale, which was the aim of our study. An initial recovery and race identity study was conducted beforehand using old spore samples from the historic "Stubbs collection", founded in 1956 by the late Dutch plant pathologist R.W. Stubbs and maintained by the Global Rust Reference Center (GRRC), Denmark, since 2010. A new method for recovery using an airbrush sprayer and Novec™ 7100 was highly successful shown by a 96% recovery of 231 isolates that had been stored for up to 45 years in liquid nitrogen (collected between 1958 and 1991) representing 34 countries. The past population structure was investigated with 212 of the pure isolates that represented six geographically spaced populations: NW Europe, the Mediterranean, East Africa, the Middle East, South Asia (Afghanistan, Pakistan and Nepal) and China. DNA was extracted from spore samples with a CTAB method, and 19 multilocus microsatellites (Simple Sequence Repeats) were used for genotyping. DAPC analysis showed that the global *P. striiformis* population consisted of seven (K=7) distinct genetic populations. Recombinant populations were found in China and South Asia whereas clonal populations were found in NW Europe, East Africa and the Mediterranean. Overall, 89 multilocus genotypes were present in the past where the highest genotypic diversity was found in the Chinese population and the lowest genotypic diversity was seen in the NW Europe population. Long distance migration in the past was detected with the resampling of the most frequent multilocus genotypes observed, and additional information was gained when these were combined with the virulence phenotypes. Analysis of the temporal dynamics of the *P. striiformis* in the past populations compared to the contemporary population from a recent study revealed divergence in all geographical population except for the NW European population, which had remained stable for more than five decades. In conclusion, an overall consistent population structure on a global scale exists for the wheat yellow rust pathogen. Our results further facilitate the understanding of the overall pathogen migration worldwide and elucidate the previous finding of the Himalayas and near-Himalayan region as a putative centre of diversity of *P. striiformis*.

Structure and regional differences in U.S. *Blumeria graminis* f. sp. *tritici* populations: divergence, migration, fungicide sensitivity, and virulence patterns

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Several aspects of the biology of USA populations of wheat powdery mildew (*Blumeria graminis* f. sp. *tritici*, or *Bgt*) have been investigated for their importance to the integrated management of this widespread and potentially damaging pathogen. For example, the virulence profiles of U.S. *Bgt* populations have been surveyed at approximately decade intervals starting in 1993. Most recently, over 580 *Bgt* isolates were collected from unsprayed commercial wheat fields in 11 U.S. states in 2013 and tested for virulence to single-*Pm*-gene differentials. *Pm1a*, *1b*, *4b*, *16*, and *36* are still widely effective, although they are not widely utilized in the U.S. for various reasons. Most other *Pm* genes with numbers lower than *Pm25* are widely defeated. Based on field and laboratory data, several resistance sources from wild relatives that were recently introgressed -- *MlAG12*, *Pm25*, *Pm34*, *Pm35*, and *Pm37* -- are highly effective. They are available in soft red winter wheat backgrounds adapted to the Mid-Atlantic and Southeastern USA. Approximately 600 more *Bgt* isolates were obtained from commercial fields in 12 U.S. states in 2014, and their virulence profiles are currently being determined.

Interestingly, some widely used *Pm* genes are not universally defeated in the USA. Although ineffective along the Atlantic coast, *Pm3a* and *Pm3b* remain somewhat or very effective in several areas west of the Appalachian Mountains (Kentucky, Nebraska, Oklahoma and Texas). This discrepancy may persist due to newly illuminated migration patterns in the U.S. *Bgt* population. Between 2003 and 2010, 238 *Bgt* isolates were collected from 12 U.S. states and evaluated for local and regional population differences, linkage disequilibrium, and migration. Isolates from the Southeast, Mid-Atlantic, and Great Lakes regions comprised a single large cluster, and they were separated genetically from the populations in Kentucky, Oklahoma and Texas. Moderate isolation by distance was detected ($R^2 = 0.19$, $P = 0.003$). One-way migration was estimated at the rate of approximately six individuals per generation from the Kentucky/Texas/Oklahoma populations toward the north and east. Altogether, the evidence suggested annual re-establishment primarily from local sources, resulting in a large-scale mosaic of overlapping local populations, but with some long-distance dispersal in a west-to-east direction. This suggests that novel virulences or fungicide resistance traits that arise anywhere in the eastern U.S. are likely to become widespread in that region, but unlikely to migrate west across the Appalachian Mountains.
In the USA, use of several fungicides on wheat is estimated to have increased, especially since 2007 when wheat prices began rising. *Bgt* is considered a high-risk pathogen for development of reduced fungicide sensitivity. We are acquiring the first comprehensive dataset on regional U.S. differences in *Bgt* sensitivity to commonly applied fungicides. From the 2013 isolate collection mentioned above, a set was chosen that originated from 11 fields in eight states grouped into four regions (Deep South, Mid-Atlantic, Great Lakes, and Plains). The sensitivity of over 150 single-spored isolates was evaluated on susceptible detached wheat leaves previously sprayed with fungicides. Five fungicides (tebuconazole, prothioconazole, pyraclostrobin, picoxystrobin, and fluxapyroxad) were evaluated at 12 rates each. Significant regional differences were found for all fungicides, although to the smallest degree for pyraclostrobin and fluxapyroxad. Isolates from the Plains were the most sensitive to all products. The experiment is being repeated with 2014 isolates. The existence of regional differences may indicate uneven emergence and development of reduced sensitivity.

Overall, the results underscore the importance of (1) accelerating the incorporation of new *Pm* genes, if possible in pyramids, into quantitatively resistant, adapted wheat backgrounds; and (2) restricting unnecessary use of fungicides on wheat in order to slow the emergence and spread of reduced sensitivity in the *Bgt* population.

The Wheat Rust Toolbox: Management and visualization of global wheat rust data

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In most recent years the UG99 stem rust race has moved out of East Africa. This poses a threat to other regions in Africa, Central and west Asia, and other regions where this pathogen is not yet found. In Europe a new population of stripe rust (Warrior and Kranich races) replaced less diverse pathogen populations during the years 2011-2014 (Figure 1). To enable the tracking and monitoring of the evolution of pathogen populations, a new web/database management and display system was developed, called the Wheat Rust Toolbox (Figure 1). This platform, hosted by the Global Rust Reference Centre, support two major initiatives, 1) the Durable Rust Resistance in Wheat project (DRRW), for managing and visualizing wheat rust pathogen data - mainly about stem rust (*Puccinia graminis*) from Africa, Central and West Asia and 2) the ENDURE wheat rust network for managing and visualizing stripe rust (*Puccinia striiformis*) race, virulence and genotype data for Europe. The presentation will provide an overview of data and tools available in the Wheat Rust Toolbox, the research infrastructure behind and how data are disseminated via several information platforms such as wheatrust.org, eurowheat.org and http://rust-tracker.cimmyt.org/. Opportunities for analyzing genotypic data (SSR and SNP) online via a web based version of the POPPR integrated with the Wheat Rust Toolbox will be presented. Overall the results show that the collation of data in a standardized way across many countries leads to more robust and fast conclusions and it stimulates collaboration between partners. Future directions for improvements of the Wheat Rust Toolbox and the need for a global virtual research environment are outlined.

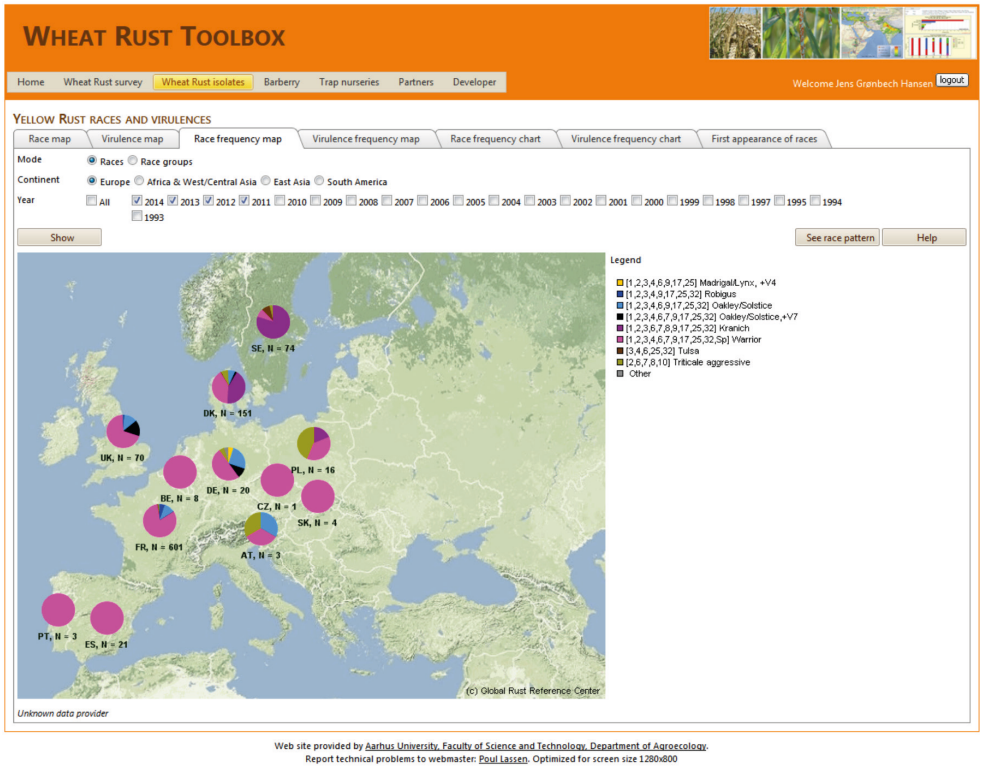

Figure 1. Race frequency map of the European stripe rust population 2011-2014.

Abstracts from oral presentation

Session 2

Population biology and epidemiology

Research progress on the role of sexual hosts for wheat rust epidemiology in China

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Wheat stripe rust, caused by *Puccinia striiformis* f. sp. *tritici*, is a remarkably destructive disease of wheat worldwide and has beenan increasing concern since discovery of sexual cycle of the stripe rust fungus based on identification of barberry (*Berberis* spp.) serving as alternate host for the pathogenin recent years. Previous studies demonstrated barberry plays an important role in providing initial inoculums to trigger the stem rust infection on wheat around the susceptible barberry in natural conditions, and in generating new races of the stem rust fungus through genetic recombination after completion of sexual stage on barberry in some of American and European countries. However, the function of barberry for wheat stripe rust is limited so far. In recent years, we made efforts to verify the role of barberry in epidemiology of wheat stripe rust in China and obtained primary results. Based on our investigations, thirty five of *Berberis* spp. were identified currently and distribute widely in China, especially northwestern and southwestern regions of China. Twenty eight of *Berberis* spp. were identified to serve as alternate hosts for *Puccina striiformis* f. sp. *tritici* (Pst), the causal pathogen of wheat stripe rust. Importantly, a total of twenty Pst samples were isolated from *Berberis* spp. with natural aecial infections that were collected from different provinces of China. Identification of these Pst samples in pathogenicity on Chinese differentials for Pst indicated that two of Pst samples were CYR32 that is known predominant Pst race, and the other were new that differ from all of known Chinese Pst races. Single uredium isolates derived from the twenty Pst samples showed diverse phenotypes on a set of single *Yr* gene lines as compared that of their parent Pst samples. This suggests that barberry plays a role in providing inoculums to cause stripe rust infection of wheat around the infected barberry and resulting in virulence variation to generate new Pst races in natural conditions in areas where the susceptible barberry and wheat co-exist. Further studies on relation between barberry and stripe rust fungus on its primary, and accessory hosts in epidemiology of wheat stripe rust are in progress.

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Why Are Alternate Hosts Important for Stem Rust, But Not for Stripe Rust in the US Pacific Northwest?

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Common barberry (*Berberis vulgaris*) has been known to serve as an alternate host for the wheat stem rust pathogen, *Puccinia graminis* f. sp. *tritici* (*Pgt*), under natural conditions in the US Pacific Northwest for a long time. The plant has been recently shown to be infected by basidiospores of the wheat stripe rust pathogen, *Puccinia striiformis* f. sp. *tritici* (*Pst*), under controlled conditions. However, it was not clear if barberry plays any role in stripe rust epidemics under natural conditions. To determine *Puccinia* spp. on barberry plants, we collected aecial samples from barberry plants in the Pacific Northwest from 2010 to 2013 and characterized by inoculation on wheat plants under controlled conditions and using molecular markers and sequences of the internal transcribed spacer (ITS) region of nuclear ribosomal DNA. All tests using single aecia clearly showed either *Pgt* or other formae speciales of *P. graminis*, but did not show any *P. striiformis*. The results strongly imply that barberry is essential for stem rust epidemics, but not for stripe rust under the natural conditions in the US Pacific Northwest.

To determine why *Pgt* is able to infect barberry plants but *Pst* cannot under the natural conditions, the viabilities of teliospores of both *Pgt* and *Pst* were investigated from 2011 to 2014 by studying their structures and determining the germination rates using telial samples collected periodically from wheat fields. After maturity, elongate telia of *Pgt* are exposed from the erupted plant epidermal tissue, but telia of *Pst* remain under the plant epidermal layer. When physically separated from plant tissue, *Pgt* teliospores could not be germinated, but *Pst* teliospores could be easily germinated under moist conditions and their respective optimal temperature conditions. Teliospores of *Pst* usually produced in July were physically degraded during winter, and their germination rate decreased from 50-90% in August to less than 1% in the following March and no germination after May. In contrast, *Pgt* teliospores usually produced in July and August remained physically intact and physiologically dormant, and could not germinate until February of the following year, and their germination rate gradually increased to 90% in May, at which time young leaves of barberry were susceptible to infection. In addition, a time-series experiment was conducted for inoculation of barberry plants with *Pst* teliospores. The results showed that *Pst* teliospores need a minimum of 32 h continual dew-forming condition to infect barberry, and infection reaches a peak after incubation of inoculated plants for 88 h. The lack of protracted moist conditions during the season of telial production effectively negates *Pst* infection of barberry plants in the Pacific Northwest.

Epidemiology of cereal rusts in the presence of their alternate hosts

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Cereal rusts (*Puccinia* spp.) are among the most studied plant pathogens. Most research focuses on the uredinial stage, since that is the spore stage that infects the crop and cause disease. However, the possibility for the fungi to complete their full life cycle has implications for the epidemiology of the disease. My research predominantly focuses on two pathosystems, the fungi causing stem rust, *Puccinia graminis* and crown rust, *Puccinia coronata*, their grass hosts and alternate hosts barberry (*Berberis* spp.) and common buckthorn (*Rhamnus cathartica*) respectively. The presence of aecia on the alternate host dramatically increases the genetic diversity of the pathogens and also affects the epidemiology of the diseases. The presence of the alternate host represents a local source of initial inoculum. This, in turn, leads to local populations that show evidence of some genetic isolation, although individuals from other locations will contribute to the genetic diversity of the local population. This may have implications in the generation of new gene combinations, which would in turn be reflected in a large number of different races. Rust fungi are mainly transported by wind, and to understand the impact of air-borne inoculum, the fungal community in the air has been studied using a variety of different spore traps, and compared with field observation to obtain a better understanding of the frequency of air-dispersed spores in different locations. To control cereal rusts reproducing sexually, measures such as removal of the alternate host (to minimize genetic variation and initial inoculum) together with a focus on durable resistance breeding (to cope with the variation in races) are necessary.

First insights into segregation for aggressiveness among sexual progeny isolates of *Puccinia striiformis*

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It was recently discovered that common barberry (*Berberis* spp.) may serve as alternate host for sexual reproduction of *Puccinia striiformis,* a fungus causing yellow (stripe) rust on several cereals and grasses. It is currently considered one of the most devastating diseases on wheat worldwide. Infection on cereals and grasses are primarily caused by asexual urediniospores and during the end of the disease cycle teliospores start to develop. Teliospores may germinate and produce basidiospores which can then infect barberry. On barberry, pycnia containing pycniospores develop on the adaxial side of the leaf and upon successful fertilization aecial cups containing aeciospores develop on the abaxial side of the leaf. Aeciospores may infect the cereal and grass hosts and produce asexual urediniospores, thus completing the life cycle. An experimental system for infection of barberry was established at the Global Rust Reference Centre, which has provided first insights into the genetics of *P. striiformis*. A selfing of an isolate of the Warrior race, which has been prevalent in Europe since 2011, resulted in 17 progeny isolates which showed segregation for SSR markers, virulence, and aggressiveness. The segregation for SSR markers and virulence has already been reported (Rodriguez-Algaba *et al.,* 2014). Here we present preliminary data about segregation for aggressiveness. An experimental system was developed for the assessment of components of aggressiveness, e.g., latent period and lesion growth. The progeny isolates were tested in three independent experiments at different times of the year in a controlled greenhouse environment. Four progeny isolates had a significantly longer latent period and higher lesion growth rate than the rest whereas two isolates had a short latent period combined with a significantly lower lesion growth rate. This behaviour was different from the parental isolate which was intermediate for both latent period and lesion growth rate. These results suggest the existence of a trade-off between latent period and lesion growth rate, which is consistent with a common hypothesis in the theory of pathogen evolution that fitness parameters may be negatively correlated. The progeny isolates constitute a unique material to gain further insight into the molecular basis of pathogen aggressiveness (Chen *et al.,* 2015).

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Correlation among life-history traits in plant-pathogen interaction and consequences for epidemic spread: a case study in the wheat leaf rust pathosystem

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Quantitative traits, referred to as aggressiveness components (from the pathogen side) or resistance components (from the host side) are usually measured in controlled conditions on individual plants, and at the scale of a single pathogen life cycle, whereas epidemic development is measured in the field and encompasses a succession of elementary life cycles of the pathogen.

Assessment of these traits is required to measure components of quantitative resistance in the host, to compare the fitness of pathogen strains, or to parameterize epidemic models. In these three cases, the question is raised of how each elementary quantitative trait contributes to the development of an epidemic, but very few information is available in the literature on this matter, and it is mostly based on theoretical studies. The objective of this study is to understand the contribution of elementary traits of pathogenicity to epidemic development in field conditions.

A set of wheat cultivars was confronted to three different leaf rust pathotypes, both under controlled and field conditions, during three consecutive years. In the greenhouse, Infection efficiency (*IE*), latent period (*LP*), lesion size (*LS*), spore production per lesion (*SPL*) and spore production capacity (*SPS*) were measured. In the field, disease severity (*DS*) was measured at three different dates.

Generalized Linear Models in a Bayesian framework were developed to estimate the quantitative traits and the field epidemic development variables for each cultivar-pathotype pair. Finally, i) the correlations between the estimated quantitative traits, and ii) the correlations between quantitative traits and epidemic development in the field, were investigated.

Most but not all the quantitative traits were related between them. Positive relationships (*IE-LP, IE-SPS, LP-SPS* and *LS-SPL*) can be interpreted as a pleiotropic effect of genes/QTLs influencing different resistance components of the host, or different aggressiveness components of the pathogen. Negative relationships (*LS-SPS, LS-LP, LS-IE* and *IE-SPL*) reflected trade-offs effects between components of host resistance, or between components of pathogen aggressiveness.

All the individual quantitative traits were correlated to the resistance in the field, except *SPL*. The strength of this relationship varied across the course of the epidemic, all traits being more strongly related with *DS* at the beginning of the epidemic. Later, the influence of *LP* and *IE* on *DS* decreased or get stabilised, whereas the influence of the sporulation traits on *DS* increased as the epidemic ended.

Traits covering all the pathogen life cycle have to be taken into account, whenever comparisons of the fitness of pathogen strains, or the resistance of plant cultivars under field conditions, are to be computed from the quantitative traits measured in controlled conditions.

Negative correlations between the traits represent a potential evolutionary constraint for the parasite. The knowledge of these relationships enables to identify combinations of resistance components prone to ensure an efficient and durable field resistance.

Abstracts from oral presentation

Session 3

Cell and molecular biology and genomics

Developing new tools for interrogating cereal invaders

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Emerging and re-emerging diseases of humans, animals and plants pose a significant hazard to public health and food security. With recent advances in sequencing technology, bacteriologists and virologists are now integrating high-resolution genotypic data into pathogen studies. However, the application of genomics to emerging filamentous plant pathogens has lagged. To address this, we are leading the genome sequencing of hundreds of isolates of the wheat yellow rust pathogen *Puccinia striiformis* f. sp. *tritici* (PST), aimed at improving our understanding of the molecular mechanisms that drive PST evolution. Furthermore, we have developed a robust and rapid "field pathogenomics" strategy to improve filamentous pathogen surveillance. We applied this method in 2013 to PST, using gene sequencing of PST-infected wheat leaves taken directly from the field to gain insight into the population structure of an emerging pathogen. Our analysis uncovered a dramatic shift in the PST population in the UK and supports the hypothesis that recent introduction of a diverse set of exotic PST lineages may have displaced the previous populations. Working with cross-institutional and industrial partners we are now developing this technique further to reduce its cost so it can be applied routinely within our UK pathogen surveillance program for agroecosystems.

To sense or not-to-sense: Expression of *Blumeria* effector repertoires on barley loss-of-function mutant hosts

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The interaction of barley, *Hordeum vulgare* L., with the biotrophic powdery mildew fungus, *Blumeria graminis* f. sp. *hordei (Bgh)*, is an ideal model to address fundamental questions in host resistance and susceptibility. Effector proteins secreted by *Bgh* suppress or induce host processes to promote nutrient acquisition and colonization. The 130-Mb *Blumeria* genome harbors ca. 540 predicted secreted effectors, designated BECs (*Blumeria* Effector Candidates) or CSEPs (Candidate Secreted Effector Proteins). Large-scale RNA-Seq of *Bgh*-infected barley resistance-signaling mutants indicates that distinct subsets of effector candidates are differentially expressed at penetration, or during haustorial formation. This suggests that *Bgh* is able to sense compromised resistance functions in the various isogenic mutant hosts and modify expression of its effector repertoire accordingly.

BEC1019, a predicted single-copy metalloprotease, is differentially expressed in haustoria among all barley loss-of-function mutants. *Barley stripe mosaic virus*-mediated gene silencing of *BEC1019 in planta* significantly reduces fungal colonization of barley epidermal cells, demonstrating that BEC1019 plays a central role in virulence. In addition, delivery of BEC1019 to the host cell cytoplasm via *Xanthomonas* type III secretion suppresses cultivar non-specific hypersensitive reaction (HR) induced by *Xanthomonas oryzae* pv. *oryzicola*, as well as cultivar-specific HR triggered by the AvrPphB effector from *Pseudomonas syringae* pv. *phaseolicola*. *BEC1019* homologs are present in 96 of 241 sequenced fungal genomes, including plant pathogens, animal pathogens, and free-living non-pathogens. Comparative analysis revealed variation at several amino acid positions that correlate with fungal lifestyle, and several highly conserved, non-correlated motifs. Site-directed mutagenesis of one of these, ETVIC, compromises the HR suppressing activity of BEC1019. We postulate that BEC1019 represents an ancient, broadly important fungal protein family, members of which have evolved to function as effectors in plant and animal hosts.

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Identification of barley powdery mildew avirulence effectors using association mapping

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Plant pathogens secret small effector proteins into host cells to facilitate host colonization, in turn plants evolve resistance proteins to recognize specific pathogen effectors and trigger host defense response, so called effector-triggered immunity (ETI). Barley resistance protein MLAs are intracellular NLRs and confer race-specific immunity to barley powdery mildew *Blumeria graminis f. sp. hordei* (*Bgh*) by recognizing *Bgh* isolate-specific avirulence effectors. The allelic MLA proteins are highly polymorphic at the C-terminal LRR domains, pointing to a diversifying selection at the effector recognition sites. This indicates a direct recognition and co-evolution between MLAs and avirulence effectors; alternative, MLAs co-evolve with host proteins, leading to an indirect recognition to avirulence effectors. Although 23 MLAs are cloned, their cognate avirulence effectors are ambiguous. Thus, the identification of avirulence effectors from *Bgh* is the key to uncovered the molecular mechanism underlining the recognition and co-evolution between MLAs and effectors. We aimed at identifying MLA-specific avirulence effectors using association mapping with a collection of 20 *Bgh* isolates. First, we determined the pathotype of each isolates on barley near-isogenic lines containing different MLA recognition specificities; then we identified *Bgh* isolate-specific SNPs using their RNAseq data in comparison with the reference genome of isolate DH14. From the association mapping, we successfully identified candidate avirulence effector of MLA7 and MLA13. Expression of the candidate genes into barley leaves by agrobacteria infiltration could trigger a specific cell-death response on the respective MLA containing barley NILs. Further validation of the candidate avirulence effectors is currently in progress.

Regulation of the hemoglobin/NO cycle in barley infected with powdery mildew or yellow (stripe) rust

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Nitric oxide (NO) is an important cellular signaling molecule in plants. It is involved in a range of physiological functions in plants. NO plays a particular central role in plant responses to biotic stresses, where it seems to function at different molecular levels including formation of nitrosylated proteins and cross-interference with various reactive oxygen species. Plant hemoglobins are important modulators of the NO signal, presumably by an oxidative mechanism including the direct reaction with O_2. When biotrophic pathogens infect plants, the pathogen and the plant are struggling to take control over gene expression towards either compatibility or incompatibility. Our previous studies have shown that artificial up-regulation or silencing of endogenous hemoglobins in plants can modulate NO levels during pathogen infection to an extent where susceptibility levels are severely changed. We here show how barley plants infected with either powdery mildew (*Blumeria graminis* f. sp. *hordei*) or yellow (stripe) rust (*Puccinia striiformis* f. sp. *hordei*) are struggling with the respective pathogen to control hemoglobin gene expression.

Membrane trafficking in plant cells attacked by powdery mildew fungi

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We are interested in plant membrane trafficking processes and how they are involved in plant interactions with microbes. For this purpose we use the powdery mildew fungi and the attacked leaf epidermal cells, which are amenable for confocal microscopy. These fungi are obligate biotrophs, introducing haustoria in the host cell as a means of acquiring nutrients.

We have previously shown that PEN1 and GNOM, a syntaxin and an ARF-GEF, function on the same pathway defending the cell against penetration and haustoria establishment. We have more recently obtained data showing that components of multivesicular body formation and secretion of exosomes are involved as well. Pathogen haustoria are surrounded by plant-generated membranes. The nature of these extrahaustorial membranes (EHM) remains enigmatic. We have addressed this question and found that the powdery mildew-associated EHM in barley cells shares features with the endoplasmic reticulum membrane (ER), although the EHM is not an extension of the ER. A defence component, which is often observed but poorly studied, is the haustorial encasement. Here a cell wall-like structure forms around the haustorium. We have uncovered a Rab GTPase required for encasement formation and for the first time been able to document that it suppresses pathogen proliferation.

Fighting on many fronts – effector-assisted breeding for multiple pathogens

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The role of necrotrophic effectors in promoting virulence can be exploited as a way to select more resistant germplasm. Resistance to necrotrophic diseases was typically found to be partial, in contrast to the major gene resistance noted in some cases for biotrophic pathogens. This has meant that breeding for disease resistance is much more demanding and explains why necrotrophic pathogens have grown in importance whilst progress in controlling biotrophic diseases was often rapid (until the emergence of the next mutant pathogen race). However the identification and production of cloned and expressed effectors of necrotrophic pathogens allows breeders to select introgressions that are insensitive. Effectors from both Parastagonospora nodorum and *Pyrenophora tritici-repentis* have been ex-pressed in microbial systems and used to identify germplasm that is insensitive to the effector Thus, in the case of multi-effector systems like *P. tritici-repentis* and *P. nodorum*, selection of cultivars insensitive to each effector promises to assists breeders improve disease resistance in an incremental, step-wise fashion.

There are several complications to consider; Effectors are not present in all isolates of the pathogen present locally; effector genes vary in expression levels and activity; effectors interfere with recognition by other effectors. We argue that whilst these factors complicate the exploitation of effectors they do not compromise our ability to achieve sustained improvements in resistance to these necrotrophs.

I will also briefly mention the barley powdery mildew epidemic in Western Australia and steps taken to end it.

