Poster 59. Haplotype analysis of the leaf stripe resistance locus Rdg2a in different barley genotypes.

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Leaf stripe disease on barley is caused by the seed-transmitted hemi-biotrophic fungus *Pyrenophora graminea* (anamorph *Drechslera graminea*). Race-specific resistance to leaf stripe is controlled by two known *Rdg* (*Resistance to Drechslera graminea*) genes, the *H. spontaneum*-derived *Rdg1a*, mapped to chromosome 2HL, and *Rdg2a*, identified in *H. vulgare* and mapped on chromosome 7HS and cloned in the resistant cultivar Thibaut. The *Rdg2a* locus contains a gene cluster of three sequence-related coiled-coil, nucleotide-binding site, and leucine-rich repeat (CC–NB–LRR) encoding genes. However, only one gene conferred resistance to isolate Dg2, against which *Rdg2a* is effective, when the susceptible cultivar Golden Promise was transformed with the *Rdg2a* candidates. The high level of sequence similarity between the three genes most likely contributed to significant rearrangements during evolution, probably derived from unequal crossing-over resulting in sequence exchange between paralogs and in the generation of recombinant genes, as well as in expansion/contraction of gene copy number. To examine the haplotype variation at the *Rdg2a* locus, the sequencing of the allelic *Mrdg2a* (*Morex rdg2a*) locus of the Dg2-susceptible cultivar Morex was carried out and revealed large rearrangements including two deletions that generated an *Rdg2a*-homolog gene. This gene most likely derived from an un-equal crossing-over between the *Rdg2a* ancestor(s) and its paralog *Nbs2-Rdg2a*. PCR analyses performed with informative markers at eight loci within the *Rdg2a* locus identified eight different haplotypes. The Thibaut haplotype was observed to be largely conserved in Dg2-resistant barley cultivars. The resequencing of the *Rdg2a* gene in barley genotypes showing the same Thibaut haplotype or the same resistant phenotype revealed high sequence similarity to Thibaut *Rdg2a*, demonstrating the widespread conservation of the gene. Nonetheless, some sequence variation were identified in at least two barley genotypes that were verified for possible differences, with respect to *Rdg2a*, in the range of resistance specificities towards different leaf stripe isolates.

Poster 60. Identification of a novel major leaf rust resistance QTL in the Swiss winter wheat cultivar Forno.

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Leaf rust (*Puccinia triticina*) is a major wheat disease which can cause significant yield losses globally. Rapidly evolving pathogen races increase the demand for more durable and race-nonspecific resistance genes. So far only four (*Lr34*, *Lr46*, *Lr67*, and *Lr68*) such durable leaf rust resistance genes have been identified. The identification and characterization of new sources of durable leaf rust resistance is important to ensure food security in the future. We earlier identified two major leaf rust resistance loci on chromosomes 1BS and 7DS in the Swiss winter wheat cultivar Forno. The resistance on chromosome 7DS was conferred by a single gene named *Lr34* that encodes ABC transporter gene.

In this study we further characterized the second resistance locus on chromosome 1BS, named *QLrP.sfr-1BS*. This locus initially explained 28–32% of the phenotypic variance using 240 recombinant inbred lines (RILs) of the cross 'Arina/Frono'. We grouped these 240 RILs into the following categories: *+QLrP.sfr-1BS/–Lr34*, *–QLrP.sfr-1BS/+Lr34*, *+QLrP.sfr-1BS/+Lr34*, *–QLrP.sfr-1BS/–Lr34*, each having 21, 26, 22, and 18, lines respectively. The selected lines were phenotyped for the three traits, area under disease progress curve for the percentage of leaf area infected infection type, and leaf tip necrosis (LTN). The average length of LTN of only the *QLrP.sfr-1BS* group was significantly lower than the group having *Lr34*, indicating that unlike *Lr34*, *QLrP.sfr-1BS* is not associated with LTN. The groups only containing either *QLrP.sfr-1BS* or *Lr34* showed the same level of resistance, whereas the group containing both *QLrP.sfr-1BS* and *Lr34* show significantly higher level of resistance, demonstrating an additive effect of the two genes. A genetic linkage map for chromosome 1BS spanning a distance of 190 cM was constructed using SSRs and RFLP markers. Composite interval mapping detected *QLrP.sfr-1BS* with a LOD score of 17, flanked by markers wmc230 and gwm18. By adding

more polymorphic SSR markers, the target interval was reduced from 40 cM to 8 cM. To further enrich the linkage map we explore the syntenic information of the reference genomes of *Brachypodium* and wheat.

Poster 61. Fine mapping of the leaf rust resistant Lr14 locus in durum wheat.

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Leaf rust is a main disease that affects durum wheat production. Resistance to this fungal pathogen is therefore a main objective for durum wheat breeding. The leaf rust resistant allele *Lr14*-Creso from the durum wheat cultivar Creso and its derivative Colosseo is one of the most important leaf rust resistance sources present in the modern durum germplasm, and it has been located in the distal portion of chromosome 7BL (Maccaferri et al. 2008. Theor Appl Genet 91:731-738), with the identification of linked SSR markers (gwm146 and gwm344) suitable for marker-assisted selection. Our target is to fine map and eventually clone *Lr14*-Creso. To this end, a set of ~100 recombinant $BC_2F_{3:4}$ isolines were developed. Additional BC_3 isolines were developed in order to confirm and to further study the phenotypic effects of *Lr14*-Creso. New SSRs and 13 conserved orthologous sequence (COS-SNP) derived markers (UBW) were developed and mapped within an interval of 8 cM that includes the QTL peak. The COS-SNP markers have been obtained by exploiting the conserved collinearity between the most distal portions of rice chromosome 6, *Brachypodium* chromosome 1, and wheat chromosome arm 7BL. Using the coding sequence of the rice and *Brachypodium* collinear genes, the corresponding wheat orthologs were retrieved, specific PCR assays (~1 kb) targeting the intron/exon boundaries of the genes were designed, amplified on the genomic DNA of the parents Colosseo and Lloyd and the amplicons cloned in pGEM®-T Easy Vector. Sequencing of the amplicons allowed for the identification of the SNPs differentiating the two homeologous copies of each gene (genome-specific SNPs) as well as the varietal-SNPs between Colosseo and Lloyd. These SNPs were then used to develop markers that, at the same time, were 7B-specific and polymorphic between the two parents. The detailed synteny analysis and the map of the region including the newly developed markers will be reported. The results are supported by an independent association mapping study carried out using a panel of 183 elite accessions (Maccaferri et al. 2010. Mol Breed 26:189-228), which allowed us to validate the presence of *Lr14* and to further improve mapping resolution.

Poster 62. Marker development for the wheat leaf rust resistance gene Lr16 using a comparative genomic approach.

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Wheat leaf rust, caused by the fungus *Puccinia triticina*, is one of the serious diseases of wheat worldwide. *Lr16* is a widely deployed leaf rust resistance gene that is effective against the North American *P. triticina* population when in combination with *Lr34*. Previous studies mapped this gene on the distal end of wheat chromosome bin of 2BS (fraction length (FL) 0.84–1.00). In the current work, we integrated a flanking marker and additional markers that are within a close proximity to the gene. Orthologous conserved markers were developed from bin-mapped expressed sequence tags (ESTs) and from ESTs identified based on colinearity with the *Brachypodium* genome. ESTs with orthologous genes in these collinear regions were used to develop new conserved markers for saturating the region. Seventy-three pairs of primers were developed, and ~30% showed polymorphism in the segregating RIL/DH populations. Efforts are now underway to integrate these polymorphic markers into genetic maps.