PsANT, the adenine nucleotide translocase of *Puccinia striiformis*, promotes cell death and fungal growth

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Adenine nucleotide translocase (ANT) is a constitutive mitochondria component that is involved in ADP/ATP exchange and mitochondria-mediated apoptosis in yeast and mammals. However, little is known about the function of ANT in pathogenic fungi. In this study, we identified an ANT of *Puccinia striiformis* f. sp. *tritici* (*Pst*), designated *PsANT*. PsANT contains three typical conserved mitochondria-carrier-protein (mito-carr) domains and shares more than 70% identity with its orthologs from other fungi, suggesting that ANT is conserved in fungi. Immuno-cytochemical localization confirmed the mitochondrial localization of PsANT in normal *Pst* invading hypha cells or collapsed cells. Over-expression of *PsANT* verified that *PsANT* promotes cell death in tobacco, wheat and fission yeast cells. Further study showed that the three conserved mito-carr domains worked together to induce cell death. qRT-PCR analyses revealed an in-planta induced expression of *PsANT* during infection. Knockdown of *PsANT* using a host-induced gene silencing system (HIGS) attenuated the growth and development of virulent *Pst* at the early infection stage but not enough to alter the virulence of the pathogen. These results provide new insight into the function of *PsANT* in fungal cell death and growth and might be useful in the search for and design of novel therapies in future.

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Yellow rust fungus effector candidate PEC6 targets adenosine kinase to suppress PAMP triggered immunity

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Yellow (Stripe) rust, caused by the biotrophic fungus, *P. striiformis* f. sp. *tritici* (*Pst*), is globally the most prevalent and damaging disease on wheat. It is well known that pathogens employ effectors to interfere with host defence. However, no effector from the yellow rust fungus has been identified until now, but genome and transcriptome sequencing has revealed many effector candidates (Cantu et al. 2013, BMC Genomics 14: 279 and Garnica *et al.* 2013, PLOS ONE 8: e67150).

We selected effector candidates, which are highly expressed in haustoria, and tested their function in tobacco (*Nicotiana benthamiana*) and wheat (*Triticum aestivum*) by delivering effectors into plant cells using the type-three secretion system (T3SS) of the EtHAn strain of *Pseudomonas fluorescens*. In *N. benthamiana*, PEC6 (*Puccinia* Effector Candidate 6) significantly suppressed the ROS accumulation, ion leakage and callose deposition induced by *P. fluorescence*. Similarly, PEC6 compromised defence in wheat and enhanced susceptibility to yellow rust. Knocking down PEC6 by virus induced gene silencing decreased yellow rust virulence. Localization analysis showed that PEC6 localized in nucleus and cytosol in wheat leaves after transient expression by particle bombardment. Yeast two-hybrid screening (Y2H) and bimolecular fluorescence complementation (BiFC) showed that PEC6 targets host adenosine kinase, which is involved in cytokinin inactivation

We speculate that PEC6 might be working in defence signalling by targeting adenosine kinase and thereby induce changes of the cytokinin pool and compromise the defence response of the host.

Awakening of ETI: Stripe rust effector candidates reveal HR on wheat *via* a bacterial type III secretion system

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Although the wheat stripe (yellow) rust disease is among the most damaging one on wheat, world-wide, there are still too many unknowns in the interaction mechanism of this pathogen with its host. Whole-genome and transcriptome sequencing are valuable approaches to pinpoint effector candidates that might be involved in PAMP triggered immunity (PTI) and effector triggered immunity (ETI), but routinely used high through-put validation methods scare for testing the biological functions of the wheat pathogen effectors. Nevertheless, one of the bacterial type 3 secretion system (T3SS) seems to be a very promising strategy, since it successfully allows the expression of the candidate effectors in the natural host, wheat. *Pseudomonas fluorescens* mediated T3SS offers auspicious and reliable effector delivery system than others due to lack of basal resistance symptoms in wheat. We analyzed the most of promising effector candidates whether they produce hypersensitive response (HR) upon their delivery into 13 stripe rust resistant (YR) differential wheat lines. We report here, for the first time, 3 of the *Puccinia striiformis* f. sp. *tritici* effector candidates produce HR due to activation of ETI.

These effectors were further characterized to assess the subcellular localizations in *planta* using *Agrobacterium* (GV3101) mediated gene transfer into *N. bentamiana*. Interestingly, one of the effector seems to be targeting plastids of the guard cells, one effector is likely to be located in cytoplasm and nucleus, another one is on nucleus and cell membrane.

An integrated approach to understanding adult plant resistance: histology and molecular features of stem rust on wheat

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Commercially implemented single gene resistance in wheat may be overcome by fungal pathogens such as *Puccinia graminis* f. sp. *tritici*. The emergence of Ug99, and variants within this race group, highlighted the necessity of durable stem rust resistance. Adult plant resistance (APR) has the potential to remain durable in the presence of new rust variants. Two entries, JIC218 and JIC542, were selected from the John Innes Centre collection for African wheat to possess APR against stem rust. Stem rust development and wheat defense responses were monitored in stems of the two JIC lines in comparison with a susceptible control (Line 37-07) under greenhouse conditions. Histological techniques such as scanning electron microscopy and fluorescence microscopy provided insight into the colonization of adult wheat plants by stem rust. Molecular techniques included quantifying fungal biomass by WGA-TITC binding fluorescence and RT-qPCR. Expression of a fungal haustorium-associated gene was also tracked by RT-qPCR. Microscopic observations and the molecular based infection timeline allowed the APR lines to be clearly distinguished from the susceptible check at 120 hours post infection. RNA sequencing was used to investigate transcriptional changes observed in the JIC lines and stem rust race PTKST after infection. A total of 3134 genes were differentially expressed during the plant pathogen interaction. Integration of these various methods allows a better understanding of APR as well as the relationship between different resistance loci and cellular responses.

Abstracts from oral presentation

Session 4

Plant breeding and resistance genetics

Second-generation biotech approaches for durable disease resistance in cereals

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Broad-spectrum, quantitative pathogen resistance is of high importance to plant breeders due to its expected enhanced durability. However, it is usually controlled by multiple quantitative trait loci and therefore, challenging to handle in breeding practice. Knowing about the underlying genes would facilitate its more targeted utilization by allele introgressions. Identified candidate genes can also be used for increasing resistance by genetic engineering. Three approaches to confer durable pathogen resistance to barley and wheat by transgene technology will be presented here.

The first approach focuses on the silencing of potential susceptibility-related genes of barley during the interaction with the powdery mildew fungus *Blumeria graminis* f. sp. *hordei* (Bgh). These genes are either silenced alone or in combinations of three, under the control of a constitutive or a pathogen-inducible promoter. In order to identify genes that affect race-nonspecific resistance of barley to the powdery mildew fungus *Blumeria graminis* f.sp. *hordei* we combined a functional-genomics approach based on genomewide transcript profiling and transient-induced gene silencing (TIGS, over 1,000 genes) with association-genetic (re-sequencing) and meta-QTL mapping approaches. This guided us to a shortlist of approximately 40 candidates with converging evidence for an important role in race-nonspecific resistance of barley. Several of those candidates enhanced resistance upon TIGS and thus might function as susceptibility factors. The second approach focuses on host-induced gene silencing (HIGS) in fungal pathogens attacking transgenic plants that carry RNAi constructs directed against transcripts of the pathogen. Proof of concept was obtained in the barley/Bgh system and in wheat attacked by the *Fusarium* head blight fungus *F. culmorum*. The third approach is based on pathogen-inducible over-expression of combinations of three defense-related genes involved in cell-wall modification or in signaling. Transgenic lines were evaluated for enhanced resistance to Bgh, rice blast, scald and spot blotch.

The prospects and limitations of these approaches will be discussed and put into the context of sustainable, knowledge-based improvement of biotic stress resistance in crop plants.

Cloning stem rust resistance from bread wheat progenitors

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The diploid grass species *Triticum monococcum* (sub sp *monococcum* and *boeoticum*) and *Aegilops tauschii*, represent the A and D genome relatives respectively of common wheat (*T. aestivum*), and they harbour diverse resistance genes which includes *Sr22*, *Sr33* and *Sr45* that are effective against stem rust races prevalent in Africa, USA, Australia and Asian continents. Through inter-specific hybridisation, these resistance genes have been successfully transferred into bread wheat and used in commercial production. As part of the objectives towards dissecting the biology and immune recognition of these genes to different stem rust races, my past and the on-going research are focused on isolating these important genes using a combination of conventional positional cloning and mutagenesis approaches.

Despite the complexity of the genomes of wheat and its wild relatives, due in part to multiple gene copies and highly repetitive DNA sequences, positional or map-based gene cloning techniques have been successful in isolating traits of agronomic importance, albeit at a relatively slow rate. This approach was used in identifying, the chromosomal region of *Ae. tauschii* harbouring *Sr33* which contained a mixed cluster of resistance gene analogs (RGAs). Mutational and complementation analysis enabled confirmation of the *Sr33* gene member. With the rapid advances of next generation sequencing combined with mutational genomics, *Sr33* mutants could be detected, and this approach has been extended to the cloning of *Sr22* (from *T. monococcum*) and *Sr45* (from *Ae. tauschii*).

Fine-mapping of a QTL for nonhost resistance to *Blumeria graminis* f. sp. *tritici* in barley

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Inheritance studies of Nonhost Resistance (NHR) are challenging in the sense that, in order to perform classical genetic analyses, crossings between a host and a nonhost plant would be required. It is rarely possible to obtain the progeny of such interspecific crosses, because of issues regarding abnormal segregation, lethality and sterility. An alternative approach is to determine the inheritance of resistance in so-called "near nonhosts" plant pathosystems. Near-nonhosts are plant species in which a low percentage of accessions show some degree of susceptibility to a particular heterologous pathogen. Barley (*Hordeum vulgare* L.) is a near nonhost to several rusts and powdery mildews infecting cereals and grasses. SusPtrit is an experimental barley line developed to accumulate susceptibility factors to the wheat leaf rust, *Puccinia triticina*. This line is at seedlings stage also slightly susceptible to the wheat powdery mildew, *Blumeria graminis* f. sp. *tritici* (*Bgt*), as evidenced by the formation of micro-colonies, that appear as tiny white spots over the surface of the leaf (Figure 1). Histological analysis showed that SusPtrit allow an unusually high level of haustorium formation.

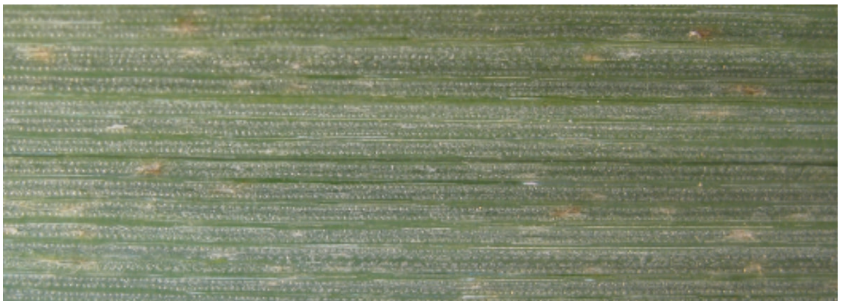

Figure 1. SusPtrit barley line about 10 days after inoculation with *Bgt*: formation of micro-colonies is visible by naked eye.

A RIL mapping population originated from the crossing of SusPtrit with cv. Vada (VxS) was screened for susceptibility to *Bgt*. The VxS RILs at seedling stage were densely inoculated with *Bgt*, and scored one week later according to the degree of micro-colony formation visible by naked eye. A major QTL was mapped on chromosome 5H (LOD score 19.9) contributing to 43% of the phenotypic variation. The QTL was named *Rbgtq1*, and the donor of resistance allele is parent Vada. The same QTL was mapped when the phenotyping was performed based on microscopic observations of degree of haustorium formation.

To fine-map *Rbgtq1*, some VxS RILs were selected that differed for the region of the QTL, and that had shown the associated elevated or reduced level of susceptibility to *Bgt*. Such contrasting lines were intercrossed and the F_2 were screened for recombinants in a region of 18.7 cM around the peak marker of the QTL (distance according to the position of the selected markers in our Barley Consensus SNP Map, Yeo *et al.* 2014). Four markers were used for the first round of genotyping, when 369 F_2 plants were genotyped using the SNP-based Kaspar technology (KBioscience, UK). Later, small amplicon genotyping method (Liew *et al* 2004) was used to screen F_3 plants for recombinants, using the LightScanner® system (Idaho Tech, USA). After the first round of phenotyping on F_3 plants it was possible to narrow down the

location of *Rbgtq1* to a region of about 2 cM on the VxS map and of about 0.5 cM according to genetic positions of the WGS assembly of Morex contigs. It was also possible to determine that the resistance allele of the QTL is dominant over the susceptible allele.

Breeding for the future: Generation of wheat plants resistant to powdery mildew by tilling

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Bread wheat (*Triticum aestivum*) is the third largest cereal cultivated in the world and the second in terms of dietary intakes. In 2012 its production reached 670 million tons. However, yield can be severely affected by the powdery mildew disease caused by the fungus *Blumeria graminis* f. sp. *tritici*. Loss-of-function alleles of *Mlo* (*Mildew resistance locus o*) gene(s) in barley, tomato, pea and Arabidopsis confer non-race specific resistance against their causing powdery mildew disease fungus. In hexaploid wheat, three orthologs (wheat homoeologs) of barley *Mlo* (*TaMlo-A1*, *TaMlo-B1*, *TaMlo-D1*) have been identified. *TaMlo-B1* was previously shown to complement barley *mlo* mutants at the single-cell level, indicating functional equivalence of these wheat and barley *Mlo* genes. Therefore, we propose to take advantage of the non-transgenic TILLING (Targeted Induced Local Lesions IN Genomes) technology to identify mutants in the respective *Mlo* homoeologs to ultimately generate a wheat *mlo* triple mutant. A mutagenized population of the spring bread wheat cultivar Cadenza was screened for the exon encoding the third cytoplasmic loop of the Mlo protein. Several missense mutations were identified for the three genomes (Fig. 1) and were functionally tested by transient gene expression in barley single cells. Only mutant variants that showed significant reduction of host cell entry compared to the wild-type *Mlo* were selected for further crosses. Currently, several combinations of *mlo* triple heterozygous mutants are in propagation. The resulting wheat homozygous lines are expected to provide durable broad-spectrum powdery mildew resistance.

Figure 1.Different spikes from wheat plants with mutations in one of the three *Mlo* homoeologs *TaMlo-A1*, *TaMlo-B1*, *TaMlo-D1*.

Durable but complex: stem rust resistance from emmer wheat

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Two emmer wheats are the source of durable stem rust resistance in bread wheat. Ilumillo emmer was crossed with Marquis in Minnesota in 1914 that later gave rise to the cultivar Thatcher (Tc) while a Yaroslav emmer x Marquis cross in South Dakota in 1916 produced the resistant bread wheat cultivar Hope. One-hundred years later, both Thatcher and Hope still carry moderate levels of stem rust resistance. The most important gene transferred from emmer into Hope was *Sr2* which confers race non-specific, partial resistance. We identified several candidate genes at the *Sr2* locus and it is possible that more than one gene is required for *Sr2* resistance. Resistance in Thatcher sourced from emmer is also complex consisting of several known race-specific genes and unknown genes that are only effective in adult plants. *Sr12* is one of the race-specific genes that originated from emmer. In several mapping studies strong field resistance in Tc was associated with the *Sr12* locus, although field races used in these experiments had virulence to this gene at the seedling stage. These results suggest that some major genes that are considered "defeated" as determined by seedling assays may still contribute to adult plant resistance. We have fine mapped *Sr12* to the centromeric region on chromosome 3BL and developed mutants that will assist in gene cloning and in dissecting complex Tc stem rust resistance at the molecular level. A KASP assay for a tightly linked SNP-based marker was developed that will facilitate selection for this gene in breeding programs.

The distribution and sequence variation of stripe rust resistance gene *Yr36* (*WKS1*) in wild emmer wheat natural populations

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Stripe rust, caused by *Puccinia striiformis* f. sp. *tritici* (*Pst*), is one of the most damaging diseases of wheat. The wheat gene *Yr36* (*WKS1*), derived from wild emmer wheat (*Triticum dicoccoides*), confers partial resistance to a broad spectrum of stripe rust races at relatively high temperatures. The structure of this gene shows unique architecture with a kinase and a putative START lipid-binding domains which are found only in the Triticeae tribe. We analyzed the distribution and the sequence variation of *WKS1* in accessions from a broad range of *T. dicoccoides* natural populations. We found that the *WKS1* is dispersed only in Israel, Jordan, Syria, and Lebanon, which are located at the Southern distribution range of *T. dicoccoides* natural populations in the Fertile Crescent. The distribution of *WKS1* in Israel is mainly clustered in the northern populations. Analysis of full length *WKS1* (> 7 kb) from 54 wild emmer wheat accessions identified very low nucleotide diversity ($\pi = 0.00019$), with higher nucleotide polymorphism in the intron regions of *WKS1* than in the coding regions. The coding region of *WKS1* included four haplotypes among all tested accessions, encoding three different putative WKS1 proteins (designated P1, P2, and P3), as compared to the published WKS1 sequence. Further stripe rust infection tests indicated that the putative WKS1 protein P1 is present in most of the partial resistant accessions. These results suggest WKS1 (P1) may be functional in conferring resistance to the stripe rust disease and can be utilized for wheat improvement.

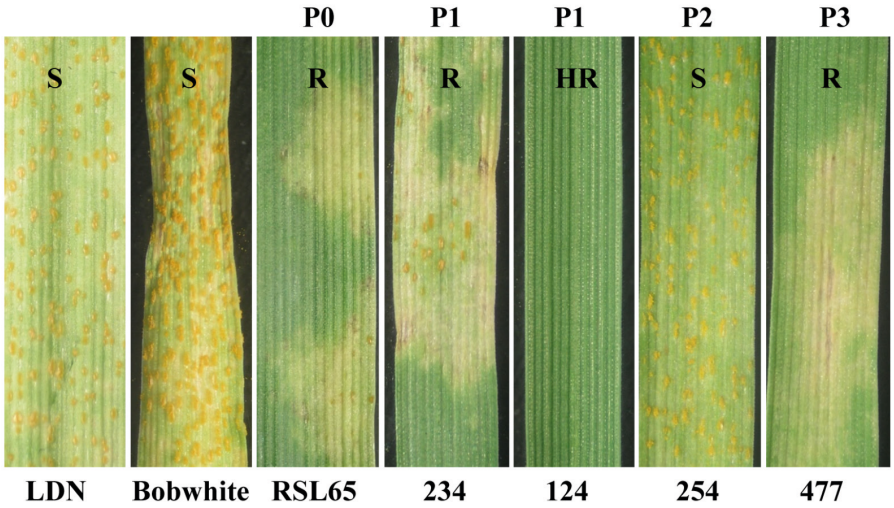

Figure 1. Stripe rust resistance phenotype in wild emmer wheat accessions carrying different WKS1 protein haplotypes. S, susceptible; R, resistant.

Genetic analysis of *Lr13* and *Ne2*

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Leaf rust, caused by *Puccinia triticina* Eriks. (*Pt*) is a worldwide disease of wheat (*Triticum aestivum* L.) that can be controlled using leaf rust resistance (Lr) genes. *Lr13* confers race-specific resistance to *Pt* at the adult-plant stage but can also be detected at the seedling stage under certain conditions. While virulence to *Lr13* is common, the combination of *Lr13* and *Lr34* has shown to be effective in providing field resistance. Another interesting feature of *Lr13* is its association with the hybrid necrosis gene *Ne2*. Hybrid necrosis occurs in plants carrying the complementary dominant genes *Ne1* and *Ne2*. When individuals carry both *Ne1* and *Ne2*, leaves begin to die shortly after they have fully elongated (progressive necrosis). In this study we further investigate the relationship between *Lr13* and *Ne2*. A doubled haploid (DH) population (n = 196) was developed from the cross of Thatcher/Thatcher-*Lr13* (a near-isogenic line carrying *Lr13*). DH lines were inoculated with *Pt* race BBBD at the adult-plant stage and the flag leaves were rated for their infection types. Each DH line was also crossed with Kubanka, a durum wheat that carries *Ne1*, and the F_1 progeny were sown and observed for progressive necrosis. Additionally, leaf tissue was collected from each DH line for genetic mapping of *Lr13* and *Ne2* using DNA markers. There were no recombinants between *Lr13* and *Ne2* in the DH population. Genetic mapping placed these genes in a chromosomal location that is consistent with previous genetic maps. Recombinant inbred lines (RILs) were developed from the crosses CSP44/WL711 and VL404/WL711. The RILs were inoculated with *Pt* isolate 104-1,2,3,(6),(7)+Lr24 the seedling stage and were also crossed with the cultivar Spica, a carrier of *Ne1*, to assess the leaf rust resistance and the hybrid necrosis phenotypes. Out of 171 RILs there were no recombinants between *Lr13* and *Ne2*. Seed of Manitou, Egret, Thatcher-*Lr13*, and Avocet R was treated with EMS to generate mutants for *Lr13*. There were eight mutants generated that lost *Lr13* activity and did not appear to carry large chromosome deletions. These mutants were subsequently crossed with Spica. All eight of these mutants also lost *Ne2* activity. Given the co-segregation of leaf rust resistance and hybrid necrosis following at least 500 opportunities for recombination in the above populations and the co-silencing of *Lr13* and *Ne2* in EMS mutants, it appears that *Lr13* and *Ne2* may represent the same locus.

Identification and and location of genomic regions controlling adult plant resistance to barley leaf rust

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Barley leaf rust caused by *Puccinia hordei* is best controlled through genetic resistance. Among the available resistance classes, adult plant resistance (APR) is considered to be more durable because of its association with additive/and or epistatic effects of multiple genes. The diversity of APR is however narrow and to date only two sources (*Rph20* and *Rph23*) are known, and there is a need to identify and map new sources. To cater this need, we mapped a DH population, Baronesse/Stirling (B/S) using three-years of phenotypic data and more than 10K DArTseq molecular markers. QTL mapping indicated involvement of three consistent QTLs on chromosome 2H, 5H and 6H closely linked with *DArT_3985732*, *DArT_3986031* and *DArT_3264010* markers at a genetic distance 70.82, 15.56 and 61.69cM respectively. The QTL detected on chromosome 5H is in the same region where the APR gene *Rph20* is located in Flagship/ND24260 population. The parent Baronesse showed positive amplification when genotyped with marker *bPb-0837* (closely linked to *Rph20*) indicating that 5H QTL corresponds to *Rph20*. The other two QTL responsible for APR are potentially new and currently being fine mapped for developing closely linked markers for gene pyramiding and marker assisted selection.

A meta analysis of partial resistance loci to powdery mildew in wheat

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Powdery mildew, caused by the biotrophic pathogen *Blumeria graminis* f. sp. *tritici* is a wheat disease of global significance.Two types of resistance are generally considered: race-specific resistance, which is usually monogenic, and race-nonspecific partial resistance that allows some infection, but slows down the disease development. Due to the often-observed short longevity of race-specific resistance, there is an increasing interest among breeders to utilize partial and race non-specific resistance, which can be facilitated by marker-assisted selection. Many sources of potentially durable partial resistance to powdery mildew have been subjected to QTL mapping studies during the last two decades. In the present meta analysis, we have reviewed the powdery mildew QTL mapping literature, with special focus on adult plant resistance of wheat cultivars and breeding lines with documented race non-specific resistance. The locations and confidence intervals of each reported QTL were projected onto the wheat consensus map. A total of 22 studies were reviewed, including a total of 96 QTL. QTL for adult plant resistance were reported on all 21 chromosomes, and the meta analysis showed a total of 39 QTL regions. Out of these, 14 regions were found to be common among at least two independent studies with documentation for race non-specificity of the resistance as shown in the following table:

Meta QTL	Resistance sources	Meta QTL	Resistance sources	Meta QTL	Resistance sources
1AS	Naxos	2BL	Massey, USG3209, RE9001, Naxos, Lumai 21	5BSc	Saar, Folke
1Ac	Oberkulmer, Bainong 64	2DL	Oberkulmer, Lumai 21, Folke, Naxos	5DL	RE714
1BL	Massey, USG3209, Saar	4BL	Forno, Avocet	6BS	Bainong 64, Folke
2AL	Forno, Massey, USG3209, Naxos	4DL	Bainong 64, RL6077	7DS	Opata 85, Fukuho-komugi, Saar, Naxos, Strampelli, Libellula, Chinese Spring
2BS	Festin, Folke, Pingyuan 50, Lumai 21	5AL	Oberkulmer, Saar, Folke		

Interestingly, all three known pleiotropic disease resistance loci *Lr34/Yr18/Pm38*, *Lr46/Yr29/Pm39* and *Lr67/Yr46/Pm46* showed up as meta QTL in this study. These are promising loci that in addition to powdery mildew resistance also will provide some protection against all three rust diseases in wheat, and should be combined with other confirmed QTL for powdery mildew resistance.

Nordic public-private-partnership in pre-breeding for disease resistance in barley

Ahmed Jahoor

Nordic Seed A/S, 4864 Holeby, Denmark

Members of Nordic Barley Pre-Breeding consortium:
Nordic Seed, Denmark
Lantmännen Lantbruk, Sweden (only in 1. Phase)
Graminor, Norway
Boreal Plant Breeding, Finland
Sejet Plant Breeding, Denmark
University of Copenhagen, Denmark (only in 1. Phase)
Swedish University of Agricultural Sciences, Sweden
Agricultural University of Iceland, Iceland
Natural Resources Institute (LUKE), Finland
Coordinator: Ahmed Jahoor

In total 180 barley lines were collected from the participating breeding companies in Denmark (Sejet Plant breeding and Nordic Seed) Finland (Boreal) Iceland (LBHI), Norway (Graminor) and Sweden (Lantmännen) (30 lines were provided from each participant). These 180 lines were genotyped using the *9K iSELECT SNP* chip 42 Simple Sequence Repeats (SSRs) markers. Meanwhile, these 180 lines were under the participants' observation phenotyping 7 major barley diseases and 12 agronomical traits underlying different climatic conditions. Barley diseases were studied under 28 different environments and the agronomical traits were studied under 92 different environments during 2012 and 2013 spring barley growing seasons. Association mapping was conducted using 7000 SNPs and disease resistance as well agronomic traits. A handful of linked markers were identified in this material. Usefulness of these linked markers have been validated at each companies. During the presentation, the population structure of the material as well as the results of association mapping will be presented. In addition, MAGIC populations are being developed for different disease resistance including eight parents for each crosses. In total four populations will be estabilished.

Abstracts from oral presentation

Session 5

Multiple disease management

Integrated management of biotrophs and necrotrophs

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In many climates, cereal production is threatened by necrotrophs and biotrophs, so effective control of two or more contrasting pathogens is required. Unfortunately, effective disease control applies an evolutionary pressure selecting for pathogen strains which are insensitive to fungicides and/or virulent. Here we consider how integration of host resistance and fungicides can help to maximize efficacy and minimise evolution.

Integration for efficacy: The need for fungicide treatment and the economic optimum fungicide dose is determined primarily by disease severity and the yield loss per unit severity (Phytopath. 91, 708-716). The former is reduced by host resistance and the latter by disease tolerance (Ann. App. Biol. 154, 159-173). If the combination of resistance and tolerance is sufficiently effective, or the environment is not conducive to epidemic development, the optimum dose becomes zero. But in practice treatment is often required. Moreover, a single fungicide mode of action or a single resistance gene seldom provide a commercially acceptable level of disease control, and single control methods often prove insufficiently durable when deployed alone. Hence fungicides are often mixed, resistance genes pyramided, and both integrated. This results in vast numbers of possible combinations of cultivars (of differing host resistance) and fungicides, from which to choose effective combinations. Fortunately, the joint action of mixtures of modes of action and the efficacy of pyramiding increasing numbers of resistance QTL (against biotrophs and necrotrophs) are both reasonably predictable from their individual efficacies, using a multiplicative survival model (Pl. Path. Doi 10.1111/ppa.12288). The same approach also predicts the joint action of sequential fungicide applications (Pl. Path. 52, 638-647). Combinations of host resistance and fungicide which should deliver the required level of control can therefore be identified by field experiments and calculation.

Integration for durability: A key determinant of durability of host resistance is the rate at which one or more virulent strains are selected for in a pathogen population, thus increasing in frequency until control is eroded. Similarly the effective life (durability) of a fungicide mode of action is determined substantially by the rate of selection for insensitive strains. Principles governing the selection of fungicide insensitive strains have been derived and tested extensively against experimental data (Ann. Rev. Phytopath. 52, 175-195). These principles show that reducing the difference in the per capita rate of increase of sensitive and insensitive strains, slows selection. The difference can be reduced by slowing the rate of increase of both strains, for example, by adding a second fungicide mode of action which is effective against both strains. By extension, it can be inferred from the governing principles that: (i) partial (rate-limiting) host resistance, which is effective against fungicide sensitive and insensitive strains should slow selection for fungicide insensitivity, and (ii) fungicide treatment, which is effective against avirulent and virulent strains, should slow selection for virulence. Complete reliance on either host resistance or fungicide is likely to be less durable than an integrated approach, but experimental evidence is required for this hypothesis.

Reconciling high efficacy and low selection: Deploying more than one fungicide mode of action in a mixture creates concurrent selection for strains which may be insensitive to either or both (Phytopath.

103, 690-707). Similarly, deploying a 'mixture' of fungicides with host resistance genes, creates concurrent selection for strains which may be insensitive and virulent. The need for high efficacy and low selection can, in principle, be best reconciled by an integrated approach, where most of the control is obtained from the control method at lowest risk of erosion by pathogen evolution. Pathogens differ markedly in their propensity to evolve insensitivity and virulence. Such contrasts could be exploited, through integrated strategies, to deliver effective and durable control of biotrophic and necrotrophic pathogens.

Resistance in winter wheat to stripe rust and virulence of the pathogen in eastern United States from 1990 to 2014

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Before 2000, stripe (yellow) rust caused by *Puccinia striiformis* f. sp. *tritici* (*Pst*) was not considered an important disease in the hard red winter wheat (HRWW, approximately 12.5 million ha annually) and soft red winter wheat (SRWW, approximately 3.3 million ha annually) regions east of the Rocky Mountains. There were no direct breeding efforts to incorporate stripe rust resistance, but the 1BL:1RS translocation with resistance gene *Yr9* was in several cultivars and the 2NS:2AS translocation with *Yr17* was in several breeding lines. These translocations were used primarily for resistance to leaf (brown) rust provided by resistance genes *Lr26* and *Lr37*, respectively. However, each translocation also conferred resistance to the regional *Pst* population described as race CDL-3 (now PST-3) with narrow virulence. In 2000, an exotic strain of *Pst* (based on AFLP fingerprint patterns) emerged. The new strain overcame *Yr9* resistance, was more aggressive, more adapted to warm temperatures, and had enhanced overwintering capabilities. These and other unknown traits conferring superior fitness allowed the new strain to immediately and permanently replace the old strain in eastern United States. Stripe rust then became the most important disease across most of both regions. The objective of this presentation is to document the effective resistance genes in regional cultivars and the virulence changes on those genes in the *Pst* population from 1990 to 2014. In 2000, the new strain caused severe disease on many cultivars and breeding lines. However, diverse cultivars and lines, including lines with *Yr17*, had no disease. The effective resistance genes in these cultivars and lines were easily selected in the field and were used by breeders to develop a large number of resistant cultivars. In 2010, *Yr17* virulence emerged in combination with *Yr9* virulence, and all contemporary cultivars were susceptible at the seedling stage. However, many cultivars and lines were highly resistant at adult stages in the field, indicating adult-plant resistance (APR). Using 12 regional cultivars as differentials and regional isolates from 1990 to 2013, five major adult-plant virulence patterns were characterized, indicating that these resistance genes are race specific. The cultivar 'Mason' was susceptible to the old strain but had effective APR to all regional isolates since 2000. SRWW had a greater number of diverse sources of effective resistance than HRWW. The findings of this study indicate that race-specific APR is protecting HRWW and SRWW from stripe rust in eastern United States. Furthermore, these findings support the use of resistances found in contemporary regional cultivars and breeding lines for identifying virulence changes that are important in the field.

Minimizing stripe rust of wheat, *Puccinia striiformis* f. sp. tritici, by an integrated pest management strategy

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Yellow (stripe) rust, caused by the fungus *Puccinia striiformis* f.sp. *tritici*, is on the rise. For some years, new races occur in Germany and infest not only wheat, but also triticale. In order to prevent further spread of yellow rust, resistant varieties are essential. To minimize the infestation, we analyse a novel approach of integrated pest management in a project funded by BMEL. The aims of this project are (1) monitoring virulences, pathotypes and diversity of German Yr populations, (2) testing the sensitivity of Yr populations to the most common fungicides, (3) identifying race-specific resistance genes in new wheat germplasm and (4) selecting new, durable adult plant resistances by biotechnological methods.

In 2014, the monitoring of the German Yr population revealed that the Warrior race is present in 69% of the samples. This race, first detected in 2011, spreads in Europe and infests wheat and triticale. Our virulence analysis suggest that only a few monogenic resistances remained effective (*Yr 5, 8, 10, 15, 24*) in Germany. We developed a miniaturized test system to investigate the fungicide sensitivity of the German Yr populations. New races and climate changes are the reasons for the need of durable adult-plant resistances. We analysed four selected wheat populations and the results of field tests suggested a wide range of genetic variation. These populations will be genotyped on a 15K Infinium wheat chip to map the positions of new durable resistance genes. Genetic control of Yr by effective adult-plant resistances will offer a cost effective and environmental-friendly strategy to reduce losses in wheat production.

Heavy Attacks of Yellow Rust in Southern Sweden for the Last Seven Years

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Yellow rust *(Puccinia striiformis)* has been monitored in wheat during the last 25 years and since 2007 severe attacks of yellow rust have been very common every year in the southern part of Sweden. The repeated appearance of new yellow rust races continues to challenge varietal resistance. In 2007 became suddenly the former resistant varieties Tulsa and SW Gnejs very susceptible to yellow rust. The heavy attacks were caused by a new race, the Tulsa race, and that was determined by MS Hovmøller, Aarhus University. Mild winters and large areas of the variety Tulsa (40%) susceptible to the new race made the situation bad. A few years later, in 2011, a new race, the Kranich race, appeared and the former resistant variety Kranich now proved to be very susceptible. The Kranich race has a broad virulence spectra and several varieties cultured, e.g. Audi and Cumulus, also proved to be very susceptible to the Kranich race. During the last years both the Kranich race and the Warrior race have been common in Sweden. However, the Kranich race has been the dominating of the two races until now.

In 2008 there were sporadic attacks of yellow rust on triticale and in 2009-2010 triticale was aggressively attacked by the new yellow rust race, a race named Triticale aggressive. It was predominantly the varieties Dinaro and Cando that were infected and very big yield losses appear in untreated crops. In 2012 there was again high incidence of yellow rust in triticale, e.g. Tulus. During the last years Kranich and Warrior races have dominated the yellow rust and they do also to a certain extent attack triticale.

Yellow rust can reduce yields by 50% or more in untreated crops. Field trials in southern Sweden have shown yield increases for three treatments, up to 9 ton/ha, in susceptible winter wheat varieties. The important timing for treatment starts at BBCH 30. In mild autumn symptoms of yellow rust have easily been found in November in many cultivars. Field trial with fungicide treatment in the autumn showed no impact on the yellow rust development in the spring or yield response. Azoles, strobilurin and morpholine fungicides are all effective to protect crops from yellow rust. In Sweden there are only a few azoles approved, propiconazole, prothioconazole and difenoconazole in mixture with propiconazole Timing of the fungicide spraying has shown to be more important than the dose. It is of outermost importance that farmer fields are monitored regularly, since new races can occur.

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Evaluation of Nepalese wheat germplasm for rust and powdery mildew resistance

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The three rusts (stripe, leaf and stem) are the major biotic stress in Nepalese wheat along with powdery mildew caused by unpredictable weather conditions. Seventy one Nepalese wheat landraces from different parts of Nepal, 14 Nepalese wheat varieties and 11 exotic check wheat lines were tested for the three rusts and powdery mildew resistance at Khumaltar, Lalitpur, Nepal. Natural epiphytotic condition was separately used for stripe rust (normal sowing), leaf rust (late sowing), stem rust (very late sowing) and powdery mildew (increasing humidity). Similarly, spreader rows were sown in screening plots and stripe rust was inoculated on spreader rows of 'Morocco'. Twenty three Nepalese wheat landraces, 9 Nepalese wheat varieties and 5 exotic wheat lines showed powdery mildew resistance at Khumaltar, Lalitpur condition. Chinese spring, Halberd and Bhrikuti were susceptible while WK1204 and BL3623 were resistance to powdery mildew. Microsatellite markers (for PM39, PM-MIAB10 and PM8) of powdery mildew resistance were used and a good association (65.12%) was observed with marker Xwmc44 (for PM39). Similarly, twenty two Nepalese wheat landraces, 6 wheat varieties and 6 exotic wheat lines were resistant to stripe rust. Leaf rust resistance was observed in 53 wheat landraces, 14 varieties and 9 exotic lines. However, 11 landraces, 6 varieties and 6 exotic lines were resistant to both stripe and leaf rust. For stem rust, 69 land races were found resistant.

Based on "Avocet" background isogenic lines; stripe rust resistance gene Yr5, Yr7, Yr9, Yr10, Yr24 and YrSp were effective in Khumaltar condition. Similarly, Sr26, Sr36, Sr31 were effective for stem rust. Yr9 marker allele was present in Bhrikuti (YR=0R), PasangLhmu (YR=0R), Annapurna-1 (YR=5R, Lr=0R) and NPGR# 6720 (YR=0R). Similarly, Annapurna-1 showed marker allele for (YrR61, Qyr. osu-5A, Sr2, Sr22 and Sr31), Bhrikuti (Yr=0R, Lr=0R; Lr34/Yr18); BL3623 (Yr=0R, Lr=0R; Yr48, Lr46/Yr29), WK1204 (Yr=0R, Lr=0R; YrR61, Qyr.osu-5A, Lr46/Yr29, Sr22 and Sr2), PVN76 (Yr=0R, Lr=10R; Lr46/Yr29, Sr2, Sr22), NPGR#10540 (Yr=0R, Lr=60S; Lr46/Yr29, Sr22) and NL1073(Sr=0R; Sr2, Sr22). Nine Nepalese wheat landraces are resistant to all three rusts; however, they did not show any of the tested marker alleles. No linked markers for stripe rust resistant gene showed any association in the newly released resistant wheat variety NL1073 (Francolin#1) although Xwmc44 (for Lr46/Yr29) showed good association (68.75%) in other lines.

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Challenge, Progress, and Perspective for the Implementation of Integrated Management of Wheat Stripe (Yellow) Rust Caused by *Puccinia striiformis* f. sp. *tritici* in China

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China is the largest wheat producer and consumer in the world. Wheat stripe (yellow) rust caused by *Puccinia striiformis* f. sp. *tritici* (*Pst*) is the most destructive foliar disease of wheat in all winter wheat regions. Epidemics of the disease have occurred at varying intensity all over the country and are notably endemic in the Northwest and Southwest wheat growing areas since 1950. Annual losses of wheat yields due to stripe rust have averaged about 1 million tons. The most severe epidemics occurred in 1950, 1964, 1990 and 2002, which caused yield losses of wheat approaching 6.0 million tons, 3.2 million tons, 2.65 million tons and 1.4 million tons, respectively. Traditionally, wheat stripe rust is considered a low-temperature disease and frequently occurs in temperate areas with cool and moist conditions. However, recent severe epidemics have occurred in warmer areas where the disease was previously infrequent or absent. This led to the presumption that *Pst* populations have adapted higher temperatures enabling it to inflict damage in previously unfavorable environments. And a noticeably increased frequency of race V26 with a combined virulence for *Yr24/26* and *Yr10* firstly detected in 2010 is the major virulence change recorded in recent years compared with the results on an annual basis. Based on the geographical conditions, wheat plantation, occurrence and dispersal of disease, wheat stripe rust in China can be divided into three major zones, namely the autumn sources of inoculums, the spring sources of inoculums, and spring epidemic areas. A major strategy of headstream management has been put forward, i.e. "integrated management of wheat stripe rust in the sources of inoculums to protect wheat safety plantation in all over the country". A series of effective measures for the control of disease, including the improving cultivar resistance, changing cultivation crops, regulating sowing date, seed-dressing with fungicides, and spraying fungicides in the initial stage of disease, has been developed. The integrated management systems based on the biodiversity have been set up in the areas of inoculum sources of *Pst*, respectively, which have been widely applying in wheat production resulting in the sustainable control of wheat stripe rust epidemics and remarkably economic efficiency. It is anticipating in an alternative strategy for limiting virulence evolution, and development of early forecast system and the ecological control measures of disease in the areas of inoculum sources of wheat stripe rust in the near future.

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How can we achieve resistance to biotrophic and necrotrophic diseases simultaneously?

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Durable resistance to a disease, even if it is only partial, is highly desirable in plant breeding because it increases the stability of crop productivity and lessens uncertainty in crop management. Some important genes for durable resistance have a broad-spectrum effect against all genotypes of a pathogen species or even several pathogens. Outstanding examples of such genes are *mlo*, which has provided almost complete, durable resistance to powdery mildew (*Blumeria graminis*) in spring barley for over 35 years, and the leaf-tip necrosis (LTN) genes such as *Lr34* and *Lr46* for partial resistance to rusts (*Puccinia* spp.) and mildew in wheat. All these genes are widely used; about half the varieties of spring barley in Europe have *mlo* mildew resistance, while *Lr34* and other LTN genes are present in many spring wheat varieties world-wide.

These genes for durable resistance to biotrophic fungi incur significant costs by increasing susceptibility to non-biotrophic diseases. We have shown that the LTN genes increase susceptibility to Septoria tritici blotch in wheat (*Zymoseptoria tritici*) in both seedlings and adult plants. This trait may be associated with an enhanced rate of senescence in wheat with LTN genes. These genes also increase susceptibility of wheat seedlings to blast (*Magnaporthe oryzae*) and Ramularia leaf spot (*Ramularia collo-cygni*). In barley, *mlo* genes (non-functional alleles of the *Mlo* gene) increase susceptibility to several non-biotrophic fungi. We have shown that enhanced susceptibility of *mlo* barley lines to Ramularia leaf spot is generated by a complex physiological process involving interactions between different pathways leading to cell death. The existence of trade-offs between responses of cereals to biotrophic and non-biotrophic fungi is further demonstrated by the suppression of powdery mildew in plants which have previously been inoculated with virulent isolates of *Z. tritici*.

These trade-offs of genes for durable resistance to biotrophs need to be mitigated in breeding programmes which aim to produce commercially desirable cereal varieties. Their practical significance may vary between regions according to the prevalence of different diseases. Research on the mechanisms by which they increase susceptibility to non-biotrophs may identify opportunities to improve control of those pathogens without losing the benefit of controlling rusts and mildews. Meanwhile, a Darwinian approach in which plant breeders use diverse germplasm and select advanced lines with a combination of desirable traits is improving control of Ramularia leaf spot in spring barley varieties with high yield, excellent quality and *mlo* mildew resistance. A similar approach may offer combined control of both biotrophic and non-biotrophic fungal diseases in wheat varieties with LTN genes. An important consideration is to have access to a range of field trial sites in which elite germplasm can be screened against all important diseases.

Effect of Sequential or Co-inoculation of *Blumeria graminis* f. sp. *tritici* and *Parastagonospora nodorum* on disease development on Wheat

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Sequential and simultaneous infection of plants by biotrophic and necrotrophic pathogens are common under field conditions. However, data on the effect of co-infection of a host plant with these two types of pathogens are limited. We conducted a controlled greenhouse experiment to study disease development on wheat plants inoculated simultaneously and sequentially with the biotroph, *Blumeria graminis* and the necrotroph, *Parastagonospora nodorum*. Spring wheat, cv. Zebra was grown in a greenhouse compartment and sprayed with aqueous spore suspensions of (i) *B. graminis* f. sp. *tritici* alone, (ii) *P. nodorum* blotch alone, (iii) *P. nodorum* followed by *B. graminis* f.sp. *tritici* after 24hr, (iv) *B. graminis* f.sp. *tritici* followed by *P. nodorum* after 24hr, (v) both *P. nodorum* and *B. graminis* f. sp. *tritici* at the same time, and with (vi) water (control). Disease severity and incidence were assessed 7, 15 and 21 days after inoculation. We found that development of powdery mildew and glume blotch symptoms significantly vary depending on the order of inoculation ($P = 0.01$). In the first trial, 15 days after inoculation, glume blotch disease severity were 21% on *P. nodorum* followed by *B. graminis* f.sp. *tritici* inoculated plants, 55% on *P. nodorum* alone inoculated plants, 56% on both *P. nodorum* and *B. graminis* f.sp. *tritici* at the same time inoculated plants, 71% on *B. graminis* f. sp. *tritici* followed by *P. nodorum* inoculated plants, and no disease developed on the control (water sprayed) plants. Similarly, 15 days after inoculation, powdery mildew severity was more than 2-fold on *P. nodorum* followed by *B. graminis* f. sp. *tritici* inoculated plants compared to the other inoculations. The observed variation in the susceptibility of the host in the sequential inoculated plants could be due to host resistance being less efficient in recognizing the mixed infections or the second arriving pathogen may become more aggressive in the presence of another competing species. Several authors suggested that competition among different pathogens for a limited food sources could lead to the evolution of more virulent and aggressive races or to a change in the reproduction rate of the pathogen. Most research on pathogen host interactions are based on inoculations with one type of pathogen, biotroph or necrotroph, but consideration of the interactions between the two is critical for disease prediction and for designing appropriate disease management strategies.

The progressive failure of VPM resistance to all three wheat rusts on the Australian continent

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The VPM resistance comprises three rust resistance genes *Yr17*, *Sr38* and *Lr37* that occur on a translocation segment derived from *Triticum ventricosum* and has been widely incorporated into common wheat germplasm in Australia. The Australian continent is divided into three wheat growing regions: the summer-dominant rainfall areas of northern NSW and Queensland (Region 1); the uniform and winter-dominant rainfall areas from NSW, Victoria, South Australia and Tasmania (Region 2); the winter-dominant rainfall area of southwestern Western Australia (Region 3). Region 3 is separated from the remaining wheat growing regions by more than 1000 km of desert.

Virulence for *Yr17*, *Sr38* and *Lr37* has evolved over time and space in the Australian wheat rust flora (Table 1). The deployment of the VPM resistance in the variety Camm in Region 3 provided the selective pressure for virulence evolution to *Yr17*, *Sr38* and *Lr37*, yet virulence only evolved for *Sr38* and *Lr37*. Wheat stripe rust pathotype 134 E16 YrA+ was introduced to Region 3 in 2002 from USA, yet no further step-wise mutations have occurred despite occasional severe epidemics. In contrast, 134 E16 YrA+ spread rapidly in eastern regions where it produced two derivative pathotypes with virulence for *Yr17*; these pathotypes caused commercial yield losses. Virulence for *Yr17* in earlier pathotypes did not impact the wheat industry.

Table 1. The distribution and lineages of pathotypes of wheat stem, leaf and stripe rusts with VPM virulence in Australia, including their current distribution across the three wheat growing regions.

	Pathotype	Year detected	Present in Australian wheat regions[1]			Derived pathotypes	Present in Australian wheat regions[1]		
Wheat stem rust (*Puccinia graminis* f. sp. *tritici*)	34-1,2,7 +Sr38	2001	1	2	3	34-1,2,7 +Sr38 +Sr21 34-1,2,7 +Sr38 +Yalta Low 34-1,2,7,10 +Sr38		2	3 3 3
Wheat leaf rust	104-1,2,3,(6),(7),11 +Lr37	2002	1	2	3				
(*Puccinia triticina*)									
Wheat stripe rust	104 E137 +Yr17	1999	1	2					
(*Puccinia striiformis*)	104 E137 +YrA +Yr17	2000		2					
f. sp. *tritici*)	134 E16 +YrA +Yr17	2006	1	2		134 E16 +YrA +Yr17 +Yr27	1	2	

[1] Numbers in bold indicate the wheat region where the pathotype was first detected.

Injury profile simulator (IPSIM), a modelling platform to design qualitative models predicting injury profiles as a function of cropping practices and production situations. Application to the wheat brown rust

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In order to reduce the reliance of agriculture on pesticides, there is a need for tools to help design agroecological management strategies of pests. In particular, the "vertical integration" (combination of several control methods) and the "horizontal integration" (simultaneous management of several pests) embedded in the Integrated Pest Management concept, require methodological developments to be successfully implemented. We propose an innovative modelling framework in order to help design qualitative models to represent the impact of cropping practices, soil, weather and field environment on injury profiles caused by multiple pests (plant pathogens, weeds and animal pests). This communication presents the basic principles of the approach and an application to wheat, the main arable crop in Europe in terms of cultivated area and to a major disease of wheat worldwide, leaf or brown rust.

IPSIM is a simple generic hierarchical qualitative modeling platform based on the DEXi software. The main assumption of IPSIM is that each injury profile that can be observed in a given field only depends on the associated cropping system and part of the production situation (described in terms of soil, climate and field environment). DEXi is thus used to easily design hierarchical deterministic Bayesian networks (i.e. with probabilities only equal to 0 or 1) based on nominal and ordinal attributes describing agroecosystems. The structure of the model and the way attributes are aggregated together are determined using expert knowledge, along with technical and scientific literature.

This platform was used successfully to develop IPSIM-Wheat-brown rust, a model that predicts brown rust severity, an important foliar disease caused by *Puccinia triticina*, as a function of cropping practices, soil, weather and field environment (Figure 1). IPSIM-Wheat-brown rust, a sub-model of IPSIM-Wheat, was designed and its predictive quality was assessed on a large dataset (1739 observed fields, over 19 regions in France and 15 years). The model proved to have a good predictive quality: 0.68 weighted

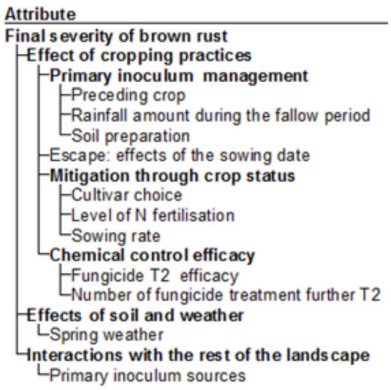

Figure 1. Structure of the IPSIM-Wheat-brown rust.

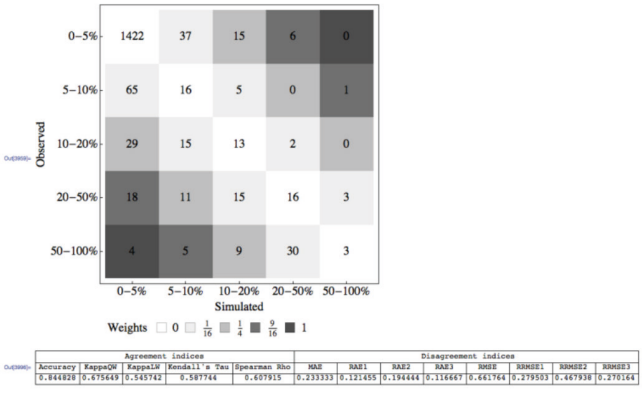

Figure 2. Confusion matrix of the IPSIM-Wheat-brown rust model and marginal distributions.

Kappa; 84.7% of the simulated classes encompassed the observed values and 94.5% had at most a difference of one class only (Efficiency = 0.41, Root Mean Square Error of Prediction = 11%; bias = 0.84%) (Figure 2). It is remarkable that these performances were obtained without any calibration.

IPSIM-Wheat-Brown rust does not aim to precisely predict the incidence of brown rust on wheat. It rather aims to rank cropping systems with regard to the risk of brown rust on wheat in a given production situation through *ex ante* evaluations. IPSIM-Wheat-brown rust can also help perform diagnoses of commercial wheat fields. Its structure is simple and combines available knowledge from the scientific literature (data and models already available) and expert knowledge. IPSIM-Wheat brown rust is now available to help design cropping systems with a low risk of brown rust on wheat and less reliant on pesticides, in a wide range of production situations. IPSIM-Wheat-brown rust is one of the sub-models of IPSIM-Wheat, a future model that will predict injury profile on wheat as a function of cropping practices and the production situation.

Abstracts of poster presentation

Race Characterization and New Virulence Spectra of Asexual and Sexual Populations of *Puccinia graminis* f. sp. *tritici* in China in 2009-2013

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Stem rust of wheat was once the most destructive disease which has been successfully under control for decades mainly through genetic methods in most countries including China, which also thanks for the pathogen race and virulence surveillance. The present survey was made using 209 *Puccinia graminis* f. sp. *tritici* isolates (thirty initially originated from *Berberis* spp.) collected nationwide during 2009-2013. A new Chinese differential host system (including 5 sets of Sr-gene lines) was used in the study. The results were shown that five race clusters (21C3, 34, 34C1, 34C6 and 34C3) or 20 races were identified and race cluster 21C3 was the most prevalent (57.9% in occurrence frequency), race 21C3CTHTM, the most dominant (24.9%), and race 21C3HTTTM, though 1.9%, was the broadest by virulence spectrum. Among those race clusters and races identified, race cluster 34C6 and eleven races including six *Sr5*+*Sr11*virulence associations were newly discovered in China. Further detailed investigation was also conducted into the race and virulence variation comparison between asexual and sexual populations. The results were indicated that all of the 30 sexual isolates were exclusively categorized into 34 clusters (34, 34C3 and 34C6) which included the new cluster 34C6, seven new races (so high as 96.6%) and five *Sr5*+*Sr11* virulence (so high as 63.3%) while the 179 asexual isolates were mostly composed of cluster 21C3 (67.5%), of 5 new races(so low as 9.7%) and of two *Sr5*+*Sr11* virulence (so low as 3.9%). It is indicated that the variation levels of races and virulence of sexual population were strikingly higher than those of asexual population and therefore that the sexual population structure was obviously different from the asexual one. In all, all of the evidences supported that sexual isolates are prone to being variable more in races and virulence. The virulence frequencies of 209 isolates were also tested with 48 single Sr genes lines. It was indicated that nine single Sr genes(*9e*, *26*, *31*, *33*, *35*, *37*, *38*, *47* and *Tt3*) were all highly effective, five (*7b*, *9a*, *9b*, *9d* and *McN*), completely ineffective, six (*30*, *35*, *36*, *21*, *39*, *22* and *5*), 57.9%-96.2% effective and the remaining 28 Sr genes, more than 50% ineffective.

Key words: New physiological race; Sexual population; *Puccinia graminis* f. sp. *tritici* ; *Berberis*; *Sr* genes.

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Yellow rust pathotypes and resistance gene postulations in Tunisian wheat

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The yellow rust, caused by *Puccinia striiformis* is one of the most common wheat diseases mostly damaging in cool and temperate regions. Epidemics on wheat have increased worldwide with the emergence of an aggressive strain and tolerant to high temperature (PstS1/S2), which reached North Africa and south of France since 2004 and, a multivirulent exotic strain (Warrior) that spread in Western Europe in 2011. The selection of resistant varieties brings to agriculture effective solutions to reduce the use of pesticides. However, races of the pathogen quickly overcome resistance genes. Therefore, the selection of varieties with durable resistance to yellow rust interests many bread and durum wheat breeders. To conduct a genetic control strategy against *P. striiformis*, it is essential to study the pathotype dynamics and the resistance genes in wheat. We determined the virulence combinations using the European and world differential sets discriminating between 23 virulence factors and the simple sequence repeat (SSR) diversity of 20 yellow rust isolates collected in Tunisia in 2014 in the major wheat growing areas. In addition, we postulated resistance genes in 28 wheat Tunisian varieties and accessions at the seedling stage in order to identify resistant genotypes and resistance genes for their valuation in breeding programs. The predominance of the race 239E175V17 at the origin of the epidemic in Tunisia in 2014. Genetic analysis revealed that this race is exotic and distinct from the Northwest European and the Mediterranean genetic groups, previously presentin Tunisia. Furthermore, resistance gene postulations showed the presence of Yr6, Yr7, Yr9 + Yr4, Yr3 and Yr25 in Tunisian wheat varieties and accessions. In addition, the commercial durum wheat varieties 'Khiar' and 'Salim' and bread wheat 'Tahent' appeared to be resistant against the local Northwest European and Western Mediterranean pathotypes, as well as against Warrior race. These varieties are thus a short-term alternative to fight against the development of yellow rust in Tunisia. These results should be confirmed by molecular and pedigree analyses of the accessions.

The function of *Berberis* spp. in *Puccinia graminis* f. sp. *tritici* in field in China

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Wheat stem rust, caused by *Puccinia graminis* f. sp. *tritici*, is one of the most destructive diseases on wheat worldwide and resulted in significant yield loss due to epidemics of the disease. Previous studies demonstrated that barberry (*Berberis* spp.) as alternate host for the stem rust fungus is an important element for contributing to epidemics of the disease in some countries, not only providing initial inoculum but also generating new races. However, in China, it has been not suggested that barberry plants play no role in wheat stem rust development and virulence variation of *P. graminis* f. sp. *tritici* so far. In this study, severe rust infections on *Berberis shensiana*, *B. brachypoda*, *B. potaninii*, *B. soulieana,* and *B. aggregata* were observed during field surveys in 2011and 2012. Based on artificial inoculation of wheat seedlings (cv. Mingxian 169) in laboratory with aeciospores from natural-infected barberry bushes, 185 samples of *P. graminis* f. sp. *tritici* were isolated. From the 27 selected samples that were tested on a set of differentials used to differentiate *P. graminis* f. sp. *tritici* races in China, 18 races were tested, of which 8 races were new and others were of Chinese 21 and 34 race groups. In addition to the information of virulence or avirulence patterns on the Chinese differentials, none of the races were virulent to resistance gene *Sr31*. The virulence frequencies based on single *Sr* genes or differentials ranged from 0 to 96%. This study indicates that the stem rust fungus can generate new races through sexual reproduction on *Berberis* spp. in natural conditions in China.

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Virulence structure of *Puccinia coronata* f. sp. *avenae* in Central and South Eastern Poland

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Crown rust, caused by *Puccinia coronata* f. sp. *avenae*, belongs to the most wide spread and harmful oat diseases and this pathogen causes severe epidemics worldwide. The number of incidence of oat crown rust in Poland, especially in Central and South Eastern part, has increased recently. One possible explanation could be a lack of resistant Polish oat cultivars. Moreover weather conditions as mild winters, late springs and wet summers support infection.

Virulence of *P. coronata* is not well known in Poland and because of this virulence of Polish oat crown rust population was studied on 28 reference lines with different *Pc* genes. Multiple samples of *P. coronata* f. sp. *avenae* were collected from about 50 different oat fields during the summer of 2014. Samples were taken from oat flag leafs both random farm fields and field plots of Polish breeding companies in Central and South Eastern Poland. Single pustule isolates were obtained from the field collections and 10 of them were multiplied in fitotron. Host-pathogen test on 10-days differentials leaves fragments under control temperature, humidity and lighting conditions were used to evaluate virulence of isolates. Assessment of leaf infection was performed after 12 days using a qualitative scale of S, MS, MR, R, and HR, where reaction phenotypes were S = susceptible, large to moderately large pustules with little or no chlorosis; MS = moderately susceptible, moderately large pustules surrounded by extensive chlorosis; MR = moderately resistant, small pustule surrounded by chlorosis; R = resistant, chlorotic or necrotic flecking; and HR = highly resistant, no visible reaction.

Resistance genes *Pc*52, *Pc*59, *Pc*60, *Pc*68, *Pc*71 and *Pc*91 were effective to all tested isolates. Only one isolate was virulent if resistance was covered by *Pc*50, *Pc*51, *Pc*70 or *Pc*94 genes. Sporadic virulence on *Pc*57 was observed. Relatively effective were also *Pc*39, *Pc*48, *Pc*58 and *Pc*104. Other *Pc* genes were defeated by the majority of the tested samples. Our results indicate that races obtained from the field plots of Polish breeding companies were the most aggressive.

Results showed that many sources of resistance are still effective against crown rust races occurring in Poland, however cumulating the *Pc* genes in one cultivar should significantly extend oat resistance.

Virulence structure of the Polish oat powdery mildew populations in 2014

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Avena sativa L. (common oat) is a plant grown around the world. In Poland, oats sown area reaches 478,572 of hectares. The largest crop regions are located in the Eastern and Central parts of the country. The common oat is a cereal sensitive to many diseases which can significantly reduce the amount and quality of yield. Powdery mildew caused by *Blumeria graminis* DC. f. sp. *avenae* Em. Marchal. is one of the most important foliar diseases of common oat in the cooler and humid regions of Europe, including Poland. In 2014 there was observed a high severity of powdery mildew symptoms in all parts of Poland. Efficiency in ensuring effective genetic resistance in cultivars depends on the proper selection of sources of resistance and knowledge of the structure and dynamics of changes in virulence frequency of powdery mildew.

The aim of presented study was determination of virulence structure of powdery mildew populations in Poland in 2014. Powdery mildew populations were collected in 10 different localizations in Poland, from every population 10 single spore isolates were obtained. Host-pathogen tests were made on 5 control lines and cultivars with different powdery mildew resistant genes and 2 fully susceptible cultivars.

In 2014 almost all isolates were virulent to *Pm1, Pm3* and *Pm6* genes. Mean virulence to *Pm1* was 77% and to *Pm6* up to 91%. Virulence to *Pm3* was also very high and reached 88% in average. On the contrary the level of virulence to *Pm7* was very low and reach 7% in average. All used isolates were avirulent to *Pm4* gene. Presented analysis showed that powdery mildew populations existing in Poland were able to break down resistance conditioned by *Pm1, Pm3* and *Pm6* genes. *Pm4* and *Pm7* genes are still effective in Polish conditions.

Virulence of *Puccinia triticina* Eriks. on *Tritcum* and *Aegilops* species

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Puccinia triticina Eriks. populations are usually studied with infected material originated from *Triticum aestivum* L., and *T. durum* Desf. However, other species of wheat and *Aegilops* can also be affected by the pathogen. Our main objective was to analyze the virulence of *P. triticina* on hexaploid, tetraploid, and diploid species of wheat and *Aegilops*.

Leaves bearing uredinia of leaf rust were collected in 2014 in the field plots of Dagestan Experimental Station of VIR (Russia) from hexaploid species *T. aestivum* L., *T. compactum* Host, *T. macha* Dekapr. et Manabde, *T. petropavlovskyi* Udacz. et Migusch., *T. spelta* L., *T. sphaerococcum* Perc., *T. vavilovii* Jakubz., *Ae. juvenalis* Thell., *Ae. trivialis* Zhuk., from tetraploid species *T. aethiopicum* Jakubz., *T. dicoccum* (Schrank) Schuebl., *T. turanicum* Jakubz., *Aegilops crassa* Boiss., and diploid *Aegilops tauschii* Coss.; in the experimental field of Institute of Cytology and Genetics (Novosibirsk, Russia) from *T. dicoccum, T. dicoccoides* (Körn. Ex Aschers. Et Graebn.) Schweinf. and *T. aestivum* and in the leaf rust nursery of A. Barayev Scientific-and-Production Centre of Grain Farming (Kazakhstan) from *T. durum* and *T. aestivum.* The *P. triticina* collections were increased on seedling of the susceptible common wheat cv. Inna and single-uredinial isolates were increased from each viable collection. All isolates were tested for virulence on the following five sets of four differentials (set 1: *Lr1, Lr2a, Lr2c, Lr3a;* set 2: *Lr9, Lr16, Lr24, Lr26;* set 3: *Lr3ka, Lr11, Lr17, Lr30;* set 4: *Lr2b, Lr3bg, Lr14a, Lr14b;* set 5: *Lr15, Lr18, Lr19, Lr20*) as adapted from the North American nomenclature for virulence in *P. triticina*[1] (Long & Kolmer, 1989). The virulence analysis was carried on detached leaves and several isolates were rechecked on intact plants.

Forty virulence phenotypes were identified among 347 isolates from wheat and *Aegilops* species. All isolates were avirulent to *Lr9, Lr19, Lr24,* and virulent to *Lr11, Lr14a, Lr17, Lr30*. Relatively strong regional variation in virulence frequency was observed on *Lr1*, *Lr2a*, *Lr2b*, *Lr15*, *Lr16*, *Lr20* and *Lr26*, whereas little variability in virulence was found on all other differentials. Average Virulence Complexity (AVC) of isolates originated from common wheat varied from 17 (Novosibirsk) and 16 (Kazakhstan) to 13 (Dagestan), while for other hexaploid species the AVC values ranged from 15 (*Ae. juvenalis*) to 11 (*T. compactum*, *T. petropavlovskyi, T. macha, T. sphaerococcum, T. vavilovii*). AVC of isolates from tetraploid species fluctuated between 13 (*T. dicoccoides, T. dicoccum* (Novosibirsk)) and 8-9 (*T. turanisum, T. durum, T. aethiopisum*), while that from diploid species *Ae. Tauschii* was 14.

Virulence phenotypes detected on the same hosts were usually similar (*Ae. trivialis*, *T. compactum*, and *T. vavilovii*). However, two groups of significantly different *Pt* isolates were collected from *T. spelta*.

Genetic diversity of 20 uredospore samples was analyzed using Kosman's assignment based approach (2-4). The UPGMA dendrogram was constructed to reflect genetic relationships among the populations. The *Pt* populations collected from the hexaploid wheat and *Aegilops* species (*T. macha*, *T. compactum*, *T. sphaerococcum*, *T. petropavlovskyi*, *T. spelta*, *T. aethiopisum*, and *Ae. trivialis*) in Dagestan are clustered into a separate group. The leaf rust populations collected from the same species *T. dicoccum* in

Novosibirsk and Dagestan were highly similar despite huge isolation by distance (more than 3000 km). Two samples collected from *T. dicoccum* and *T. dicoccoides* in Novosibirsk were "identical". Genetically similar collections of isolates from *T. aestivum* in the Western Siberia (Novosibirsk) and Northern Kazakhstan were significantly distinguished from those detected in the Caucasus region (Dagestan). Two populations from *T. durum* in Kazakhstan and *T. turanicum* in Dagestan were different from all other collections.

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Comparison of *Puccinia triticina* Eriks populations from the Northwest of Russia and Northern Kazakhstan for virulence and SSR-markers

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Wheat leaf rust, caused by *Puccinia triticina* Erikss., is a widespread disease of wheat in the Northwest of Russia and Northern Kazakhstan. These two regions are located at distance over 3500 km and have an independent source of fungus infection. In the northern part of Kazakhstan pathogen appears in the period of wheat booting and intensively spreads by the beginning of the grain formation. The urediniospores of fungus drifted by air currents from adjacent Central Asian countries considered the one of the source of infection. In the Northwestern region of Russia leaf rust is usually found at the stage of anthesis ending-early ripening. The main source of infection is aerial from the European part of Russia. Taking into account the boundering location of Northwest of Russia it presumes also the spore drifting from with Western Europe. This possibility bases differences in phenotypes composition of *Pt* populations in the northwest region from other Russian in some years.

The present research used virulence analysis and molecular genotyping for identification of difference between *P. triticina* populations originated from geographically distant locations of Northwest of Russia and Northern Kazakhstan.

The experimental samples were presented by leaf rust infected leaves from four northwestern regions of Russia collected in the period between 2008 and 2014 and account for 122 single pustule isolates from Novgorod, 92 from Leningrad, 41 from Pskov, and 54 from Kaliningrad. The northern Kazakhstan collection involved samples from three districts of Akmolinsk province collected in in 2009 and 2014, and consisted of 41 single pustule isolates. Each isolate was given a five letter code based on virulence/avirulence to each of the five sets of four differentials (set 1: *Lr1, Lr2a, Lr2c, Lr3*a; set 2: *Lr9, Lr16, Lr24, Lr26*; set 3: *Lr3ka, Lr11, Lr17, Lr30*; set 4: *Lr19, Lr20, Lr14a, Lr18*; set 5: *Lr2b, Lr3bg, Lr14b, Lr15*) as adapted from the North American nomenclature for virulence in *P. triticina* (Long, Kolmer; Phytopathology, 1989). The virulence analysis of *P. triticina* was carried on detached leaves and several isolates were rechecked on intact plants.

The part of isolates was genotyped with use of microsatellite primers (Duan *et al.*; Szabo, Kolmer; Molecular Ecology Notes, 2003, 2007) including 32 samples from northwestern Russia (seven belonged to Novgorod, 6 to Pskov, 11 to Leningrad, and 8 to Kaliningrad) and 10 from northern Kazakhstan. Allele size was determined using Genetic Analyzer ABI Prism 3500 (ABI-Hitachi, Japan).

None of the isolates across northwestern Russia and northern Kazakhstan had virulence to leaf rust resistance genes Tc*Lr9*, Tc*Lr19,* Tc*Lr24*. Frequencies of phenotypes with virulence to Tc*Lr11*, Tc*Lr14a*, Tc*Lr14b*, Tc*Lr16*, Tc*Lr17*, Tc*Lr18* were more than 90%. Relatively strong regional variation in virulence frequency was observed on other *Lr*-lines. There was some regional differentiation of virulence phenotypes within the four Northwestern regions of Russia. Phenotypes that are avirulent to *Lr2a, Lr2b, Lr2c* were predominately found in Pskov, Leningrad and Novgorod populations as well as avirulent to *Lr3a*, *Lr3bg*, *Lr3ka* in Kaliningrad ones. The high similarity for virulence and phenotypes composition was observed between Pskov, Novgorod and Leningrad populations, while the isolates from Kaliningrad region that is located closer to Western Europe slightly varied from other tested Russian

populations. Among 309 studied isolates from Northwest of Russia there were 47 virulent phenotypes. In the Northern Kazakhstan 4 phenotypes were identified among 41 analyzed isolates. According to the result, both wheat-growing regions possess virulent phenotype THTKT that refers to the most common (20.6%) of all isolates. The northern Kazakhstan populations were significantly differ from northwestern Russia on virulence. Application of SSR-markers defined also the difference between northern Kazakhstan and northwestern Russia *Pt* isolates. Moreover, the some higher difference was observed between Kazakhstan and Kaliningrad population compared with rest tested samples of Russian Federation (Pskov, Novgorod and Leningrad).

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Molecular Evolution of *Blumeria graminis*

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Blumeria graminis is a fungal pathogen which causes powdery mildew on wild grasses and cereals in temperate environments world-wide. If left uncontrolled, it can cause significant loss of yield in crops such as wheat and barley. We are researching molecular evolution of the fungus in relation to three topics of current interest. Firstly, we aim to resolve the controversy about the timing of divergence of special forms of *B. graminis*. This will involve sequencing several regions of the genome to produce a phylogenetic tree of isolates from crops and wild grasses, including samples from across Europe and elsewhere in the world if possible. Two key questions highlighted by Troch *et al.* (2014, Mol. Plant Pathol.) are the origin of *B. graminis* f.sp. *avenae* on cultivated oats and the rate of evolution of the *B. graminis* genome. Secondly, we are studying the molecular evolution of fungicide resistance in *B. graminis*, particularly how variation in the sequence of CYP51 affects the responses of *B. graminis* f.sp. *tritici* (wheat mildew) to newer and older triazoles (see Cools *et al.,* 2011, Appl. Env. Microbiol. for CYP51 variation in *Zymoseptoria tritici*). Thirdly, in order to understand how virulence evolves in *B. graminis,* we are studying the DNA and predicted protein sequences of two avirulence (*AVR*) genes in *B. graminis* f. sp. *hordei* (barley mildew; Ridout *et al.,* 2006, Plant Cell).

Monitoring of variability in wheat rust pathogens by international trap nurseries

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Wheat rusts have caused massive yield losses of wheat wherever it occurred, but in recent years it has been effectively controlled through breeding for rust resistance genes. Wheat rust populations can be highly diverse for virulence phenotypes. Changes in pathogen virulence have rendered some resistances ineffective, resistant cultivars have generally been developed ahead of significant damage. Knowledge on virulence variation in the natural pathogen population helps breeders in proposing efficient resistance strategy to rusts.

Rusts trap nurseries are targeted for wheat growing areas and are planted at locations where rusts is known to occur naturally each year. Trap Nursery consists of isolines with resistance genes, genetic stocks for additional Yr, Sr and Lr genes, selected differentials, wheat varieties carrying combinations of important resistance genes, and important commercial varieties currently grown in different regions and it is designed to collect information on epidemiology and physiologic specialization of rusts, behavior of resistant and susceptible varieties, tested under different environmental conditions.

In 2013-2014 years two sets of International Yellow Rust Trap Nurseries -7st IYRTN and 8thIYRTN were evaluated within different geographic zones of Georgia: Shida Kartli plain(Borjomi region), Kvemo Kartli plain(Asureti) and Kolkheti lowland (Kobuleti).

2013 crop season was marked by dry conditions in Georgia and yellow rust developed very weakly than in 2014. However, in 2013 moderate level of disease incidence (20-40%) was indicated on Morocco. Low infection was recorded on susceptible entries. 66% and 79% of tested entries showed moderate resistance (MR) in 2013 and 2014, respectively. Data of trap nurseries over two years indicated that several known resistance genes Yr6, Yr7, Yr8, Yr18 have limited utility as host lines carrying them displayed susceptibility in both years. The varieties Ciano 79(Yr27), Attila (Yr27+), Opata 85(Yr27+Yr18), Lal Bahadur/Pavon (Yr29) and Lemhi (Yr21) with severities from 30MS to 70MS had ineffective adult plant resistance. Lines with genes Yr1, Yr2, Yr5, Yr 10, Yr15 and Yr25 showed high resistance at most sites. Cultivar Pastor that carries Yr31 in combination with slow rusting resistance genes remains highly effective in all locations many years.

Nearly all commercial varieties were resistant and moderately resistant in 2013 and 2014 in all sites, but in 2010 the cultivars Cham 8, Sardari, Alamaut and Bohouth 6 showed moderate susceptibility (40MS).

Two sets of Stem Rust Trap Nurseries (8th ISRTN, 9th ISRTN) included 85 entries were also assessed under natural infection in two sites (Kobuleti, Borjomi) during 2013-2014 growing seasons. In 2013 wheat stem rust was severe than in 2014. Its severity varied between 10MS-60MS and 1MS-10MS in 2013 and 2014, respectively. In Kobuleti 73% of entries were found highly resistant to stem rust with "0" or trace level of infection. No significant difference was found in results from Borjomi region (Eastern Georgia), where stem rust severity was higher than in Kobuleti. About 70% and 10% of lines showed resistance and moderate resistance to stem rust respectively. Virulence to lines with genes Sr 7a, Sr 7b, Sr8a, Sr 9a,

Sr 9b, Sr9d, Sr21, Sr24, Sr25, Sr26, Sr27,Sr28,Sr29, Sr31, Sr32, Sr33, Sr34, Sr36, Sr37 have not been detected.

The existence and severity of leaf rust natural infection were assessed in 2014 on the 85 entries of 5th ILRTN-14 in Kobuleti. Resistance of 60 % of entries was indicated. 34,1% among them showed R type. Good resistance to leaf rust have been expressed on lines with genes Lr2b, Lr9, Lr18, Lr19, Lr21, Lr 10+ 27+31, Lr28, Lr29, Lr37. It should be mentioned that these genes have remained effective to leaf rust pathogen for a long time in Georgia.

Thus, important resistance sources including the cultivars with different resistance Sr, Yr and Lr genes have been revealed based on nursery data.

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Occurrence of yellow rust on wheat and effect on grain in the Czech Republic

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Yellow rust of wheat caused by *Puccinia striformis,* Wested is highly destructive in some years and causes considerable yield losses in Czech Republic. Breeding of resistant varieties is an effective approache to minimize yield and financial losses.

In 2014 there were severe outbreaks of yellow rust in many areas in Czech Republic Virulence analysis showed most of this was due to the Warrior/Ambition races. There were some difference in occurrence of yellow rust in natural and artificial infection in breeding nurseries.

The total yield losses were found to be dependent on the varieties and yield potential of the crop. In 2014 long season epidemics affected yield, with losses of up to 55% in susceptible varieties, 34% in group moderately susceptible/resistant varieties and up to12% in resistant varieties.

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Temperature aptitude of invasive strains of *Puccinia striiformis* f. sp. *tritici*

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Stripe (yellow) rust, caused by *Puccinia striiformis* f. sp. *tritici* (*Pst*), is one of the most devastating diseases in bread wheat. In 2004, an invasion of clonal lines *Pst*S1/S2 affected several continents including Europe. This strain possesses few virulence factors and is adapted to high temperature. Another invasion was observed in 2011 in Northwestern Europe. The multivirulent strain Warrior replaced the French population of *Pst* in 2012 and was found in warm and cold areas and can develop on a wide range of wheat varieties. We assessed thermal aptitude of 18 *Pst* invasive strains on two susceptible varieties cv-Victo and cv-Cartago compared to older *Pst* strains by measuring the efficiency of infection (IE) at 5 temperatures (5-23°C) and latency period (LP) under 2 temperature regimes (warm/cold). Isolates differed for their IE and LP as a function of temperature. No infection was observed above 23°C. The best IE was observed at 10°C for the 2 varieties. At 20°C, only some isolates were able of infecting wheat varieties with low IE. LP was shorter on cv-Victo than on cv-Cartago and was shorter under the warm regime than under the cold regime. Warrior isolates acted as generalist for IE and LP on both varieties, with an efficient aptitude under cool temperatures (10 and 15°C) and a reduced efficiency under warmest (20°C) and coldest (5°C) temperatures. On the other hand, the other isolates behaved as specialists such as the northern French reference isolates adapted to cold regime, and southern French reference isolates (*Pst*S3, 6E16), *Pst*S2 and 2 recent strains adapted to warm regime. Based on these IE experimental data, we predicted the fitness of these isolates in Northern vs. Southern Europe, modelling a polycyclic infection efficiency under a warm or a cold climate scenario.

Genetic Structure of *Puccinia striiformis* from wheat and triticale in Sweden

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Uredinia of *Puccinia striiformis* were collected from wheat and triticale during the period 2012-2014. DNA extracted from these samples were tested with 15 different microsatellite markers. Analyses with Genalex, *Structure*, and the R program poppr revealed clonal populations of this pathogen, although minor variation in allele size was detected for several of the markers. No clear evidence for sexual reproduction was found in this material, despite the widespread presence of *Berberis* spp. which would permit sexual reproduction. Different methods for clustering these genetically related individuals are presented and compared.

Molecular description of *Blumeria graminis* f. sp. *hordei* isolates

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The air-born fungus *Blumeria graminis* f. sp. *hordei* (*Bgh*) is a causal agent of barley powdery mildew. The pathogen attracts substantial attention due to its destructiveness. However, molecular diversity studies based on "house-keeping" genes do not provide sufficient resolution when applied to isolates from geographically limited regions. This study focused on developing a more efficient genotyping system capable to discriminate between closely related isolates. Whole genome sequence data were employed to design a panel of molecular markers based on microsatellites and insertion sites of transposable elements which represent an abundant part of the genome. A genotyping marker panel comprising 16 SSR, 14 SNP and 2 ISBP/RJM markers was applied on a set of 97 isolates originating from the Czech Republic, 50 Australian isolates and a collection of 11 isolates representing global *Bgh* diversity. The marker panel provided significant resolution of studied isolates, most of them showing unique genotype profiles. The analysis of phylogenetic relationship performed by neighbor-joining algorithm for 97 Czech isolates resulted in 87 separate clades and revealed high diversity of the pathogen population within a small geographical area. After supplementing with data on virulence of individual isolates, this study might open new opportunities of studying the host-pathogen relationship and patterns of the pathogen spatial distribution. This work has been supported by the Czech Ministry of Education, Youth and Sports (grant awards LD14105, LO1204).

Virulence and Molecular Characterization of the Wheat Stripe Rust Pathogen (*Puccinia striiformis* f. sp. *tritici*) in the United States and Other Countries

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A set of 18 *Yr* single-gene lines has been established for virulence characterization of *Puccinia striiformis* f. sp. *tritici* isolates, and an octal system has been used to designate races in addition to numerical names. A total of 138 PSTv races have been identified, including 1 race first from Canada, 4 from Chile, 11 from China, 13 from Ethiopia, 15 from Italy, 3 from Nepal, 4 from Pakistan, 3 from Turkey, and 84 from the US. The virulence numbers of the races range from 0 to 16 with the medium as 7. Virulence to *Yr1* has been detected in 44% of the races and to *Yr6* 77%, *Yr7* 71%, *Yr8* 56%, *Yr9* 62%, *Yr10* 23%, *Yr17* (62%), *Yr24* 20%, *Yr27* 38%, *Yr32* 20%, *Yr43* 43%, *Yr44* 72%, *YrSP* 12%, *YrTr1* 30%, *YrExp2* 67%, *YrTye* 29%, and no virulence has been detected for *Yr5* and *Yr15* from the races. Virulences to *Yr6*, *Yr7*, *Yr8*, *Yr9*, *Yr17*, *Yr43*, *Yr44*, *YrTr1*, *YrExp2* and *YrTye* have been detected in all countries (Algeria, Australia, Canada, Chile, China, Ethiopia, Hungary, Italy, Kenya, Nepal, Pakistan, Russia, Spain, Turkey, the US and Uzbekistan) with isolates tested. Virulence to *Yr1* has been detected in the isolates from Australia, Chile, China, Ethiopia, Hungary, Italy, Kenya, Nepal, Pakistan, Turkey, the US, and Uzbekistan. Virulence to *Yr10* has been detected in isolates from Chile, China, Ethiopia, Hungary, Kenya, Nepal, Pakistan, the US and Uzbekistan. Virulence to *Yr24* has been detected in the isolates from Australia, Chile, Ethiopia, Hungary, Italy, Nepal, Pakistan, the US, Turkey and Uzbekistan. Virulence to *Yr27* has been detected in the isolates from Canada, Chile, China, Ethiopia, Hungary, Kenya, Nepal, Pakistan, the US, Turkey and Uzbekistan. Virulence to *Yr32* has been detected in the isolates from Chile, China, Ethiopia, Hungary, Pakistan, the US, Turkey and Uzbekistan. Virulence to *YrSP* has been detected in the isolates from China, Italy, the US, Turkey and Uzbekistan. Virulences to *Yr5* and *Yr15* have not been detected from any of these countries. For comparison and continuation, the typical isolates of the 138 races are identified as 108 PST races using the previous set of 20 wheat cultivar differentials. Virulence variations among the 138 PSTv races can be mostly explained by single-step mutations. Dynamic analysis of the PSTv races identified in the US indicates a trend toward races with fewer virulences.

In a study using co-dominant simple sequence repeat (SSR) markers, a total of 273 genotypes were identified from 292 *P. striiformis* f. sp. *tritici* isolates from 18 countries. The heterozygosities of the isolates ranged from 0 to 75% with an average of 45%, and mean heterozygosities in individual countries ranged from 35% in Australia to 60% in Kenya. The genotypes were clustered into two genetic lineages and an admix group. Lineage 1 consisted of 55% of the isolates from all of the countries; lineage 2 consisted of 35% of the isolates mostly from China and Uzbekistan; and the admix group consisted of 10% of the isolates, mostly from Asian countries. Generally, the results obtained from Bayesian statistics, principal component analysis and cluster analysis consistently revealed the lack of geographical differentiation among country-wise collections. Identical genotypes were observed between proximity countries as well as isolates from distant countries of different continents, suggesting introduction or migration. Partition of molecular variance suggested that most variation occurred within countries followed by among international regions. Genetic variation among countries within regions was low but significantly different. Collections from China and Central Asia had significant but low level of genetic differentiation with collections from North American and South America. Either low or non-significant genetic differentiation between other regions may explain the low geographic structuring of the stripe rust pathogen worldwide. Using SSR markers, isolates collections from wheat, barley, and grasses in

the US were characterized into three genetic groups, one of typical wheat stripe rust pathogen, one of typical barley stripe rust pathogen, and a third group containing highly heterozygous isolates virulent on both wheat and barley differentials. SSR characterization of specifically collected stripe rust samples from wheat fields in the US Pacific Northwest where barberry plants can be found indicate that the *P. striiformis* f. sp. *tritici* population is mostly, if not absolutely, asexual, suggesting a minimal or no role of alternate hosts to stripe rust.

Virulence of *Puccinia coronata* f. sp. *avenae* in Canada; 2010 to 2013

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Oat grown in the eastern prairie region of western Canada and eastern Canada can be vulnerable to crown rust caused by *Puccinia coronata* Corda f. sp. *avenae* Eriks. Host genetic resistance has long been used in Canada to control crown rust. The effectiveness of host resistance is dependent on the virulence genes in the pathogen population. The objective of this work was to characterize the virulence of Canadian populations of *P. coronata* f. sp. *avenae* collected over 2010 to 2013 using the North American standard differential set (Chong et al. 2000) supplemented with differentials possessing the following *Pc* resistance genes; *Pc91*, *Pc94*, *Pc96*, *Pc97*, *Pc98*, *Pc101*, *Pc103-1* and *Pc104*. Crown rust samples were collected on wild oat and commercial oat plants in farmers' fields during annual surveys and from lines grown in research plots and uniform rust nurseries. Annually, 169 to 307 single pustule isolates (spi) from the eastern prairies and 16 to 31 spi from eastern Canada were assessed for virulence. Virulence was observed to *Pc38*, *Pc39*, *Pc56* and *Pc68* in more than 50% of spi in most years throughout Canada. No virulence to *Pc58*, *Pc59*, *Pc94*, *Pc98*, *Pc101* and *Pc103-1* was detected in eastern Canada. Virulence to *Pc94* was found at low levels in the eastern prairies in 2010 and 2011, but was not detected in 2012 and 2013. Virulence to *Pc91* was undetected in 2010 and 2011 in the eastern prairies, but was detected in 4% of spi in 2012 and in 12% of spi in 2013.

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Microsatellite characterization of South African *Puccinia striiformis* races

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Wheat stripe rust (*Puccinia striiformis* Westend.) was first detected in South Africa in 1996 in the Western Cape province. This isolate shared virulence similarity with race 6E16A-found in East and North Africa, southern Europe, the Middle East and western Asia. Since first detection, stripe rust has adapted and spread to most wheat producing areas of South Africa. Four races have been found. The genetic relationship between these races was determined using 16 microsatellite markers. Two Kenyan isolates used as out-groups shared 48% genetic similarity with the South African races. While race 6E22A+ was 74% similar to the other three South African races, these races, 6E16A-, 6E22A- and 7E22A- were 84% similar. Network analysis confirmed this close genetic relationship, thereby supporting the stepwise addition of virulence by these races. With no confirmed aecial infections on *Berberis* spp in South Africa, it is suggested that stepwise mutations and foreign introductions are the main drivers of diversity in the South African stripe rust population.

Latest in leaf rust virulence in durum wheat in Spain

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Leaf rust, caused by the fungus *Puccinia triticina*, is an important foliar disease in durum wheat in Spain. Many resistant cultivars have relied on *Lr14a* gene that has been effective for more than three decades. This resistance has recently been overcome in many locations of the world as in France. In a study on virulence on three years (2009-11) with 75 isolates collected from different durum wheat fields in Spain, seven races were found, which could clearly be classified within two groups (Table 1).

The first group comprised five similar races, two of which amounted for more than 85% of the total of the isolates. The second group only comprised 4% of the isolates and included two races that were different in virulence from the former group. These races are considered of 'bread wheat' type and they have minor importance regarding damage on durum wheat. Races of both groups differed for the virulence to *Lr1, Lr3, Lr3bg*, and *Lr30* genes. All races were virulent on isolines containing genes *Lr2c, Lr10, Lr14b, Lr20, Lr23*, *Lr33*, and *LrB*, and avirulent on the ones with *Lr3ka, Lr9, Lr11, Lr13, Lr16, Lr17, Lr19, Lr22a, Lr24, Lr25, Lr26, Lr28, Lr32, Lr35, Lr36, Lr37, Lr38*, and *Lr42*.

In 2013 many cultivars carrying *Lr14a* gene displayed an unusual high leaf rust severity in many fields in Spain. Four diseased leaves samples were collected from durum wheat cultivars carrying *Lr14a* gene in two Spanish sites in Spain (Cadiz, south and Girona, north). Characterization of virulence showed that the four isolates resembled to those identified during the 2009-2011 period, but with added virulence on *Lr14a* gene, and all durum cultivars carrying this gene. All these four isolates indeed formed a new race that could be designated as DBB/BS (Table 2). At the moment, other sources of resistance available on durum wheat such as *Lr27+Lr31*, *Lr3*, and *Lr61* are still effective to leaf rust in Spain.

Table 1. Distribution of 75 isolates of *P. triticina* collected on Spanish durum wheat in 2009, 2010, and 2011.

Year	DBB/BN1	DBB/DN	DBB/CN	DBB/FN	DBB/CS	FBC/PS	PBC/PS	Total
2009	10	1	4	0	0	0	1	14
2010	7	0	15	1	1	2	0	28
2011	11	1	17	4	0	0	0	33
No.	28	2	36	5	1	2	1	75
%	37.3	2.7	48	6.7	1.3	2.7	1.3	100

1 The nomenclature of races followed Singh et al. (2004), but with some modifications. Sets of Lr genes for naming: (1) *Lr1, Lr2a, Lr2c, Lr3; (2) Lr9, Lr16, Lr24, Lr26; (3) Lr3ka, Lr11, Lr17, Lr30; (4) Lr3bg, Lr13, Lr15, Lr18; and (5) Lr10, Lr14a, Lr23, Lr27+Lr31.*

Table 2. Infection types (IT) at fifth leaf stage on seven durum genotypes with known *R*-genes inoculated on three leaf rust races collected on durum wheat, and the new race observed in 2013.

Resistance gene	Races and Infection type (IT)[1]			
	Spanish races from 2009 to 2011			New race in 2013 (on cultivar Don Jaime, *Lr14a*)
	DBB/BN	DBB/CN	DBB/CS	DBB/BS
Somateria (*Lr14a*)	2	2	2	**4**
Colosseo (*Lr14a*)	2	2	2	**4**
Don Jaime (*Lr14a*)	;	;	;	**4**
Jupare (*Lr27+31*)	0	;	;	;
Guayacán (*Lr61*)	;	;	;	;
Storlom (*Lr3*)	0;	0;	0	;
Camayo (*LrCam*)	1	2	2	1

[1] IT data according to Stakman scale (Stakman et al. 1964). IT>2, susceptible reaction; IT<=2 and ';', resistant reaction.

References

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Recent Changes in the UK Wheat Yellow and Brown Rust Populations

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Managing the cereal rust diseases currently relies on the use of fungicides and varietal resistance. Host resistance can be rapidly overcome by mutations in the pathogen population and for this reason virulence surveys have been established worldwide to give early warning on any changes. The UK Cereal Pathogen Virulence Survey (UKCPVS) was established in 1967 following an unexpected outbreak of yellow rust on the variety Rothwell Perdix. The UKCPVS currently monitors the wheat yellow and brown rust populations as well as the wheat and barley powdery mildew populations. A watching brief is maintained on barley yellow rust.

In 2011 a new yellow rust race was detected, named the Warrior race after the variety it was first found on. Initial differential tests suggested that the race was another stepwise mutation with an accumulation of virulence for *Yr7* in addition to the combination of virulence to *Yr6*, *Yr9*, *Yr17*, *Yr32*. Other characteristics of this race however suggested something different from previous race changes with an increase in telia production seen under field conditions. In addition this new race was seen simultaneously in multiple locations throughout Europe (www.wheatrust.org) in contrast to the more gradual appearance of new variants seen previously. Subsequent genotypic analysis of isolates (Hubbard et al., 2015) demonstrated that the new race was an exotic incursion. Since the original incursion the "Warrior" population has continued to dominate the samples found in the UK. In the past two years isolates have been identified which bear the hallmark of the Warrior pathotype but differ in their reaction to other key differentials, including to the original host variety Warrior. The increasing complexity of the isolates has led the survey to re-evaluate the naming strategy of isolates collected.

The UK wheat brown rust population is currently diverse with at least 13 pathotypes detected. Recent changes have reflected varietal choice. The Stigg race arose in response to the deployment of the resistance gene *Lr24* in the varieties Stigg and Warrior in the UK and possibly others in Europe. In 2014 higher than expected levels of brown rust were seen on the bread making variety Crusoe. Although Crusoe was only rated as moderately resistant, levels of disease were higher than expected. The majority of isolates collected by the UKCPVS were of a pathotype which was similar to the Glasgow race of brown rust, last seen in the UK in 2006, suggesting a re-emergence of an older pathotype. In addition, isolates were virulent on more differentials than had been seen previously indicating more complex pathotypes. The full effects of the re-emergent race will be seen in adult plant trials currently under observation.

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Association Among Virulence, Temperature Tolerance and Triadimefon Resistance of *Blumeria graminis* f. sp. *Tritici*

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Evolution of plant pathogen populations was affected by many factors, such as host resistance, fungicide applying, and environment factors. To explicit the association among virulence, temperature sensitivity, and azole resistant of *Blumeria graminis* f. sp. *tritici* (*Bgt*), 129 isolates collected from nine provinces/cities in China was tested. Furthermore, the relationships among them were figured out.

Virulence gene diversity showed that index was highest in Sichuan Province (0.2241) and lowest in Zhejiang province (0.0968). Triadimefon sensitivitiy showed that median EC_{50} was 109.97 mg/L with the coeffective 107.2， and the mean resistance factor (RF) was 52.62. The resistant frequency of all isolates to triadimefon was up to 99.21%. Temperature sensitivity showed that the range of ET_{50} was 21.34-24.46°C and the mean ET_{50} was 23.14°C. Among those isolates, ET_{50}s of 58.76% isolates were within 23 - 24°C range, and ET_{50}s of 3 isolates were above 24°C, which was defined high temperature tolerance.

Fitness of high temperature tolerant and sensitive isolates were characterized. The latent period of temperature tolerant isolates in 23°C was as same as in 18°C, and was lower in comparison with sensitive isolates. Wheat infection and conidiation of temperature tolerance was higher compare to sensitive isolates. Take all data together, the fitness of high temperature tolerance in higher temperature (23°C) were higher compare with temperature sensitive isolates.

The association among triadimefon-sensitivity, temperature sensitivity and virulence diversity of *Bgt* isolates showed that there was a logarithmic function (r=0.2404, *P*=0.0096) relationship between EC_{50} and numbers of virulence genes of *Bgt* isolates, and a negative correlation between temperature sensitivity and virulence diversity.

Those data suggested that high temperature tolerance and azole resistant isolates appeared in the fields. The fitness of temperature tolerance in high temperature was higher in comparison with sensitive isolates. The stronger virulence of the population was, the more resistance to triadimefon was, but the less sensitive to temperature. These data may provide a reference for reasonable utilization of resistance varieties, as well as the use of triazole fungicides.

Berberis holstii is functional as an alternate host of *Puccinia graminis* in Ethiopia

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Berberis holstii, native to highlands of East Africa, is known to be susceptible to *Puccinia graminis* and *P. striiformis* through artificial inoculations. However, it is not known whether these pathogens complete their sexual cycle in the region. In an attempt to understand the role of *B. holstii* in pathogen variations and disease epidemiology of wheat stem and stripe rusts, we investigated the functionality of *B. holstii* as an alternate host. Natural aecial infections on *B. holstii* were observed and samples were collected in August in Mt. Kenya and Narok (Kenya), and June to December in North Shewa (Ethiopia) since 2008. Collected aecispores were inoculated onto a panel of cereal species, including Line E and 'Morocco' wheat, 'Hiproly' barley, 'Prolific' rye, and 'Marvelous' oat. For the majority of aecial samples, aeciospore viability was lost during shipment and storage; thus inoculations were not successful. Using relatively fresh samples collected in North Shewa in two different seasons (2012 and 2014), inoculations resulted in stem rust infections on Line E, Hiproly, and Marvelous. DNA assays using real-time PCR confirmed the presence of *P. graminis* in these samples. While it is likely that the rust pathogen infecting Line E and Prolific is *P. graminis* f. sp. *secalis (Pgs)*, inoculation and DNA assays did not provide sufficient resolution to distinguish *Pgs* from *P. graminis* f. sp. *tritici* (*Pgt*). Stem rust infections observed on Marvelous were assumed to be caused by *P. graminis* f. sp. *avenae*. Experiments are in progress to characterize isolates derived from these samples, and to determine if other rust fungi are present in these samples. Based on these preliminary data, we conclude that *P. graminis* completes its sexual cycle in Ethiopia. The contribution of the sexual cycle to the observed variation within the *Pgt* populations in the region remains unclear.

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Utilizing a natural population of inter-specific barberry hybrids in New England to characterize the mechanism(s) of *Puccinia graminis* resistance in *Berberis thunbergii*

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In the northeastern United States, outside the boundaries of the 20th century federal barberry eradication zone, both common barberry (*Berberis vulgaris*) and Japanese barberry (*B. thunbergii*) are found in great abundance, to the extent that both are considered invasive species. Much less common and relatively less studied is their interspecific hybrid, *B.* ×*ottawensis*, which has been produced in the ornamental horticultural industry but which also occurs naturally. Since *B. vulgaris* is a competent host of *Puccinia graminis* and *B. thunbergii* is not, *B.* ×*ottawensis* presents a unique system for characterizing the genetic mechanism(s) underlying what appears to be non-host resistance to *P. graminis* in *B. thunbergii*. In this study, a natural population of about 1,000 individuals (mixed *B. vulgaris*, *B. thunbergii*, and *B.* ×*ottawensis*) in Sheffield, MA, was investigated. While wide morphological variation was observed among and within the populations of all three species at the site, the most pronounced variation was observed among *B.* ×*ottawensis* individuals. A subset of the population was selected for genotyping by sequencing (GBS) and evaluated for reaction to *P. graminis* via controlled inoculations. Resistance was found to segregate clearly among *B.* ×*ottawensis* individuals; and GBS was shown to be a viable means of generating molecular markers in these species, despite the lack of a reference genome. These results suggest that *P. graminis* resistance in *B. thunbergii* can be genetically mapped, and mapping populations are currently under development to accomplish this goal. The genomic resources developed in this work may facilitate both barberry surveillance efforts and ornamental barberry testing programs. Furthermore, knowledge of the mechanism(s) of *P. graminis* resistance in the alternate host has the potential to inform efforts to breed for stem rust resistance in wheat.

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Pathotyping activities of wheat stem rust at Global Rust Reference Center

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Wheat stem (black) rust caused by *Puccinia graminis* f. sp. *tritici* (*Pgt*) has historically been associated with severe epidemics across the world. After the defeat of long lasting stem rust resistance due to *Sr31* in Uganda in 1998 (*Ug99*), several attempts have been made to track the diversity and changes in stem rust populations around the world. The *Ug99* race group has been reported in 12 countries, including Uganda, Kenya, Ethiopia, Yemen, Sudan, Iran, South Africa, Eritrea, Tanzania, Mozambique, Zimbabwe, and Rwanda. Considering the risk of additional spread of Ug99, the Global Rust Reference Center (GRRC) is part of global efforts to increase the capacity for pathotyping *Pgt* samples from at-risk areas in Central and West Asia and North Africa (CWANA) as well as in Europe. The stem rust activities at GRRC are conducted under quarantine greenhouse and laboratory conditions with financial support from the Danish Government and the Borlaug Global Rust Initiative (www.globalrust.org). Since 2011, a total of 269 stem rust samples were recovered out of 428 received from collaborators in Azerbaijan, Egypt, Ethiopia, Iran, Iraq, Kenya, Lebanon, Nepal, Rwanda, Sudan, Tanzania, Turkey, Uganda, Yemen and Zimbabwe. At present, around 150 have been purified as single pustule isolates and pathotyped. The samples were recovered and multiplied on susceptible varieties Morocco and McNair, while the pathotyping was carried out using the North American differentials lines carrying resistance genes *Sr5, Sr6, Sr7b, Sr8a, Sr9a, Sr9b, Sr9d, Sr9e, Sr9g, Sr10, Sr11, Sr17, Sr21, Sr24, Sr30, Sr31, Sr36, Sr38, SrTmp,* and *SrMcN*, respectively. The final pathotype was assigned a five letter race code based on its reaction on the differential hosts. Diversity at the molecular level is explored by qPCR assays developed by the Cereal Disease Lab, Minnesota, USA, where additional SNP genotyping is in progress. The activities enable us to track the changes in the stem rust population in CWANA and in Europe, which may have direct implication for wheat production in these regions.

Characterization and Genetic Analysis of a Rice Mutant Exhibiting Compromised Non-host resistance to *Puccina striiformis* f. sp. *tritici* (*Pst*)

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Wheat stripe rust, caused by *Puccina striiformis* f. sp. *tritici* (*Pst*), has beenthe most devastating disease of wheat production in China. Since the rapidvirulence variation of *Pst*, the cultivar resistance was frequently surmounted andsustainable control of stripe rust needs development of new resistance resources.Increasing effort has focused on nonhost resistance characterized by its broadspectrum and stability. In previous studies,we got a rice mutant *crr1* (compromised resistance to rust 1) that was more vulnerable to*Pst* infection. More and larger infection sites were produced on *crr1* compared with wild type Nipponbare when inoculated with *Pst*.*Pst* fungus can develop haustoria in mutant rice leaves, while no sporulation were observed.Several defense-related genes(such as *OsPR1a*, *OsLOX1*,*OsCPS4* and et. al.) were found to be induced in *crr1*as well as in wildtype plants, and the resistance to adapted *Magnaporthe oryzae* was not affected in *crr1*,suggesting a different molecular mechanisms underlying host and nonhost resistance. Furthermore, using F2 segregating population derived from two crosses of *crr1*×ZH11and *crr1*×MDJ8, the target gene was mapped between markersID17 and RM25792 with a physical distance of 200kb on chromosome 10 in rice. Genome resequencing revealed three SNPs between mutant and wild type rice in this region. However, none of them was responsible for the phenotype of *crr1*. We postulated that the mutant phenotype of crr1 was caused by epigenetic mutation.

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MOST WANTED – Host targets of wheat yellow rust effectors

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Wheat yellow rust (*Puccinia striiformis* f. sp *tritici* (PST)) is one of the most devastating diseases of wheat worldwide and more recently re-emerged as a major constraint on UK agriculture. The devastating impact of this disease gives a deep sense of urgency to improve our understanding of the molecular basis of PST pathogenicity to develop better-informed management and breeding strategies. In particular, the characterization of structure, function and evolutionary dynamics of secreted effector proteins can help guide and prioritize breeding efforts. To date, our knowledge of the effector repertoire of cereal rust pathogens is limited and little is known about the targets of filamentous pathogen effectors and the mechanisms they use to modulate immunity and host metabolism.

We recently identified a suite of candidate effectors for PST in an effectoromics pipeline, that were prioritised for future study using known effector criteria such as the presence of a secretion signal, expression during infection and expression in haustoria. A subset of these effectors was selected for functional validation in a yeast-two-hybrid screen. Selected PST effector genes were cloned and are currently being screened for potential host interacting partners against a cDNA library generated from wheat leaves infected with PST.

By identifying host proteins that interact with these putative effectors we aim to uncover the underlying physiological processes that may be targeted and manipulated by the pathogen to promote disease progression.

Identifying transcripts associated with aggressiveness in wheat yellow rust by transcriptomic sequencing

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Yellow rust (*Puccinia striiformis*) is currently one of the most prevalent and damaging disease on wheat, which may threaten global food security. This is emphasized by new strains adapted to warmer temperatures, and being more aggressive in general, which have spread rapidly in many wheat growing areas in recent years. More detailed knowledge is needed for understanding rust biology and epidemiology, e.g., the characteristics of aggressive isolates. Since 2011, the isolate DK09/11 of the "Warrior" race is considered 'aggressive' and spreading rapidly in Europe. In this study, progeny isolates arising from a selfing of the isolate DK09/11 on *Berberis vulgaris* (Rodriguez-Algaba J, et al., 2014) were selected for transcriptomic analysis. Four progeny isolates and the parent isolate DK09/11 showing different levels of aggressiveness were point inoculated on wheat leaves and harvested at three different time points (5, 7 and 9 dai) for RNA-sequencing. By using next-generation sequencing technologies, transcript expression profiles under different growth stages will be analyzed to reveal molecular mechanisms underlying aggressiveness.

Rodriguez-Algaba J, Walter S, Sørensen C K, Hovmøller MS & Justesen AF. 2014. Sexual structures and recombination of the wheat rust fungus *Puccinia striiformis* on *Berberis vulgaris*. *Fungal Genetics and Biology* 70, 77-85.

Characterization of recombinant *Lr34* protein: Identification of transport substrate and functional insights

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Wheat is one of our primary food sources. Fungal diseases like rusts cause heavy crop loss in wheat and other cereals across the world. Plant breeders use naturally occurring resistance genes to fight these diseases. However, new fungal strains rapidly emerge and defeat these genes. For almost a century, the wheat Lr34 gene has conferred stable resistance against rust diseases, making it one of the most important resistance genes. While sequence homology of the cloned Lr34 gene predicted that it encodes a putative ATP binding cassette (ABC) transporter protein belonging to the ABC G subfamily (also known as Pleiotropic Drug Resistance or PDR), its target transport substrate and mechanism of action remains enigmatic. In an effort to understand this transporter we designed several DNA constructs of the *Lr34* gene and expressed them in yeast (*Saccharomyces cerevisiae*). We report the successful expression and purification of functional recombinant Lr34 protein. We carried out *in vitro* proteoliposome translocation assays and identified the transport substrate of the Lr34Sus protein. We also report the identification of related metabolites from flag leaves of Lr34-expressing wheat plants and discuss the functional relevance of these metabolites to disease resistance and Leaf Tip Necrosis (LTN) phenotype caused by expression of Lr34.

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Whole transcriptome sequencing and effector expression analysis of virulent *Puccinia striiformis* f. sp. *tritici* infected wheat on custom designed microarray chips

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The whole genome transcriptome sequence of germinated spores of *Puccinia striiformis* f. sp. *tritici* (PST) isolate from Turkey (J05684M2; vir2, vir6, vir7, vir8, vir25, virA, virEP, virVic, virMich) had made sequenced by BGI. As a reference sequence PST-78 isolate whole genome sequence was used and transcriptome sequences of the isolated were compared. The candidate effectors were identified by selecting short and signal peptide containing sequences and together with some wheat genes. Custom microarrays were constructed and screened using Agilent array system. Microarray data were analyzed by using BRB-Array Tools (v4.4.0), which is an integrated package for the visualization and statistical analysis of microarray gene expression data. Quantile normalization that is one of the most widely adopted methods for analyzing microarray data was used as normalization method. Our data consisted of 3 subgroups, which are 24 hpi, 10 dpi (three biological repeats of virulent PST infected Avocet-S and mock infected Avocet-S) and 10 dpi (two biological repeats of avirulent PST infected Avocet-Yr10 and mock infected Avocet-Yr10). Class Comparison tests were performed to find out differentially expressed mRNAs between mock and infected samples for each subgroup (2 FC, $p \leq 0.05$). The genes of 1901, 3555 and 1885 were found to be differentially expressed for 24hpi infected vs. mock, 10 dpi infected vs. mock and 10 dpi resistant infected vs. mock comparisons, respectively. 229 genes (226 PST + 3 wheat) for 24hpi, 817 genes for 10dpi (634 PST + 183 wheat) and 162 genes (43 PST + 119 wheat) for 10dpi resistant that were found to be differentially expressed more than 10 folds were extracted for clustering analysis. Hierarchical clustering of the differentially expressed genes (10 FC, $p \leq 0.05$) was performed by using average linkage as the clustering method in Cluster (v3.0) program and Java TreeView (v1.1.6r4) was used for visualizing the cluster results.

A Link Between Chromatin Remodelling and Plant Pathogen Defence

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Septoria leaf blotch, caused by the fungal pathogen *Zymoseptoria tritici*, is an important foliar disease of wheat. A distinguishing feature of the infection process is a long period of symptomless growth, before a sudden switch to necrotrophy and the formation of lesions in the host tissue. This switch to the necrotrophic growth phase is associated with large scale transcriptional reprogramming in both pathogen and host. However, there is currently no information on which components bring about these transcriptional changes in the host.

We have identified a novel wheat gene, *TaR1*, which is associated with histone binding and chromatin remodeling, and is upregulated during *Z. tritici* infection. Knockdowns of this gene, using Virus Induced Gene Silencing, cause chlorosis and necrotic lesions to develop earlier on infection. However, fewer picnidia are formed on these plants, and fewer spores are produced. This suggests that the wheat gene identified is key for effective fungal development.

TaR1 has been shown to bind to specifically methylated histones, in a manner similar to a number of other reader proteins in plants, which act to recruit chromatin modifiers onto specific areas of DNA. While the full mechanism by which TaR1 brings about remodelling is still unknown, it is clear that in its absence the large scale transcriptional changes, required for effective infection, are interrupted.

Morphology of uredinia and urediniospores of the fungus *Puccinia recondita* f. sp. *secalis* occurring on winter rye in Poland

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In Europe winter rye is mainly cultivated in Germany and Poland as a crop for bakery and animal nutrition. In both countries leaf rust is the most common and the most damaging disease of rye (*Secale cereale* L). Among all winter rye pathogens, leaf rust *Puccinia recondita* f. sp. *secalis* causes the highest yield losses, which may reach 60-80%.

The aim of this work was to collect the brown rust isolates from rye genotypes grown in different locations of Poland and assessment of plant infection degree. The rust infection was scored based on a scale from 0, healthy plants, to 5, severely infected plants. The results of the two years' observations (2013 – 2014) in the two places indicated varied susceptibility of rye genotypes to brown rust. In the years 2013 and 2014 occurred early and strong infection winter rye crop infestation by brown rust (*P. recondita* f. sp. *secalis*). Most of inbred lines were very susceptible to infection by *Puccinia recondita* f. sp. *secalis,* so disease index 5 reached. But same single lines does not show any symptoms. Rye leaves with uredinia and urediniospores of *Puccinia recondita* f. sp. *secalis* were collected during both years from 167 different rye genotypes: inbred lines and reference cultivars. Fungal isolates originated from three experimental locations, including Great Poland, West Pomeranian and Mazovian voivodeships. Additionally brown rust isolates from a striking wild form of Secale sp. growing on WULS plots were evaluated.

In laboratory light and scanning microscope observation of collected rust isolates were done. Single test sample represented 5 rye leaves, on each leaf 5 uredinia were chosen and from each uredinium 10 urediniospores were evaluated. Many morphological features of uredinia (such as shape, colour, dimensions and presence or absence of paraphyses) and urediniospores (such as shape, colour, wall colour, dimensions, number of germ pores, spine length and distance between spines) were investigated. Observations under scanning electron microscope led to some informations about possibility in distinguishing isolates of rust particularly on the basis of the morphology of urediniospore.

Between uredinia and urediniospores collected from the leaves of the reference cultivars and inbred lines differences in their size and morphology were observed. The sizes of average uredinium from rye inbred lines were 793,58 x 295,79 µm diameter and slightly differed between the rust isolates. The averge size of urediniospores from inbred lines were 13,17 x 12,44 µm. The dimensions typical of uredinia from references cultivars were 910,72 x 283,15 µm and the average size of urediniospore were 13,22 x12,95 µm. The spores were ovoid, globoid or angular, evenly echinulated. The confirmed sizes of uredinia, as well as the sizes and morphology of urediniospores were in full agreement with literature data for *P. recondita* f.sp. *secalis.* It has been observed that the dimensions of uredinia collected from a striking wild form of Secale sp. were larger than that from the reference cultivars. When the average measurements of uredinia from wild species from the genus S*ecale* sp. were 1621,33 x 675,172 µm. In the case of wild species form the genus *Secale* sp. the average sizes of urediniospore were 12,25 x 11,44 µm.

Except morphological characteristic additionally molecular methods were used for distinguish collected rust isolates among themselves. Results obtained from Direct PCR didn't help in determining differences between the analyzed population of rust isolates.

Functional diversification of plant NOD-like receptors and signaling through the N-terminal coiled-coil domain

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Intracellular nucleotide-binding oligomerization domain (NOD)-like receptors (NLRs) are basic elements of innate immunity in plants and animals. Plant NLRs intercept the actions of diverse pathogen virulence factors (effectors). The polymorphic barley mildew A (*MLA*) locus encodes coiled-coil (CC) type NLRs, conferring isolate-specific resistance against the barley powdery mildew *Blumeria graminis* f. sp. *hordei* (*Bgh*) pathogen. In the case of 23 cloned *MLA* alleles, each recognizing a different isolate-specific *Bgh* effector encoded at AVR_A loci, diversified selection sites are localized at C-terminal LRR domain. The invariant N-terminal CC domain forms a rod-shaped homodimer in the crystal structure and expression of this domain alone is sufficient to trigger host cell death response. We have recently isolated AVR_{A7} and AVR_{A13} effectors by a pathogen transcriptome-based association mapping. Those effectors belong member of the previously defined CSEPs (Candidate Secreted Effector Proteins). Interestingly two AVRs are neither allelic nor homologous to each other and their homologs are not found in the closely related wheat powdery mildew, *Blumeria graminis* f. sp. *tritici* (*Bgt*). Functional diversification of allelic receptors has been interpreted as evidence for direct binding of effectors to cognate receptors. Furthermore iteration of effector-receptor adaptations (i.e. evolutionary arms race) could generate allelic series of cognate effectors and receptors. However since AVR_{A7} and AVR_{A13} are not allelic effectors, our data imply that an alternative mechanism has shaped the allelic series of MLA receptors. These NLRs may monitor the integrity of a convergent host cellular target of those effectors in combination with the polymorphic LRR domain.

Two distinct Ras genes from *Puccinia striiformis* exhibit differentiated functions in regulating fungal growth, virulence and programmed cell death

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As important regulators of signal transduction pathways, Ras GTPases have been demonstrated to be important for pathogenesis and programmed cell death (PCD) in several pathogenic fungi. However, their functions in obligate biotrophic pathogens are currently unknown. Here, we report our findings for two distinct Ras genes (*PsRas1* and *PsRas2*) from the wheat stripe rust fungus *Puccinia striiformis* f. sp. *tritici* (*Pst*), an important obligate biotrophic pathogen worldwide. Both *PsRas1* and *PsRas2* have conserved protein sequences among different *Pst* isolates but clearly distinct expression profiles in *Pst* infection. Transient silencing of *PsRas1* or *PsRas2* indicated that *PsRas2* but not *PsRas1* contributed significantly to *Pst* fungal growth and virulence in wheat. Interestingly, *PsRas2* could not complement the defect in the vegetative growthof the *Fusarium graminearumras2* mutant, supporting the functional difference in *Ras2* between *Pst* and *F. graminearum*. The putative roles of *PsRas1* and *PsRas2* in PCD were investigated with the aid of a plant system. Transient expression of *PsRas1* but not *PsRas2* in plants induced PCD, which was dependent on all of the conserved components of Ras GTPases in PsRas1. The PCD showed similar morphological characteristics to the plant hypersensitive response (HR). In addition, it required the participation of plant MAPK cascades, which are also involved in HR, indicating that *PsRas1*-triggered PCD in plants is similar to HR. Our findings suggest that *PsRas1* and *PsRas2* exhibit differentiated functions in regulating fungal growth, virulence and PCD, thus facilitating the understanding of rust pathogenicity and the search for novel pathogen control strategies.

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Preliminary studies of leaf rust resistance in a collection of Iranian wheat cultivars

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Leaf rust, caused by *Puccinia triticina* is a widespread disease of wheat causing substantial yield losses in wheat producing regions around the world. Breeding resistant cultivars is the most efficient method to control this disease. For this purpose, a total of 32 Iranian wheat cultivars were tested with three pathotypes at seedling stage. Following that, these genotypes were also tested for the presence of *Lr10, Lr19, Lr24 and Lr37* using specific STS molecular markers. Preliminary results categorized the genotypes into four groups. First group had only the cultivar Rasol which was resistant to all pathotypes while seven cultivars (group two) *i.e.* Pishtaz, Alborz, Golestan, Hirmand, Darya, Star and Kave were resistant to two pathotypes. Fifteen cultivars (group three) were resistant to only one pathotype and the remaining nine cultivars (group four) were susceptible to all pathotypes.

Molecular marker testing showed that none of the cultivars carried *Lr19*. Although Rasol was resistant to all three pathotypes, none of the above-mentioned genes was present in it, indicating that its resistance is due to other resistance genes. Among genotypes in group two, Pishtaz, Golestan, Darya and Hirmand had all three genes in combination except Alborz and Kave which had *Lr37* in combination with *Lr10* or *Lr24*. In this group, Star had no detectable resistance gene(s). In the third group, the cultivars Akbari, Sistan, Moghan, Shoale, Karkhe & Kavir had all three genes in combination. The cultivars Shiroodi & Atrak carried *Lr37* in combination with *Lr10* or *Lr24,* respectively. Azar 2 & Shahi Backcross carried *Lr24* singly while Roshan Backcross & Sabalan had *Lr10* singly. None of these resistance genes was detected in Ghods & Niknezhad indicating other resistance gene(s) responsible for their resistance to one of the pathotypes. Almost all genotypes in the fourth group carried *Lr24* except the cultivars Omid and Sardari. The former carried none of these genes while the latter carried *Lr37* and *Lr10* together. The cultivars Shiraz and Azadi carried *Lr24* singly while other susceptible cultivars had it in combination with *Lr10*. Hamoon was the only susceptible genotype which had three genes (*Lr10*, *Lr24* & *Lr37*) in combination. This combination also exists in second and third groups indicating the presence of other resistance gene(s) in these groups as they were resistant to at least one pathotype.

This study provided preiliminary results on the resistance of some Iranian wheat genotypes and the presence/absence of few genes in them using molecular markers. These results indicated that most of them carry the resistance genes *Lr10*, *Lr24* & *Lr37* for which virulence has been detected. On the other hand, the gene *Lr19* does not exist in Iranian genotypes, therefore it would be useful to use this gene in breeding programs. Further use of more *Pt* pathotypes and molecular markers could let us to have a better understanding of Lr resistant genes in Iranian wheat cultivars.

Biochemical and molecular evaluation of rust resistant bread wheat lines

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Incorporation of different resistant genes/quantitative trait loci (QTL) against the three wheat rusts (stem, leaf and stripe rust) into a single wheat line using gene-pyramiding and marker-assisted selection (MAS) allows protection against these diseases. However, gene-pyramiding can cause decreases in baking quality characteristics which are important for the milling and baking industry as well as consumers. The aim of the study was to evaluate rust resistant lines for good protein content using molecular marker data as well as biochemical data generated over a two year period. Lines were evaluated for the presence of five rust resistant genes/QTL (*Lr19, Lr34/Yr18/Sr57, QYr.sgi.2B-1, Sr2* and *Sr26*) for two consecutive breeding cycles. These lines were also subjected to sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) and size-exclusion high performance liquid chromatography (SE-HPLC) to determine various protein quality characteristics based on single seed analyses. Results indicated that the most resistant lines did not necessarily had the best bread-making qualities and vice versa. Results also indicated that lines were firstly selected based on rust resistance followed by selection for protein quality alleles and lastly the large polymeric proteins percentage (LUPP%). The best rust resistant lines to be used in future breeding programmes were identified based on both rust resistance and quality characteristics.

Wheat leaf rust resistance breeding in Russia

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In Russia breeding for resistance to leaf rust (*Puccinia triticina* Erikss.) has been carrying out for over half a century, but the disease has not lost its significance until now. In the period between 2005 and 2014 the State Register of Breeding Achievements permitted for use 147 new varieties of winter wheat and 88 of spring wheat. Resistance evaluation to leaf rust of new cultivars and identification of *Lr*-genes is a conventional practice of the All Russian Institute of Plant Protection (VIZR). It is shown that among the new varieties 3% of winter and 37% of spring wheat possess seedling resistance. Along with this, over 40% of winter wheat varieties had different levels of field resistance.

With the use of molecular markers the spring wheat varieties appeared to have *Lr19* and *Lr9*. The varieties carrying *Lr19* (L503, Samsar, L505, Volgouralskaya, Dobrunya, Yuliya, Ecada 6, Kinelskaya Niva, Lebedushka, Omskaya 37, Omskaya 38, Ecada 113, and Tulaikovskaya 108) cover 7% of total number of spring cultivars listed in the State Register. Most of them are recommended for cultivation in the Volga region. The first cultivar L503 having *Lr19* was included into the State Register in 1993 and thereafter the number of cultivars with this gene has been increasing. In the middle of 1990s when the sowing area under these varieties exceeded 100 thousand ha the effectiveness of *Lr19* was lost. Currently virulence to *Lr19* is recorded in the regions of cultivation of varieties with this gene, as well as outside. According to some researchers, it is effective to use combination of *Lr19* with other *Lr*-genes, such as *Lr26*. Couple of cultivars (Omskaya 37 and Omskaya 38) are intensively cultivated in the West-Siberian region providing the resistance to leaf rust by means of this sort of combination.

Varieties with a gene *Lr9* form 10% of the total number of spring wheat (Terciya, Tuleevskaya, Sonata, Duet, Chelyaba 2, Pamayati Ruba, Udacha, Alexandrina, Novosibirskaya 44, Kinelskaya otrada, Chelyaba yubileinaya, Sibakovskaya yubilainaya, Chelayaba stepnaya, Altaiskaya 110 , Apasovka, Novosibirskaya 18, Sibirskii al'yans, Sibirskaya 17, and Zauralochka) and 1% of winter wheat (Splav, Nemchinovskaya 24, and Nemchinovskaya 17) in the State Register. Mostly, they are recommended for cultivation in the Western Siberia and Ural regions (the proportion accounts for 18 and 14%, respectively). The first cultivar Terciya carrying this gene has begun to be cultivated in the Western Siberia since 1995 and in 2008 it was appeared virulent isolates. At this time, the gene *Lr9* has lost its effective strength in Western Siberia, but remains quite resistant in the European regions.

The high resistance to leaf rust was identified in the spring wheat varieties Belyanka, Favorit, Tulaikovskaya 5, Tulaikovskaya 10, Tulaikovskaya 100, Tulaikovskaya zolotistaya, Chelyaba 75 and winter one Poema. With making use of molecular markers a series of genes (*Lr9, Lr19, Lr24, Lr25, Lr28, Lr29*, *Lr38, Lr41*, and *Lr47*) were not found out at this material. These varieties (except of Chelyaba 75 and Poema) were created with inclusion of *Agropyron intermedium*, while cv. Chelyaba 75's pedigree consisted of *Aegilops speltoides* that was recommended for cultivation in the Ural region. Genotyping the later cultivar by use of molecular markers it was found the presence of *Lr1*, *Lr10*, and presumably *Lr66*, while the other ones (*Lr35, Lr28, Lr47*, and *Lr51*) descended from *Ae. speltoides* were not identified.

Thus, the percentage of spring wheat varieties with seedling resistance determined the high and partial effectiveness of major gene accounts for 20% in the State Register, moreover, one third of them carries effective nonidentical to known Lr-genes.

The situation with winter wheat varieties looks differently. More than half of the studied cultivars originated from North-Caucasian breeding have been characterized with a certain level of resistance at the adult plants stage in the field conditions. In addition, molecular screening did not reveal the known effective APR (*Lr35, Lr37, Lr21*), but defined the range of ineffective genes (*Lr1, Lr10, Lr26*, and *Lr34*) that can be met singly and in various combinations. It can be assumed that the resistance of these varieties at the adult plant stages is provided by gene combination overcomes the efficiency. Among recommended winter wheat varieties for cultivation in the North-Caucasian region the genes spread at the current ratios: 20% belongs to *Lr26*, 25% to *Lr34*, 15% to *Lr10*, 5% to *Lr1*. Despite the challenge of variety creation with the different combinations of race-specific *Lr*-genes, which completely or partially lost their effectiveness, there is a significant attention is dedicated to this type of research world over, because of possibility to improve the genetic protection of wheat and stabilize the pathogen population by reducing its reproductive capacity, but not complete elimination.

New strategy to obtain durable resistance to powdery mildew and leaf rust in wheat crop?

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Plants have evolved highly complex defence against potential pathogens. Nonhost resistance (NHR), defined as the immunity displayed by all genotypes of a plant species towards all pathotypes of a potential pathogenic species, is the most common form of disease resistance in plants. It may be based on Pathogen-Associated Molecular Pattern (PAMP)-Triggered Immunity (PTI) and Effector-Triggered Immunity (ETI).

PTI is activated upon the recognition of conserved pathogen components referred as PAMPs by Pattern Recognition Receptors (PRRs) in plants, and often hampers haustorium formation. However, pathogens are able to suppress PTI by releasing effectors into plant cells. This is called Effector-Triggered Susceptibility (ETS). Another option for plants, referred as ETI, is based on the specific recognition of pathogen effectors by resistance proteins and generally leads to hypersensitive response (HR) after haustorium formation.

This durable immunity of plant genotypes to a broad-spectrum of potential pathogens suggests that NHR is highly relevant for agricultural applications. However, its genetic basis still remains unclear.

The current research project aims to a better understanding of NHR in barley through two complementary approaches:

- One, based on forward genetics, involves the fine mapping of a 80 cM QTL in barley which has shown to confer resistance to several heterologous rusts including the wheat leaf rust *Puccinia triticina;*
- The other one, built on reverse genetics, aims to functionally characterize two barley Receptor-Like Kinases (RLKs) genes which have shown, from previous research at IPK (Gatersleben, DE) using transient system, to play a role in NHR to the wheat powdery mildew (*Blumeria graminis tritici – Bgt)*. The current project aims to validate this by stable over-expression studies. The transfer of these genes into wheat and the assessment of the resulting wheat transgenic lines for response to *Bgt* inoculation might determine whether genes involved in NHR in barley can confer host resistance when transferred to wheat.

These two approaches will lead to identify new components involved in basal resistance and to better understand nonhost resistance. This knowledge may be applied in breeding programs for resistance.

An Intergrated Strategy for Rust Resistance in Kenyan Wheat: Understanding Pathogen Virulence and Screening for New Resistance Sources

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Wheat (*Triticum aestivum* L.) yellow rust, caused by *Puccinia striiformis* f. sp. *tritici* (*Pst*), is one of the most devastating foliar and ear diseases of wheat. The appearance of new and more aggressive races has resulted in severe yield losses in recent years. Central to addressing the challenge of wheat yellow rust is the development of more effective breeding strategies that incorporate virulence analysis for a better understanding of Pst. The current study was aimed at understanding *Pst* pathogenicity and exploiting this understanding in the design of effective breeding strategies that maximize the potential for durable disease resistance. This involved the characterization of the pathogenicity of *Pst* for races prevalent in Kenya, and evaluation of Triticeae germplasm collection for *Pst* resistance. To achieve this, Six hundred Watkins landraces were evaluated for wheat rust resistance during the off-season and main-season at a screening site in KALRO, Njoro in 2013. We have identified fourty resistant lines with disease severity ranging between 0 to 40%. Lines identified to be resistant for stem and yellow rust will be utilized in targeted breeding to develop double rust resistance lines. We have also initiated virulence tests on Pst isolates collected during the disease surveys in Kenya at present and in the past. These isolates are being recovered and the testing on yellow rust differential sets at GRRC, Denmark, is in progress.

Figure 1. Resistant and susceptible landraces to yellow rust.

Crossing block winter facultative wheat genotype reactions to stripe, leaf and stem rusts

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Bread wheat is important cereal crops and rusts (*Puccinia* spp.) are the most significant disease decrease wheat yield and quality in Turkey. On the other hand crossing block materials determined important for rust disease. The aim of the study was determining of the reactions of the 245 Crossing block winter facultative (CBWF) genotypes to rusts. These genotypes were developed by the International Winter Wheat Improved Project (IWWIP-TCI).

For this goal, adult plant and seedling test were conducted for yellow rust while only seedling test were conducted for leaf and stem rust. Evaluations were carried out at the research facilities of CRIFC at İkizce and Yenimahalle in Ankara locations in the 2014 season.

For adult plant reactions; the genotypes were observed with local *Pgt* populations (avirulent on *Sr24, Sr26, Sr27*, and *Sr31*) and the genotypes were inoculated with local *Pst* populations (virulent on *Yr2, 6, 7, 8, 9, 25, 27, Sd, Su, Avs*). Stripe and stem rusts development on each entry were scored using the Modified Cobb scale when the susceptible check cv. Little Club had reached 80S infection severity in June and August in 2014. Coefficients of infections were calculated and values below 20 were considered to be resistant. For seedling test; the seedling was inoculated with local *Pgt*, *Pst* and *Pt* (avirulent on *Lr9, Lr19, Lr24,* and *Lr28*) populations. Stripe, leaf and stem rust development on each entry were scored after 14 days with 0-9 and 0-4 scale for yellow rust and leaf and stem rust respectively.

The crossing block winter facultative materials determined to reaction to stripe, leaf and stem rust. Ninety three (43%) (seedling stage) genotypes and 136 (56%) (adult stage in the artificial epidemic) genotypes were resistant to local *Pst* and 78 (32%) (seedling stage) genotypes and 26 (11%) (adult stage in the natural epidemi) genotypes were resistant to local *Pgt,* and 89 (36%) (seedling stage) were resistant to the local *Pt*. Resistance genotype used to cross materials for resistance breeding programme.

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Resistance to Yellow Rust of Wheat Genotypes from IWWPMN Collections

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The causal agent of yellow rust *Puccinia striiformis* f. sp. *tritici* has become a global problem since the emergence of new races, which caused most cultivated resistant varieties in the world to lose control over this disease. In Serbia it occurred sporadically until the production year 2013/14, and was an insignificant and minor wheat disease. Significant incidence was detected in 1997 in some genotypes in the genetic collection at the locality of Rimski Šančevi. Since the pathogen has not been a major issue in the production of wheat in Serbia, there has been no research of the virulence of its population and sources of resistance. Therefore, the aim of this study was to evaluate the resistance of genotypes from IWWPMN (International Winter Wheat Powdery Mildews Nursery) collections under natural infection and to determine the sources of resistance to *P. striiformis* f. sp. *tritici*.

Resistance to the yellow rust causal agent was assessed under conditions of natural infection in 646 genotypes from IWWPMN at the experimental field of the Institute of Field and Vegetable Crops at the locality of Rimski Šančevi. The basic plot size was 1 m^2. Evaluations were conducted on 28 May 2014. Disease severity (%) was evaluated according to the scale from 0 to 100% based on the modified Cobb's scale, and the infection types from 0 to 9. All studied lines fell into six categories of resistance according to the severity and the infection types.

The category of very-resistant (VR) lines included 301 (46.6%) lines, resistant (R) 84 (13.0%), and medium-resistant (MR) 95 (14.7%) lines. In the sensitive categories which include categories of medium (MS), susceptible (S), and very-sensitive (VS) there were 132, 31 and 3 lines, respectively. In total, the categories of resistant lines consisted of 480 (74.3%) lines, and categories of sensitive consisted of 166 (25.7%) lines.

The highest level of resistance to the causal agent of yellow rust was found in the following lines: FR 81.11; FR 81.19; FR 83.2; FR 87-2; FR 89.5; FR 89.6; FR 91.6; SC 861949; SC 850328; SC 850559; GA 83139-1C41; OR CR 8606; GP 4067-530; AK 386-3-31; D 16; TP 114; 1356-54; ZG 91-84; and ZG 471-80.

This study showed that the large number of lines are resistant to the causal agent of yellow rust. However, the study of the fungus populations' virulence and Yr resistance genes efficiency are necessary for a more detailed resistance analysis of varieties and lines.

Leaf rust and powdery mildew in Polish winter wheat

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Leaf rust, caused by *Puccinia recondita* f. sp. *triticina*, and powdery mildew, caused by *Blumeria graminis* f. sp. *tritici*, are the two most important diseases of wheat worldwide. Every year, these two diseases cause significant agricultural production losses, the extent depending on the weather conditions. The varieties of wheat that are grown in Poland are still susceptible to the natural populations of *P. recondita* and *B. graminis*.

Pyramiding resistance genes for leaf rust and powdery mildew combined with the MAS is very effective process to obtain a new cultivar resistant to these diseases. Moreover, in breeding programs there are several examples of the successfully introduction resistance genes into a single cultivar and molecular markers are commonly used in this process.

The strategy of accumulation a new effective resistance genes originating from new sources of resistance into one genotype is the best way to obtain a new cultivar with durable and effective resistance resulting in more stable yields. In the plant breeding are many examples for such process. However an introduction Lr41, Lr47, Lr55, Pm21, Pm36, Pm37 resistance genes in wheat is reported first time in presented study.

The second objective is of our study is to clarify the location Lr55 gene in wheat genome and mapping closely linked molecular markers suitable for markers assisted selection (MAS).

The third goal of this research is to determine the effectiveness of resistance genes for powdery mildew and leaf rust in relation to the populations *B. graminis* and *P. triticina* currently occurring in Poland.

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Effective leaf rust resistance gene combinations involving *Lr34* or *Lr67*

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Leaf rust, caused by *Puccinia triticina* Eriks., is one of the most common and destructive diseases of wheat in most location where it is cultivated. The leaf rust resistance gene *Lr34* has conditioned durable resistance worldwide. It is particularly effective in combination with other resistance genes. The gene combinations involving *Lr34*, can often be much more resistant that what would be expected from the same genes when they occur individually. The leaf rust resistance gene *Lr67* was recently discovered and appears to work in a similar manner as *Lr34*. Less is known about how *Lr67* reacts in combination with other resistance genes. In this study we developed six different double haploid populations, each segregating for either *Lr34* or *Lr67* and a second resistance gene, to analyze the level of resistance in progeny containing either one of the resistance genes or their combination. The second resistance genes in these crosses were either *Lr13*, *Lr16* or *Lr32*. Individually *Lr13* is ineffective, while *Lr16* is partially effective and *Lr32* is highly effective. The six populations resulted from the following crosses: Thatcher (Tc)-*Lr13*/Tc-*Lr34*, Tc-*Lr13*/Tc-*Lr67*, Tc-*Lr16*/Tc-*Lr34*, Tc-*Lr16*/Tc-*Lr67*, BW196R-*Lr32*/Tc-*Lr34*, BW196R -*Lr32*/Tc-*Lr67*. These populations consisted of 70-130 double haploid lines each, which were tested for leaf rust resistance in inoculated field nurseries for three years, 2012, 2013 and 2014. In each year the Thatcher near isogenic lines with either *Lr34* or *Lr67* had intermediate levels of leaf rust resistance. For the two crosses involving *Lr13*, both the populations with *Lr34* or *Lr67* demonstrated transgressive segregation in which some progeny lines with *Lr13* and either *Lr34* or *Lr67*, were more resistant than any of the parental lines. Similarly, in the two populations involving *Lr16*, progeny lines with *Lr16* and either *Lr34* or *Lr67*, were much more resistant than the *Lr16* line or those with only *Lr34* or *Lr67*. The BW196R-*Lr32* line only had relatively low levels of leaf rust severity, but a number of lines in which *Lr32* was combined with either *Lr34* or *Lr67* had very low levels of leaf rust infection over the three years of this study. We conclude that both *Lr34* and *Lr67* can combine with other resistance genes in a synergistic manner to condition a high level of leaf rust resistance. This is true for partially or highly effective resistance genes such as *Lr16* or *Lr32*, respectively. However, this is also true for very ineffective resistance genes such as *Lr13*. This has implications in resistance breeding since relatively simple gene combinations involving *Lr34* or *Lr67* can have relatively high levels of resistance, that are likely durable. *Lr34* and *Lr67* appear to behave very similarly towards stem rust, caused by *Puccinia graminis* Pers.:Pers., and stripe rust, caused by *Puccinia striiformis* Westend, as they do to leaf rust, so the potential for very effective gene combinations involving these genes exists for control of these other rusts as well. Resistance genes *Lr13*, *Lr16* and *Lr34* are all very common in Canadian wheat germplasm, so there is opportunity to combine these genes in future crosses.

Mlo resistance to powdery mildew in winter barley in Poland

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Winter barley is an important cereal crop and it is grown in all agricultural regions of Central and Western Poland. The powdery mildew caused by *Blumeria graminis* f. sp. *hordei* is one of the most frequently observed disease on winter barley which can cause considerable yield losses. The disease can be controlled with fungicides but fungicides are ecologically not desirable and their frequent use speeds up the evolution of fungicide resistance. The use of resistant cultivars is the most effective method to control powdery mildew and the incorporation of new genes for resistance to powdery mildew into barley cultivars has been very useful in combating powdery mildew.

The resistance conferred by most of new resistance genes has not been maintained for more than a few years with some exceptions. One of these exceptions is the Mlo resistance. Mlo resistance has become a very important source of powdery mildew resistance in barley because there is no known virulence for these genes. However, many factors e.g. temperature, water stress or light intensity may affect the expression of this gene. Since 1979 (registration of cultivar 'Atem') the Mlo resistance has been deployed in more than 150 cultivars throughout Europe in spring barley**.**

The objective of this study is to use of Mlo resistance to control powdery mildew on winter barley in Poland. This research aim to use molecular markers closely linked to mlo gene suitable for markers assisted selection (MAS). The practical aim of presented investigation is to use of MAS in introduction of Mlo resistance into background of winter barley germplasm with valuable economical characteristics in Polish agricultural conditions.

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Characterisation of QTL conferring resistance to yellow rust in the UK wheat MAGIC population

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Yellow rust (YR), caused by *Puccinia striiformis* f. sp. *tritici*, is one of the most important foliar diseases of wheat worldwide. Breeding for resistance, in combination with pathogen surveillance and fungicide application, has been the most effective method of controlling YR outbreaks. Nevertheless, resistance to YR is a complex quantitative trait to dissect. Multi-parent populations, through multiple founders and several rounds of genetic recombination provide a platform for high resolution mapping of complex traits. The UK 8-parent wheat Multi-parent Advanced Generation Inter-Cross (MAGIC) population generated at NIAB (Mackay *et al*., 2014) has previously been used to undertake screens for YR resistance. While parental lines show a range of susceptibility at both the seedling and adult stages, preliminary analyses identified transgressive YR resistance, controlled by numerous quantitative trait loci (QTL), with a major resistance locus on 2D. Two inoculated UK field trials, as well as additional seedling tests of different YR isolates at the seedling stage, will be conducted to further investigate the genetic basis of resistance amongst the MAGIC population. These investigations will be aided by the use of emerging wheat genomics tools, and the ability to generate 'pseudo' near isogenic lines from residual heterozygosity present in the MAGIC progeny.

Reactions of some Turkish white bread wheat materials in preliminary yield trials set-2 to and stripe, leaf, stem rust

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Bread wheat is one of the most important cereal crops in Turkey. Rusts (*Puccinia* spp.) are the most significant disease decrease wheat yield and quality in the Central Anatolian Plateau. The purpose of the study was determining of the resistance of the 180 Turkish winter white bread wheat genotypes in preliminary yield trials set-2 (WBW-2) developed by the department of Wheat Breeding, Central Research Institute for Field Crops (CRIFC) to rusts. For this purpose, adult plant and seedling test were conducted for yellow rust while only seedling test were conducted for leaf and stem rust. Evaluations were carried out at the research facilities of CRIFC at İkizce and Yenimahalle in Ankara in the 2014 season.

For adult plant reactions; the genotypes were inoculated with local *Pst* populations (virulent on *Yr2, 6, 7, 8, 9, 25, 27, Sd, Su, Avs*) and ; the genotypes were observed with local *Pgt* populations (avirulent on *Sr24, Sr26, Sr27*, and *Sr31*). Stripe and stem rusts development on each entry were scored using the modified Cobb scale when the susceptible check cv. Little Club had reached 80S infection severity in June and August 2014. Coefficients of infections were calculated and values below 20 were considered to be resistant. For seedling test; the seedling was inoculated with local *Pgt*, *Pst* and *Pt* (avirulent on *Lr9, Lr19, Lr24,* and *Lr28*) populations. Stripe, leaf and stem rust development on each entry were scored after 14 days with 0-4 and 0-9 scale for leaf-stem rust and yellow rust, respectively.

Fifty two (29%) (seedling) genotypes and 81 (45%) (adult stage) genotypes were resistant to local *Pst,* 38 (21%) (seedling) were resistant to the local *Pt* and 69 (38%) (seedling) were resistant to the local *Pgt*, The resistance materials selected to stem, leaf, and stripe rust and advance yield trials set.

This study was financed and supported by General Directorate of Agricultural Research and Policy of the Ministry of Food, Agriculture and Livestock of Turkey (Project no: TAGEM/TBAD/13/A12/P01/002).

Some wheat lines developed by another culture to reactions of stem, leaf and stripe rusts

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The breeding period time is shortened by another culture technique. The development materials of the rust diseases of test material must be made quickly. Because this material has not been determined previously disease reactions tests. The purpose of the study was determining of the reactions of the 20 lines (IVD-1) and 4 standards cultivars genotypes to rusts. These genotypes (20 lines) were developed by the Central Research Institute for Field Crops (CRIFC) Department of Wheat Breeding Unit.

For this aim, adult plant and seedling test were conducted for yellow rust while only seedling test were conducted for leaf and stem rust. Evaluations were carried out at the research facilities of CRIFC at İkizce and Yenimahalle in Ankara locations in the 2014 season.

For adult plant reactions; the genotypes were inoculated with local *Pst* populations (virulent on *Yr2, 6, 7, 8, 9, 25, 27, Sd, Su, Avs*). Stripe rust development on each entry were scored using the modified Cobb scale when the susceptible check cv. Little Club had reached 80-100S infection severity in June - July 2014. Coefficients of infections were calculated and values below 20 were considered to be resistant. For seedling test; the seedling was inoculated with local *Pgt* (avirulent on *Sr24, Sr26, Sr27,* and *Sr31*), *Pt* (avirulent on *Lr9, Lr19, Lr24,* and *Lr28*) and *Pst* populations. Stripe, leaf and stem rust development on each entry were scored after 14 days with 0-9 and 0-4 scale for yellow rust and stem-leaf rust and, respectively.

The test materials (20 lines) determined to reaction to stripe, leaf and stem rust. Three (15%) (seedling stage) genotypes and 5 (25%) (adult stage in the artificial epidemic) genotypes were resistant to local *Pst* and three genotype were resistance both seedling stage and adult plant stage . On the other hand 2 (10%) (seedling stage) genotypes were resistant to local *Pgt,* and all materials (100%) (seedling stage) weren't resistant to the local *Pt*. Resistance genotype used to cross materials for resistance breeding programme.

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Characterization and Genetic Analysis of a Rice Mutant Exhibiting Compromised Non-host resistance to *Puccina striiformis* f. sp. *tritici* (*Pst*)

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Wheat stripe rust, caused by *Puccina striiformis* f. sp. *tritici* (*Pst*), has beenthe most devastating disease of wheat production in China. Since the rapidvirulence variation of *Pst*, the cultivar resistance was frequently surmounted and sustainable control of stripe rust needs development of new resistance resources. Increasing effort has focused on nonhost resistance characterized by its broadspectrum and stability. In previous studies, we got a rice mutant *crr1* (compromised resistance to rust 1) that was more vulnerable to*Pst* infection. More and larger infection sites were produced on *crr1* compared with wild type Nipponbare when inoculated with *Pst*.*Pst* fungus can develop haustoria in mutant rice leaves, while no sporulation were observed. Several defense-related genes(such as *OsPR1a*, *OsLOX1*,*OsCPS4* and et. al.) were found to be induced in *crr1*as well as in wildtype plants, and the resistance to adapted *Magnaporthe oryzae* was not affected in *crr1*,suggesting a different molecular mechanisms underlying host and nonhost resistance. Furthermore, using F2 segregating population derived from two crosses of *crr1*×ZH11and *crr1*×MDJ8, the target gene was mapped between markersID17 and RM25792 with a physical distance of 200kb on chromosome 10 in rice. Genome resequencing revealed three SNPs between mutant and wild type rice in this region. However, none of them was responsible for the phenotype of *crr1*. We postulated that the mutant phenotype of crr1 was caused by epigenetic mutation.

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Genetic and physical mapping of *Rphq11*: a QTL conferring resistance to *Puccinia hordei* in barley

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Barley leaf rust, caused by *Puccinia hordei,* is one of the most destructive diseases of barley. Control is possible by deployment of quantitatively inherited partial resistance. One of several QTLs for partial resistance, *Rphq11*, was mapped against *P. hordei* isolate 1.2.1 in seedlings of the mapping populations L94 x 116-5 and in Steptoe/Morex, and located at the middle of chromosome 2HL. The resistance allele was contributed by Steptoe. *Rphq11* explains 34% of the phenotypic variance in partial resistance. A 58 cM fragment has been introgressed from Steptoe into SusPtrit (susceptible to *P. triticina*, but also highly susceptible to *P. hordei*), resulting in a near-isogenic line, SusPtrit-*Rphq11*.Using a high-resolution NIL-F2 population consisting of 3953 individuals, we mapped *Rphq11* to a 0.15 cM interval between WBE129 (co-segregated with WBE305) and WBE307, and it co-segregated with WBE144.. The corresponding syntenic regions in rice and *Brachypodium* are respective 30 kb and 39 kb, and contain 8 and 9 predicted genes (listed in Table 1), respectively. The SusPtrit BAC library was screened by using the sequence of WBE144, WBE129 and WBE307. A BAC contig consisting of 6 BAC monoclones was constructed. BAC fingerprinting results show that the *Rphq11* BAC contig spans approximately 160-200 kb. Sequencing and annotation of the BAC contig is being carried out presently.

Table 1. Genes in rice and *Brachypodium Rphq11* sentenic regions.

Markers of barley	Genes in rice syntenic region	Genes in *Brachypodium* syntenic region	Description
WBE307	Os04g46910	Bradi5g17960	actin-depolymerizing factor
	Os04g46920	Bradi5g17970	zinc knuckle domain containing protein
WBE144	Os04g46930	Bradi5g17980	serine racemase, putative
	Os04g46940	Bradi5g17990	copper-transporting ATPase 3, putative
	Os04g46950		expressed protein
WBE129	Os04g46960	Bradi5g18000	glutathione peroxidase domain containing protein
		Bradi5g18010	cis-zeatin O-glucosyltransferase 1-like
		Bradi5g18020	cis-zeatin O-glucosyltransferase
	Os04g46970	Bradi5g18030	cis-zeatin O-glucosyltransferase
WBE305	Os04g46980	Bradi5g18040	cis-zeatin O-glucosyltransferase

Fine genetic mapping of the powdery mildew resistance gene, *PmG3M*, derived from the wild emmer wheat (*Triticum dicoccoides*)

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Powdery mildew is one of the most destructive diseases of wheat caused by the parasitic fungus *Blumeria graminis* f.sp. *tritici* (*Bgt*). Wild emmer wheat (*Triticum turgidum* ssp. *dicoccoides*), the tetraploid progenitor of cultivated wheat, is a valuable source for novel disease resistance genes. A novel dominant gene, *PmG3M*, derived from wild emmer wheat was discovered, that confers broad-spectrum resistance to powdery mildew. The *PmG3M* was genetically mapped on the distal side of chromosome arm 6BL of wheat, by means of mapping population produced by crossing of the *T. turgidum dicoccoides* (accession G305-3M) donor line with a susceptible *T. turgidum durum* (cv. Langdon).

In the current study we focused on the development of a high resolution genetic and physical maps that will enable to clone *PmG3M*, by means of advanced comparative genomics approaches.

Using the survey sequencing approach, the ”Genome Zipper” and the strong collinearity to the sequenced small cereal genomes, such as rice, *Brachypodium*, sorghum and barley, we were able to develop and map 14 new markers (ten CAPS, three SNPs and one STS) and to refine the genetic map of the *PmG3M* on 6BL to 3.8 cM between the closest markers. Based on the current genetic map, the collinear genomic region spanning the *PmG3M* locus is only 107 Kb in rice, and 2.8 Mb in *Brachypodium*.

Further studies are underway to develop a high resolution physical map of the *PmG3M* gene region on chromosome 6BL as a basis for positional cloning of this highly important gene.

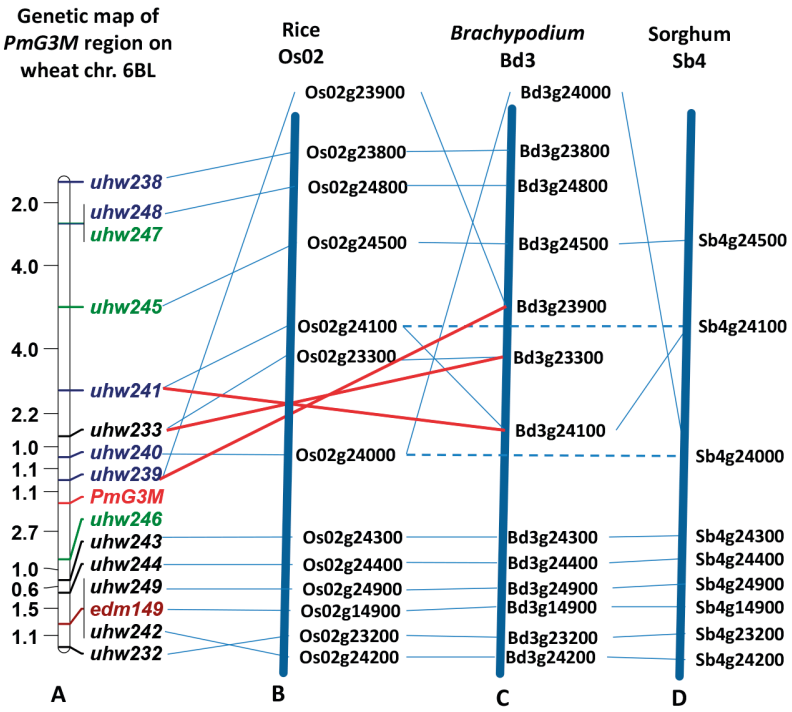

Figure 1. Genetic map of chromosome arm 6BL of wheat containing powdery mildew resistance gene *PmG3M* and anchored markers for the physical map of the chromosome arm 6BL, sequence of *Brachypodium* chromosome Bd3, rice Os02 and sorghum Sb4. (A) Genetic map of the *PmG3M* gene region on wheat chromosome arm 6BL. Markers are shown on the right with map distances on the left. Molecular markers and syntenic genes codes designed by different colors: the *PmG3M* locus by red, markers developed by means of collinearity of syntenic regions of *Brachypodium,* rice and sorghum by black, markers developed by using the GenomeZipper approach by blue, markers developed by means of collinearity of barley syntenic regions by green, well known markers by brown. The regions collinear to *PmG3M* gene region on chromosome 6BL of wheat shown in rice Os2 (B), *Brachypodium* Bd2 (C) and sorghum Sb4 (D). The read lines which connected genetic map (A) and *Brachypodium* (D) are representing transversion on Bd3 chromosome.

Gene mapping stem and leaf rust resistance genes in wheat

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Stem and leaf rust are both important fungal disease of wheat. Stem rust caused by *Puccinia graminis* Pers.:Pers. f.sp. *tritici* Eriks. & E. Henn., has historically caused severe yield loss. Stem rust has been under control due to the use of resistance genes in wheat cultivars. In 1999, a new race of stem rust was discovered in Uganda (known as Ug99, or TTKSK), which has virulence to widely used resistance genes. Ug99 is characterized by its virulence to *Sr31*, a widely used resistance gene. Due to Ug99's unique virulence a large proportion of the world's wheat cultivars are susceptible. TTKSK has spread from Uganda and there are now 7 races. Few resistance genes remain effective and those that are will not stay effective forever. Leaf rust is caused by *Puccinia recondita* Rob.ex Desm f.sp. *tritici*,. It is the most widespread rust on wheat with a 5-15% yield loss which adds up to a substantial loss. Resistance genes are the most economical method of control. There are over 70 leaf rust resistance genes characterized, many of which have been utilized in wheat breeding. Most of these resistance genes are race-specific and only remain effective for about 10-20 years, therefore new resistance genes need to be found. The purpose of this study is to map new resistance genes in the wheat line 6SEPMON23, which is believed to be a source of new resistance to both stem and leaf rust.6SEPMON23 is a DH population, which stem and leaf rust seedling tests have been performed. These tests show that there are two resistance genes against TTKSK, and one for leaf rust races MGBL and MBRJ. The resistance genes will be located through linkage analysis using a modified version of bulk segregant analysis using the 90K Infinium SNP chip.

Revealing the diversity of quantitative resistance loci to *Puccinia triticina* to breed for more durably resistant bread wheat varieties

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Quantitative resistance is often considered more durable than major resistance genes because it exerts a lower selection pressure on the pathogen. The genetic diversity of quantitative resistance can also play an important role in enhancing durability of resistance as it leads to a complexity that is difficult to overcome for the pathogen. Quantitative resistance alters the expression of different traits of the host–pathogen interaction, and those traits can be associated to different genes and quantitative trait loci (QTL), implicated in a range of molecular mechanisms. Many breeding programs are now implementing selection on quantitative resistance with the objective to accumulate quantitative resistance loci (QRL) in modern varieties and to enhance the durability of their resistance against cereal rusts. Measuring disease severity as a whole in field experiments is important, because it allows an assessment of the global effect of QRLs on epidemics. However, field assessments are not sufficient to characterize the diversity of the QRLs found in terms of the individual traits of the pathogen life cycle that are affected by the resistance and of the underlying molecular mechanisms. Our objective was to find diversified QRLs against leaf rust in French wheat breeding material. We measured the level of resistance against leaf rust in the field in a panel of 86 wheat genotypes. Then, we selected a set of 13 genotypes having high levels of quantitative resistance in the field to measure in the greenhouse various resistance related traits against 3 isolates of *P. triticina* differing in aggressiveness. Based on the different resistance traits being affected by the pathogen in the greenhouse, we chose a subset of 6 genotypes to develop 5 segregating populations, with effectives ranging from 99 to 181 lines. For one population (Apache\Balance), we search for QRLs in the greenhouse as well as in the field and we observed that all the QRLs found in greenhouse conditions were also involved in field resistance. Most of the time, a QRL was associated to one or two traits only. This work revealed that the different resistance traits are mostly governed by different sets of genetic factors, and that a high genetic variability for quantitative resistance is available in modern wheat varieties. The other 4 mapping populations were evaluated for resistance to leaf rust in the field only. In each population, seven to eighteen new QRLs were identified. The construction of a consensus map for these populations showed that most of the QRLs found were located in different genomic regions. Fine mapping of the most interesting QRLs will allow the identification of tightly linked markers to use in marker assisted selection. Our hypothesis is that the selection of appropriate combinations of QRLs, taking into account both the size of their effect and the resistance traits affected, will enhance the level of field resistance of wheat varieties in a durable manner.

Molecular Mapping and Identification of Wheat Genes for Effective All-stage Resistance and High-temperature Adult-plant Resistance to Stripe Rust

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Growing resistant cultivars is the most effective, easy-to-use, economical and environment-friendly strategy for sustainable control of wheat stripe rust, caused by *Puccinia striiformis* f. sp. *tritici*. New genes for effective resistance and their tightly linked markers are needed to quickly incorporate diverse genes into cultivars for adequate and durable resistance. In the recent years, we have identified numerous genes in wheat cultivars and landraces for either all-stage (AS) resistance or high-temperature adult-plant (HTAP) resistance. The general procedure starts with developing recombinant inbred populations through single-seed decent. The mapping populations are phenotyped for stripe rust reactions in multiple field locations and years under natural infection and/or inoculation with selected races of the pathogen under controlled conditions depending upon the type of resistance. Genotyping techniques we have used include resistance gene analog polymorphism (RGAP) coupled with nulli-tetrasomic and deletion lines, simple sequence repeat (SSR) and single nucleotide polymorphism (SNP) markers. In addition to bi-parental mapping populations, we have also used selected wheat germplasm/cultivar panels for mapping stripe rust resistance genes using the genome-wide association study (GWAS) approach.

We have mapped 14 genes for AS resistance. *Yr45* was identified from Afghanistan spring wheat PI 181434 and mapped to chromosome 3DL. *Yr53* was transferred into spring wheat lines from Ethiopian durum wheat PI 183627 and mapped to chromosome 7BL. Also transferred from Ethiopian durum wheat PI 331260 and PI 480016 into spring common wheat lines, both *Yr64* and *Yr65* were mapped to chromosome 1BS. *YrSP* from European winter wheat Spaldings Prolific was confirmed to chromosome 2BL and determined to be at different locus from genes reported on the same chromosome including *Yr5*, *Yr7*, *Yr43*, *Yr44* and *Yr53*. One gene was mapped to chromosome 3DL in Ethiopian spring wheat PI 195097. Two genes were mapped to chromosomes 5BL and 7BL in Pakistani spring wheat PI 182126. Three genes were mapped to chromosomes 5BL, 5DL and 6BL in European winter wheat cultivar Druchamp for race-specific AS resistance, which also has HTAP resistance. Similarly, three genes were mapped to chromosomes 2AS, 3AL and 5BS in Pakistani spring wheat PI 182103 that also has HTAP resistance.

For HTAP resistance that is usually non-race-specific and durable, we have mapped 14 genes or quantitative trait loci (QTL). *Yr52* was identified from Indian spring wheat PI 183527 and mapped to chromosome 7BL. *Yr59* was identified from Iraqi spring wheat PI 178759 and also mapped to chromosome 7BL. *Yr62* was identified from Portugal spring wheat PI 192252 and mapped to chromosome 4BL. Three QTL were mapped to chromosomes 4DL, 5BS and 7BL in Pakistani spring wheat PI 182103. Eight QTL were mapped to chromosomes 1BL (2 QTL), 1DS, 2BL, 3AL, 5AL, 5BL and 6BL in winter wheat Druchamp. In addition, a GWAS was conducted on 1,000 spring wheat accessions phenotyped in multiple locations and races in the greenhouse. Genotyping was done using 4,585 SNPs in the wheat Infinium 9K assay. Ten QTL that consistent across various stripe rust evaluation experiments were identified and mapped to chromosomes 1BL, 1DS, 2AS (2 QTL), 3BS, 4AL, 4DL, 5AL, 6BL and 6DS.

Tightly linked markers, which are mostly within 5 cM, were identified for the above mapped genes. Many of the markers were tested on wheat germplasm/breeding lines for their high polymorphism across

various wheat backgrounds. New germplasm lines with better agronomic traits have been developed for some of the mapped resistance genes and many other resistance lines. The new germplasm lines should be easier to use in breeding programs and the markers should be useful in marker-assisted selection for incorporating the new resistance genes into wheat cultivars.

Mapping and validation of powdery mildew resistance loci from spring wheat cv. Naxos with SNP markers

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The German spring wheat cultivar 'Naxos' has shown high levels partial resistance to powdery mildew at the adult plant stage and the absence of race-specific resistance at the seedling stage. QTL mapping in the SHA3/CBRD x Naxos population identified resistance loci with stable effects across environments on 1AS and 2DL and two minor loci on 2BL and 7DS (Lu et al., 2012). The main objectives of the present study were to validate the powdery mildew resistance QTL from 'Naxos' in new genetic backgrounds and identify tightly linked SNP markers for powdery mildew resistance breeding. Two F_6 RIL populations from the crosses 'SABUF/5/BCN/4/RABI//GS/CRA/3/*Ae. tauschii (*190)' x 'Naxos' and 'Avocet' x 'Naxos' were evaluated over two years (2012 and 2013) at two locations (Hamar and Ås) in south-eastern Norway. The first population was genotyped with the Illumina iSelect 90K wheat chip and a total of 10,372 polymorphic SNP markers were used to develop linkage maps based on 131 RILs. The major QTL on 1AS and the minor QTL on 2BL reported in 'SHA3/CBRD' x 'Naxos' were confirmed by QTL mapping in this new population. In addition, two major QTL were identified on 2AL and 7BL. The phenotypic variations explained by the four major QTL on 1AS, 2AL, 2BL and 7BL from 'Naxos' were 18.9, 12.0, 11.2, and 11.3%, respectively, for the mean severity data across environments. Additionally, a QTL on 3AS from 'SABUF/5/BCN/4/RABI//GS/CRA/3/*Ae. tauschii (*190)' was found that explained 12.0% of the phenotypic variance. KASP assays were developed for the most closely linked SNP markers to the Naxos QTL on 1AS, 2AL, 2BL and 7BL and genotyped on 140 RILs from the Avocet x Naxos population. Three of these QTL – on 1AS, 2AL and 7BL – were found to segregate in this population but only the 1AS QTL was significant. However, this population also segregated for the *Lr46/Pm39* locus on 1BL (with resistance from Naxos) which showed a major effect. When this was taken into account, and QTL mapping performed on the subpopulation lacking *Lr46/Pm39*, all three QTL showed significant effects. The results suggest that *Lr46/Pm39* and the KASP markers linked to the QTL on 1AS, 2AL and 7BL can be useful in marker-assisted selection.

Acknowledgments
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Marker assisted screening of nepalese wheat genotypes and advanced lines for resistance to different races of wheat rust species

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Primers tightly linked to genes of interest are valuable tools in rust resistant wheat breeding. This research, conducted at Biotechnology Division, NARC; Nepal was focused to detect source of rust resistant genes and further utilize as an important weapon in gene pyramiding. A total of forty gene-specific primers consisting of Lr, Yr and Sr associated gene were applied for screening thirty wheat genotypes. DNA extraction, PCR Amplification, Gel Electrophoresis and autoradiography were carried out. Based on the value of band size, the presence of concerned resistance gene was predicted and binary scoring was done. Genealex and NTSYSpc2.1 softwares were applied for detail analysis. Some genes like Lr29, Lr51, YrCH42, Sr36, were detected in many genotypes but some genes like Lr47, Sr39, Sr24 were absent. Among tested genotypes, Chyakhura-1 possesses highest number of rust resistant genes and Annapurna-4 bears lowest number of genes. Dendrogram, PCA and jacard similarity coefficient matrix revealed relatedness among genotypes regarding presence of rust resistance genes. Highest value of Jaccard coefficient was obtained between genotypes Danphe-1 and Danphe-2 (0.87) showing their greatest resemblance. UPGMA dendrogram grouped thirty genotypes into distinct four clusters. Allelic frequencies calculation and Chi-square tests for H-W equilibrium were carried out for each marker. Percentage of Polymorphic loci was 88.57 and grand mean of observed heterozygosity was found to be 0.193 (S.E 0.057). Mean value of Shannon's informative index and fixation index (F) were 0.869 (S.E 0.076) and 0.617 (S.E 0.104) respectively. Thirty polymorphic markers were found highly significant in chi-square test.
Key words: Genotypes, Primers, Marker assisted screening, Polymorphism.

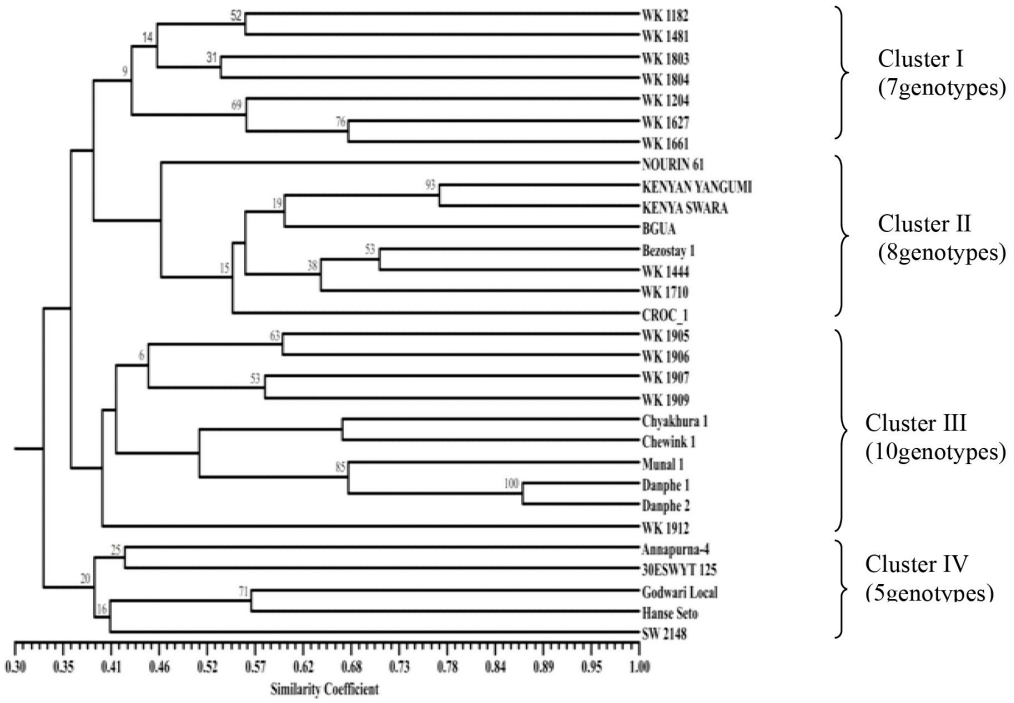

Figure 1. Grouping of genotypes into clusters as revealed by UPGMA dendrogram.

Genetic analysis of stripe rust resistance in spring wheat

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Stripe rust caused by *Puccinia striiformis West.* f. sp. *Tritici*) is a very devastating foliar disease of wheat and has caused substantial yield and quality losses in wheat across much of western Canada in at least four of the past 12 years. Growing resistant cultivars is the most economical and environmentally safe approach to reduce the use of fungicides and to reduce crop losses due to this disease. However, with the occurrence of new virulent races, deployed resistance genes can be rapidly rendered ineffective. Continuous identification and characterization of new resistance genes is essential to minimize economic losses. In order to identify novel sources of resistance to stripe rust and facilitate their utilization through marker assisted breeding, we developed three doubled haploid (DH) and several other segregating populations in spring wheat. The source of resistance in these populations are Sadash and AAC Innova (both registered cultivars) and several uncharacterized germplasm lines. These populations were screened in a stripe rust disease screening nursery under natural infection during 2013 and 2014. Among the DH populations, one was segregating for single gene while the other two showed complex inheritance with more than two genes. Among the segregating populations, four showed a single dominant gene in F_2 and $F_{2:3}$ progenies while one showed a single recessive gene. These populations are being advanced to develop $F_{2:6}$ recombinant inbred lines (RILs). The DH populations will be utilized for genotyping using 90K SNP Infinium iSelect assay as well as phenotyping over environments to map these genes on wheat chromosomes. The results of the genetic analysis of these populations for stripe rust resistance will be presented.

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Identification of wheat germplasm resistant to tan spot toxin Ptr ToxA using molecular markers

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Kazakhstan is one of the largest wheat producers in Central Asia. Cultivation of wheat varieties in monoculture with stubble burning to reduced tillage contributes to the accumulation of infectious potential and development of leaf spotting diseases (LSD), septoria and tan spot, in Kazakhstan to epidemic proportions. Under conditions favorable for the development of these diseases, crop losses exceed 50%. Genes conferring resistance to toxins are involved in the reduction in sensitivity to toxins. As there was no breeding work for the tan spot in the past, most wheat varieties released for commercial production are susceptible to the diseases. Therefore identification of germplasm resistant to tan spot is a very important task. Molecular markers accelerated the development of wheat cultivars with superior resistance by rapid identification of related genes. This study was aimed to screen and identify new wheat germplasm resistant to tan spot. The *Tsn1* gene confers sensitivity to the proteinaceous host-selective toxins Ptr ToxA produced by the tan spot fungus *Pyrenophora tritici-repentis* (Friesen *et al*., 2005). Two SSR markers tightly linked to the *Tsn1* gene have been used in this study. The markers Xfcp1 and Xfcp394 delimit a 0.4 cM interval including *Tsn1*. Using these markers the recessive tsn1 allele conferring toxin insensitivity was found in the number of accessions. Out of 97 wheat accessions, 38 sources including 20 Kazakhstani cultivars and advanced lines and 18 foreign wheat entries had polymorphic band same as both molecular markers Xfcp1 and Xfcp394, linked the recessive tsn1 allele conferring toxin Ptr ToxA insensitivity. Estimation of seedling resistance to tan spot confirmed the results of PCR for the presence of genes insensitivity to the toxin Ptr ToxA. A number of advanced lines showed high yield potential combined with resistance to *Pyrenophora tritici-repentis*. Currently in preliminary nursery 5 breeding lines are tested as a candidate to the new wheat cultivars, combining resistance to tan spot and high yield potential.

Effect of field management strategies on yellow rust in organic grown triticale and wheat

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Organic grown wheat and triticale have become very insecure in recent years due to severe attacks of yellow rust caused by the pathogen *Puccinia striiformis*. Before 2009 winter triticale was the most grown cereal crop in organic farming in Denmark due to high level of disease resistance, stable yields and high levels of competitiveness to weeds. This situation changed rapidly in 2009 when organic farmers experienced yield losses up to 90-100% as a result of severe attacks caused by a new race of yellow rust. Following these attacks the triticale variety Tulus was identified as resistance to the new race and in 2011/2012 this variety covered approximately 75% of the Danish triticale area in organic farming. In March/April 2012 the situation suddenly changed again when Tulus became highly susceptible at the early growth stages. However, later in the season plants recovered and showed a high level of adult plants resistance. Because of these experiences many organic farmers stopped to grow triticale. Nevertheless, if disease could be avoided triticale would still be a highly valuable crop in organic farming. This project was therefore launched with the aim to provide new strategies to prevent severe attack of yellow rust in organically grown triticale, and in wheat which can be an alternative to triticale.

Two parallel field trials, one with two varieties of triticale and one with two varieties of wheat, were conducted at an organic farm to study the effect of plant density, row spacing and nitrogen level on yellow rust severity. The trials were fully factorized and laid out as completely randomized block design with 4 replicates. Disease was established in early spring by planting pots with seedling infected with yellow rust in each experimental plot. Leaf area index were assessed three times and disease level four times during May and June 2014. Results for the final grain yields were also obtained for each treatment. Our results showed that a high level of nitrogen fertilization led to a higher disease severity in both wheat and triticale. The effect of plant density and row spacing was also significant in plots of the triticale variety Gringo for which the highest disease levels was observed. Leaf area index was affected by all the four experimental factor i.e. plant density, row spacing, nitrogen level and variety.

Development of an Integrated System for Control of Stripe Rust in the United States

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Epidemic of stripe rust, caused by *Puccinia striiformis* f. sp. *tritici*, occurs throughout all wheat-growing regions in the United States, especially more frequently in the Pacific Northwest due to the disease favorable weather conditions and growing both winter and spring wheat crops. Growing resistant cultivars and applying needed fungicides are the major approaches for control of the disease. Cultural practice can have effects on the disease. To support breeding programs throughout the country, more than 25,000 germplasm/breeding line entries are screened for stripe rust resistance at multiple locations under natural infection of the pathogen and entries in various uniform regional nurseries and varietal trails are also screened with selected races in the greenhouse at both seedling and adult-plant stages under low-temperature (4-20°C) and high-temperature (10-30°C) conditions every year. Infection type and severity data, together with summarized type of resistance and rating of resistance levels, are provided to breeding programs for releasing new cultivars with combination of adequate and durable resistance to stripe rust, resistance/tolerance to other biotic and abiotic stresses, and other desirable agronomic traits. Commercially grown cultivars are included in monitoring nurseries planted in various locations for monitoring resistance changes and evaluated in randomized split block experiments for yield losses and fungicide responses. The data of yield loss due to stripe rust and increase by fungicide application, together with relative yield loss and rating, of each cultivar are provided to growers for selecting resistant cultivars to grown. In the Pacific Northwest, every commercially grown cultivar has some level of resistance, and the overall resistance including all-stage resistance and various levels of high-temperature adult-plant resistance in the cultivars have been able to reduce yield losses from potentially 10-90% to 0-20%, and fungicide application has further reduced yield losses to below 3% in the recent years. New fungicide treatments are tested every year for efficacy and best timing, resulting in registration of numerous fungicides for growers to choose and reduction of fungicide cost. To improve effectiveness of fungicide application and reduce unnecessary application, we developed a set of models for predicting stripe rust epidemic levels in the term of potential yield loss. Using the model, we make first stripe rust forecast based on November and December weather conditions as early as in January and second forecast in early March based on the weather conditions during the entire winter before herbicide application and spring wheat planting. Field survey is conducted from November and throughout the growing season. Based on forecasts and field surveys, rust updates and recommendations are provided through e-mail, telephone and websites to growers for choosing resistant cultivars to grow and to make decisions on whether and when to use fungicides based on the type and level of a cultivar, the timing of disease development and pressure, and other cultural practices such as herbicide application.

Practical disease management in winter wheat in Denmark

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Wheat (*Triticum aestivum*) is the most important cereal crop in Europe. Wheat productivity is, however, restricted by a number of fungal diseases, including yellow rust (*Puccinia striiformis*), powdery mildew (*Blumeria graminis*), septoria tritici blotch (STB) (*Zymoseptoria tritici*), tan spot (*Drechslera tritici-repentis*) and Fusarium head blight (*Fusarium* spp) as some of the major problems.

Yellow rust epidemics depend on pathogen survival during off-season and winter, varietal resistance and virulence dynamics in yellow rust. Because of the epidemic nature of yellow rust, the control thresholds tend to be low under European cropping conditions. Control action is recommended as soon as yellow rust is found in susceptible varieties (1% plants with attack) where 3-4 treatments may be necessary. Fortunately breeders have been good at providing cultivars with little or no risk of yellow rust epidemic. For cultivars with adult resistance a certain overreaction takes commonly place as farmers react instantly to the occurrence of rust and find it difficult to trust adult resistance as a stable resistance.

For powdery mildew, varietal susceptibility is also important, but late sowing on sandy soil has also been observed to increase the epidemic risk significantly, almost regardless of varietal resistance. The thresholds for powdery mildew control are 10-25% infected plants during tillering and elongation. Most commonly only one treatment against powdery mildew is economical as yield responses for control are relatively low once the crop has past heading. Septoria tritici blotch (STB)is the disease in wheat, which seen over the years is regarded as most severe and yield reducing. Severity of STB is mainly driven by rain and periods with high humidity. Resistance variety to STB is very incomplete and control is in most seasons relying on 1-3 fungicide treatments.

Major differences in disease prevalence and economic importance are seen between areas and years, which are encouraging the use of adjusted fungicide control strategies, which rely on input from disease monitoring, at national level or preferably at field level. For all diseases correct timing and choice of efficient fungicides and dosages are very important for optimizing the economic net return. Number of optimal fungicide treatments varies from typically from 1-4 treatments depending on region, climate and cultivars grown.

Implementation of EU regulation 1107/2009, which include cut off criteria and substitution looks like the number of fungicides available in future could be reduced significantly, minimizing the possibilities for chemical control of diseases. This will increase the need for development and implementations of IPM. However with the level of diseases present in most seasons and regions, control measures are still relying on significant input of fungicides, if economical yield losses are to be avoided. Another treat against future use of fungicides is increasing problems with fungicide resistance. Control of major diseases relies on very few groups of chemicals and the need for diversity is well known, as a mean to minimize development.

Identification of leaf and stem rust resistant germplasm from Kazakhstan-Siberia spring wheat network

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Kazakhstan-Siberian zone cultivates wheat on the area of 20 mil. ha, being one of the leading regions in supplying with stability of grain production in Kazakhstan and Russia. Meanwhile, biotic and abiotic factors limits the effect on the potential of the Kazakhstan-Siberian wheat varieties. Particularly, the drought and diseases turn out to be the major reasons of the yield shortage. Explicit result of breeding for resistance depends on the presence of initial material carrying the effective resistance genes. Special importance in this issue belongs to the ecological and geographical tests of perspective samples. For this support, the Kazakhstan-Siberian Network on Wheat Improvement (KASIB) was developed under the aegis of CIMMYT and scholars of Kazakhstan and Russia. Taking into consideration the occurring mutations with the predominant races in this region and the possibility of new virulent pathotypes entrance leads to loss of resistance in the varieties; thereby one of the way of rust control involves field phenotypic evaluation of the perspective breeding material. The 14th KASIB nursery consisting of 49 spring bread wheat cultivars and lines was tested for identification of resistance to prevailing leaf (*P. triticina*) and stem (*P. graminis*) rusts races with following selection for sources of resistance. The material was studied in the 3 locations of KASIB network – Kazakh Scientific and Production Center of Arable Farming and Crop Production, Kazakhstan (KazSPC AFCP, 43°13'N / 76°40'E), Omsk State Agricultural University, Russia (OSAU, 55°1′N / 73°18′E), and Chelyabinsk ARI, Russia (ChARI, 54°55'N / 60°46'E).

In the regions of the south-east of Kazakhstan (KazSPC AFCP) and the Ural of Russia (ChARI) the most resistant samples to leaf rust accounts for 91.3% and 87.0%, respectively, belonged to mid-maturity group, while Western Siberia (OSAU) appeared the late-maturity samples (45.4%). The same nursery was analysed for stem rust virulence in the Western Siberia (OSAU) conditions where the mid-maturity cultivars (52.2%) demonstrated the highest resistance compared with other maturity groups. Breeding material originated from the Kurgan province, Western Siberia, and Northern Kazakhstan provided the group resistance in combatting with leaf and stem rust in 3 KASIB locations. Early-maturity wheat samples including the local checks of three regions and cv.Astana 2 demonstrated the effectiveness to races of both rusts species varying within resistance (R) to moderately resistant (MS) with disease severity between 5 and 10% (tab.1).

Table 1. Resistant and susceptible wheat samples to *P.triticina* and *P.graminis* of the 14th KASIB on maturity groups.

Resistance and maturity group KazSPC AFCP		Leaf rust			Stem rust	Disease assessment
		ChARI	OSAU	OSAU		
Resistant	Early-maturity	Local checks, Astana 2 (Kazakhstan)				R-10 MS
	Mid-maturity	Ekada 148 (Kazakhstan), Lutescens 1147, Lutescens 555/01-10-1, Sigma (Russia)				0-20S
	Late-maturity	Fiton 82, Fiton C-54, Tselinnaya Niva (Kazakhstan), Lutescens 205/03-1, Lutescens 7/04-26, Lutescens 141/03-2 (Russia)				0-20S
Susceptible	Early-maturity	Pamyati Azieva, Saratovskaya 29 (Russia)				20S - 100S
	Mid-maturity	Lutescens 1519, Lutescens 1764 (Kazakhstan), Lutescens 66 B (Russia)				
	Late-maturity	Lutescens 22 (Kazakhstan), local checks late (Kazakhstan, Russia)				

The presence of adult plant resistance genes and/or all-stages resistance genes in the cultivars and lines of mid and late-maturity groups describe avirulence of two rust species races. In all studied geographical areas high resistance to leaf rust was identified in cv. Ekada 148 (PC "Fiton", Kazakhstan) showing a zero reaction type at ChARI, Russia and «R» at KazSPC AFCP, Kazakhstan. Most probably, the close link between *Lr*- and *Sr*-resistance genes located in this cultivar provides the resistance "5 MR" to stem rust races at OSAU, Russia. A series of wheat samples appeared to be susceptible in each group. The disease covered 20-100% of the plant. According to field screening the lines Lutescens 1519, Lutescens 1764, Lutescens - 66 B, Lutescens 22 of mid and late-maturity groups can be discarded from the breeding process. In this regard, the experimented wheat samples of all maturity groups showed the group effectiveness to *P.triticina* and *P. graminis* pathotypes in various geographical regions of KASIB program and can be applied as a valuable source of rust resistance in breeding.

Distribution of stem rust (*Puccinia graminis* f. sp. *tritici*) races in Ethiopia

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Wheat is one of the most important cereal crops of Ethiopia. Stem rust caused by Puccinia graminis f. sp. tritici is amongst the biotic factors which can cause up to 100% yield loss if susceptible cultivar grown and epidemic occurs. The highland of Ethiopia is considered as a hot spot for the development of stem rust diversity. This study was carried out to determine virulence diversity and race distribution of P. graminis f. sp. tritici in Ethiopia. Eighty (80) wheat stem rust samples were collected in 2013 cropping season from Oromia, Amhara and Tigray region. Of the samples collected, 66 were analyzed on to the 20 stem rust differential lines. A total of 9 races were identified, which includes TTKSK, TTKTF, TTKTK, JRCQC, TKTTF, TTKSC, TRTTF, SRKSC and RRKSF. Race TTKSK was dominant and widely distributed in the Oromia and Amhara regions with 52% frequency; it was not isolated in Tigray region. The most virulent and new race, TKTTF which causes localized stem rust epidemic in Bale and Arsi was predominantly distributed in Oromia region with 36.4% frequency value. Most of the genes possessed by the differentials were ineffective against one or more of the tested isolates. Only stem rust resistance gene 24 was found to confer resistance to all of the races isolated in this study. This gene could be used in combination with other genes through gene pyramiding in breeding for resistance to stem rust in Ethiopia.

Key words: Stem rust race, Puccinia graminis f. sp. tritici, Stem rust resistance genes.

Table 1. Races of *P.graminis* f.sp.*tritici* and their virulence spectrum in Amhara, Oromiya and Tigray regions of Ethiopia in 2013.

Race	Virulence spectrum (ineffective Sr resistance genes)	No	%
Oromia			
TTKSK	5,21,9e,7b,11,6,8a,9g,9b,30,17,9a,9d,10, 31, 38,MCN	27	47
TTKTF	5,21,9e,7b,11,6,8a,9g,9b,30,17,9a,9d,10,TMP,38,MCN	1	2
TTKTK	5,21,9e,7b,11,6,8a,9g,9b,30,17,9a,9d,10,TMP,31,38,MCN	1	2
JRCQC	21,9e,11,6,9g,17,9a,9d,MCN	1	2
TKTTF	5,21,9e,7b,6,8a,9g,36,9b,30,17,9a,9d,10,TMP,38,MCN	24	42
TTKSC	5,21,9e,7b,11,6,8a,9g,9b,30,17,9a,9d,10,MCN	1	2
TRTTF	5,21,9e,7b,11,6,9g,36,9b,30,17,9a,9d,10,TMP,38,MCN	1	2
	Total	57	100
Amahara			
TTKSK	5,21,9e,7b,11,6,8a,9g,9b,30,17,9a,9d,10, 31, 38,MCN	7	87.5
SRKSC	5,21,9e,11,6,9g,9b,30,17,9a,9d,10,MCN	1	12.5
Total		**8**	**100**
Tigray			
RRKSF	5,21,7b,11,6,9g,9b,30,17,9a,9d,10,38,MCN	1	100
G total		**66**	**100**

Novel and Classical Resistance Genes Provide Nonhost Resistance to Wheat Stripe Rust in *Brachypodium distachyon*

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The plant pathogen *Puccinia striiformis* f. sp. *tritici* (PST), commonly known as wheat stripe rust, is an obligate biotroph with a wide host range. In addition to the agronomically important grass species wheat, barley and rye, it can also rarely infect the taxonomically distant *Brachypodium distachyon*. As a model grass species with a sequenced genome, a high rate of recombination, and advantageous morphological characteristics, *B. distachyon* is an excellent system to study the genetic basis of PST resistance in an intermediate nonhost species. We found that most *B. distachyon* accessions were completely resistant to various UK and Australian PST isolates, whereas some accessions showed a range of susceptibility symptoms. Three populations were used to establish the genetic architecture of this intermediate nonhost resistance. Inoculation with three UK and one Australian PST isolates identified three major QTLs, designated *Yrr1* to *Yrr3*. Interestingly, *Yrr1* was narrowed down to a 75 kb gain of function interval on chromosome 4, which does not contain any known resistance gene homologs. However, classical resistance genes encoding nucleotide binding site, leucine-rich repeat domains (NBS-LRRs) are the candidates underlying *Yrr2* (chromosome 4) and *Yrr3* (chromosome 2). Strikingly, isolate specificity was observed to *Yrr2*, highlighting the conservation of typical aspects of host-pathogen interaction in this system. These results suggest the involvement of both novel and classical resistance pathways in intermediate nonhost resistance.

Seasonal variation in yellow rust incidence in Afghanistan during last few years

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Wheat is the most important food crop of Afghanistan. It accounts for close to 80% of cereal's acreage and production. Afghans rank among the highest consumers of wheat at about 250 Kg/capita/annum and derive up to 60% of their average caloric intake from wheat. Last decade has witnessed several wheat R&D interventions in the country to push national wheat production which is now close to five million tonne for past few years. These efforts led to the release of over 15 new wheat varieties during last 10 years or so increasing the number of varieties in seed chain to over 20 at present. Yellow rust is the major wheat disease in the country and does cause appreciable yield losses during years of sever incidence. Efforts are being made to regularly screen seed chain varieties against yellow rust in a 2010-11 season initiated National Rust Screening Nursery. The nursery comprises of all seed chain varieties and new test entries of advance breeding trials.

Table 1. Differential yellow rust incidence on seed chain varieties.

No.	Variety	2013-14	2012-13	2011-12
1	Mazar-99	TR	20M	TR
2	Parva-02	TR	5R	0
3	Solh-02	TR	10R	10 MR
4	Ghori-96	30S	80 S	40 MS
5	Pamir-94	0	5MR	40 MS
6	Muqawim-09	TR	40MS**	0
7	Sheshambagh-08	5R	10R	20 MR
8	PBW-154	30S	80S	40 MS
9	Dorokhshan-08	20MS	10MR	5 MS
10	Gul-96	0	20MR	40 S/ 60S*
11	Lalmi-01	5MR	10MR	0
12	Lalmi-02	0	10MR	TR
13	Chonte#1	0	10R	0
4	Rana-96	40S	20MR	TR
15	Baghalan-09***	60S	10MR	0
16	Lalmi-03	5MR	20MR	TMR
17	Darulaman-07	20MS	20MR	TR
18	Daima-96	30S	40MS	0
19	Koshan-09	5R	60MS**	0
20	Herat-99	60S	100S	60 S

Depending on facilities and feasibility, the nursery is sown at a number of Agricultural Research Institute of Afghanistan (ARIA) research stations located throughout the country. The nursery was sown at five, eight and 10 research stations in 2011-12, 2012-13 and 2013-14 seasons, respectively. Genotypes

were sown in two rows of two meters each with an infector line after every twenty rows. Recommended agronomic practices were adopted to raise a good crop. The yellow rust was scored following modified Cobb's scale (Peterson, 1948). The highest yellow rust score observed on a genotype anywhere in Afghanistan in a season is reported in Table 1. Seasonal variation in yellow rust occurrence is very much evident from the information given in the table. Though four varieties have been observed to be susceptible during all the three years, there are also varieties like *Pamir 94*, *Muqawim 09*, *Dorokshan 08*, *Gul 96*, *Rana 96*, *Baghlan 09* and *Koshan 09* showing differential reaction over years. The seasonal variation has perhaps mainly been because of prevalence of different races or differential inoculum load over seasons. Varieties observed susceptible only in one year remain under wait and watch but those found consistently susceptible are recommended for removal from seed chain.

*: Leaf rust; **: Single location: ***: Single year

Microphenotype of stripe rust infection in wheat lines containing different resistance loci

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Worldwide significance of stripe (yellow) rust, caused by *Puccinia striiformis*, can be attributed to a highly variable virulence profile, adaptation to different production regions, dispersal potential, and systemic colonisation and destruction of wheat foliage. Theoretically, durable protection to stripe rust can be achieved by combining several resistance QTL in a single variety. However, attainment of durability depends on the deployment of resistance genes and/or QTL that have the best potential to endure an evolving pathogen. In previous investigations of a Kariega (resistant) x Avocet S (susceptible) doubled haploid (DH) population two major QTL for adult plant stripe rust resistance on chromosomes 2B and 4A, and the pleiotropic *Lr34/Yr18/Sr57* locus on 7D, were mapped. One way of characterising resistance gene expression is through detailed investigation of host-pathogen interactions. To investigate the expression of resistance at histological level, 16 DH lines containing QTL individually or in combination, together with the parents, were sampled from stripe rust-infected field plots over two seasons. Flag leaves were prepared for fluorescence microscopy and number of haustorium mother cells, colony length and host cell necrosis were measured. Analysis of variance showed that lines differed significantly for all traits measured. Haustorium mother cells ranged from a mean of 1.68 per colony in line MP68 (4A+7D) to >30 per colony in MP16 and MP145 (no QTL). Large colonies occurred in MP148 (4A) (2307 µm) as opposed to MP65 (2B+4A+7D) (53 µm), the latter also expressing most host cell necrosis (hypersensitivity index=3.78). In general more resistant stripe rust phenotypes, characterised by less colonisation, occurred in lines carrying two or three QTL.

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Solatenol™, an SDHI fungicide setting new standards in disease control

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Solatenol™ is a new broad spectrum foliar fungicidally active ingredient discovered and developed by Syngenta. It is the third Syngenta succinate dehydrogenase inhibitor (SDHI), and the second in the benzonorbornene amide subclass.

The strong affinity to the target enzyme succinate dehydrogenase results in very high intrinsic activity. Combined with a strong association with the plant's wax layer, it provides reliable, long lasting disease control even under unfavorable, e.g. wet weather conditions. Solatenol™ has been tested on many important crops and has shown excellent activity on a wide range of destructive plant pathogens.

In mixture with azoxystrobin, Solatenol™ is registered and sold as Elatus™ in the major South American soybean markets (Brazil, Paraguay, Uruguay, Argentina and Bolivia).

In addition to its excellent performance against the soybean rust, Solatenol™ is also active on a broad spectrum of wheat (*Zymoseptoria tritici, Puccinia striiformis, Puccinia recondita*) and barley diseases (*Puccinia hordei, Ramularia collo-cygni, Rhynchosporium secalis, Pyrenophora teres*). During the rust epidemic in 2014 in Europe, it has shown outstanding and longer protection than the current standards.

Solatenol™ is also very active against key diseases in other crops including pome fruits (*Venturia inaequalis*), potatoes (early blight), vegetables (powdery mildew, early blight, anthracnose), corn *(Puccinia sorghi, Cercospora zeae-maydis*), peanuts (*Puccinia arachidis, Sclerotium rolfsii*).

Solatenol™ is safe to the crop when applied alone and in mixtures, e.g. with DMI, QoI compounds, or a range of other partners. Solatenol™ has been submitted for registration in key markets and on multiple crops, including use against cereal rusts.

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Field screening for resistance to leaf rust pathotypes in the Kazakhstan germplasm bank of spring wheat

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The spring wheat yield heavily depends on influence of biotic and abiotic stresses in the Northern Kazakhstan (NK). The researchers have repeatedly referred the significant role of grain loss to wheat leaf rust (*Puccinia triticina (Pt)*). Cultivated varieties are tend to be susceptible to Kazakhstan leaf rust pathotypes; and therefore the creation of highly-yield and resistant to diseases cultivars appears to be the important step in sustainable crop production. In order to apply new and useful sources of resistance in forthcoming breeding process the field evaluation of 88 bread wheat cultivars and lines was conducted in 2013 and 2014. The studied material included samples from local and foreign breeding – Kazakhstan (33), Russia (24), Ukraine (16), and other (15) used from genepool collection of A.I.Barayev Research and Production Center of Grain Farming (Barayev RPC GF). All wheat samples were sawn in the optimal time of the region (May, 25-27th) in artificially created epidemic in the field plot of Barayev RPC GF. The spore material consisted of 6 particular *Pt* pathotypes as well as one unidentified population was provided by Research Institute of Problems of Biological Safety (Zhambylskaya prov., Kazakhstan). The inoculation was manually carried out in the stage of stem elongation-booting using the mixture of pathotypes. Within season two scorings were recorded with 10-12 days period based on types of reaction (scale of Mains and Jackson) and severity (scale of Peterson). The current study included a big number of samples from different provinces of Kazakhstan, the vast majority of them (81.8%) demonstrated the highest types of reaction "4" to used pathotypes in both years. The host-pathogen interaction has found only a few highly-resistant cultivars Skarlet, Stepnaya 75, Ekada 113 (Western-Kazakhstan breeding) and Fiton s 50chs, Eritrospermum 35 (NK breeding). The opposite situation was observed with foreign germplasm. The cultivars Anyuta (Belarus), Bonbona (Poland), Bonpain (France), Quattro (Germany), Rolla (Sweden), A 9392 (USA), and Tincurrin (Australia) carry the resistance genes effective on the territory of NK. Used pathotypes were avirulent to 70% of Russian and Ukraine collection of wheat cultivars. Many western-Siberian (Russia) cultivars and lines showed incompatibility with pathotypes in the host-pathogen relation.

Table 1. Bread wheat samples resistant to leaf rust in the germplasm bank of Barayev RPC GF.

Countries-originators	Samples name	Pathotypes	
		2013	2014
Kazakhstan	Skarlet, Irim, Stepnaya 75, Ekada 113, Fiton s 50 chs, Eritrospermum 35	THK/RJ, QBQ/GB, TTT/QJ	Race 77, KTP/C, TJC/CD, unidentified Kazakh population
Russia	Liniya TGU-1, Omskaya 41, Lutescens 844, Lutescens 697, Sibirskiy Alyans, Saratovskaya 66, Saratovskaya 70, Novosibirskaya 18, Novosibirskaya 31, Eritrospermum 95/07, Lutescens 89-06, Lutescens 172-01, Lutescens 151/03-85, Lutescens 311/00-2-6, Eritrospermum 23390, Lutescens 23490		
Ukraine	Anshlag, Gordinya, Vishivanka, Evdokiya, Ostinka, Solomiya, Korinta, Torchins'ka, Etyud, Skorospilka 98		
Other	Casavant, CHSS 98Y03555 T, Fulgua II, Anyuta, Bonbona, Bonpain, Quattro, Rolla, A 9392, Tincurrin		

According to two-year assessment, it is recommended to take into consideration a genetic work for race-specific resistance in Kazakhstan, where the use of foreign wheat germplasm carrying the effective resistance gene can significantly contribute to rust control.

Triticum militinae introgressions into bread wheat affect host responses to powdery mildew challenge

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Resistant plant phenotype can be achieved either by introduction of R-gene-mediated race-specific resistance or non-race-specific QTL-mediated resistance. In contrast to race-specific resistance that can be overcome by a mutation in avirulence gene of the pathogen, non-race-specific quantitative resistance is durable. However the mechanisms of quantitative resistance remain obscure. We used bread wheat lines carrying introgressions from *Triticum militinae* (2n=28) on 1A, 4A, 5A, 5B and 7A chromosomes and two powdery mildew *Blumeria graminis* f.sp. *tritici* (Bgt) isolates to microscopically identify the effects of resistance QTLs on Bgt development dynamics and host cell responses (Fig.). Line having a combination of all five QTLs exhibits the slowest powdery mildew progression, line with QTL on 4A (main QTL) chromosome outperforms the background cv.Tähti, a combination of four QTLs on 1A, 5A, 5B, 7A (minor QTLs) chromosomes is not different from background. Introgression of all five QTLs effectively decreases primary and secondary host cell penetration by the Bgt, main QTL negatively affects secondary penetration, minor QTLs decrease primary penetration and do not have any significant effect on secondary penetration efficiency. No significant difference between penetration efficiency of two Bgt isolates was found. Inability to establish compatible interaction was accompanied by host cell death and hydrogen peroxide production (Fig. D). Data provided by this study contributes to understanding of quantitative disease resistance mechanism.

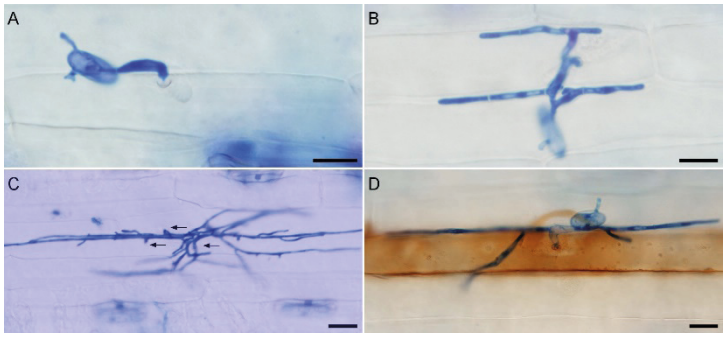

Figure 1. Powdery mildew developmental stages. Powdery mildew mycelia 24, 48, 72 hours post infection (**A**, **B** and **C** respectively**)** of susceptible cultivar Tähti. DAB staining indicative of hydrogen peroxide production in haustoriated cell of wheat line with five T. militinae introgressions 72 hours post infection (**D)**. Arrows indicate multiple penetration sites with haustoria. Scale bars (**A**, **B** and **D**) 20µm, (**C**) 40µm.

About the foundation

After 1945, as Europe recovered from the devastation of war, plant pathologists with interests in the cereal rusts were keen to re-establish contacts with each other across Europe. At that time there was concern about the possible spread of stem rust, on a pathway from the Mediterranean area northwards in Europe. There was also concern about the spread of yellow (stripe) rust. There were several informal meetings between European workers concerned with these diseases. There was agreement among them that a more comprehensive system for exchange of information was desirable. In 1964 the first combined cereal rusts conference was held in Cambridge at the Plant Breeding Institute. Some famous names attended, including E. C. Stakman, and these are listed under a photograph of the participants in the Proceedings. The front page of the Proceedings records the meeting as comprising the Third European Yellow Rust Conference, First European Brown Rust Conference and Third European Colloquium on Black Rust of Cereals.

The purposes of the Foundation, given in the legal document by which it is registered were as follows:

- to organize international conferences on problems related to the rust diseases of cereals, with special reference to Europe and the Mediterranean area;
- to promote international co-operation on the study of cereal rusts in particular, and where necessary of cereal diseases and pests in general;
- to promote scientific research on cereal rusts in particular, and where necessary, of cereal diseases and pests in general, and further the practical application the scientific results;
- to edit or to promote the editing of an information bulletin on pests and diseases of cereals, with special reference to cereal rusts.

A debt is owed by the rust workers of today to the foresight of those early post-war workers who recognised the need for an organisation for exchange of information about cereal rusts.

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