Towards cloning the powdery mildew resistance gene QPm.tut-4A introgressed to bread wheat from T. militinae.

Monika Kladivová ¹, Barbora Klocová ¹, Diana Posti ², Irena Jakobson ², Hilma Peusha ², Kadri Järve ², Ljudmilla Timofejeva ², Yuqin Hu ³, Yong Zhang ³, Frank M. You ³, Ming-Cheng Luo ³, Ajay Kumar ⁴, Shahryar F. Kianian ⁴, Hana Šimková ¹, Jan Šafář ¹, Jaroslav Doležel ¹, and **Miroslav Valárik** ¹.

¹ Centre of the Region Haná for Biotechnological and Agricultural Research, Institute of Experimental Botany, Sokolovská 6, CZ-77200 Olomouc, Czech Republic; ² Department of Gene Technology, Tallinn University of Technology, Akadeemia tee 15, Tallinn 19086, Estonia; ³ Department of Plant Sciences, University of California, Davis, CA 95616, USA; and ⁴ Department of Plant Sciences, North Dakota State University, Fargo, Loftsgard Hall 470G, Fargo, ND 58108, USA.

Introgression of agronomically important genes from wild relatives is one of the most effective means to improve wheat gene pool. However, introgressions often introduce collinearity and recombination aberrations. Recently, resistance to powdery mildew from the tetraploid wheat Triticum militinae was introgressed to hexaploid wheat cultivar Tähti. The locus with the major contribution in both seedling and adult stage was mapped on distal end of 4AL chromosome arm to the region delimited by wmc232 and wmc313 markers (~10 cM) and denominated as QPm-tut-4A. In the original mapping population from a cross of Tähti by resistant introgressive line 8.1, the region includes 12 markers. However, their order could not be resolved using 1,200 haplotypes. To resolve this obstruction, a combination of traditional approaches and recent advances in wheat genomics were used. For marker ordering, a 4AL-specific radiation hybrid panel and three additional recombination based mapping populations were employed. For efficient marker development, 4AL chromosome-specific BAC library was constructed, fingerprinted, and ordered into contigs. The region was anchored to the rice, Brachypodium, and Sorghum chromosome 6, 1, and 10, respectively. All genes from the collinear regions were mapped to our mapping populations. Marker development was enhanced using MDA amplified DNA from the 4AL chromosome arm of Chinese Spring, and the same arm carrying the translocation in combination with survey sequences of chromosomes 7A and 7D. Using these resources, the *OPm-tut-4A* gene was delimited in 0.2 cM region flanked with the gpw356 and gpw3079 markers. The remaining three SSR markers (barc70, gwm832, and gpw3556) completely linked to the QPm-tut-4A locus were used to identify three BAC contigs that comprise 158 BAC clones and cover about a 2 Mb region. From the contigs, 114 BAC ends were sequenced and used for marker development. The physical map, radiation hybrid lines, markers, and the remaining BAC-end sequences will be used to saturate the QPm-tut-4A map and, finally, clone the gene. This work has been supported by the MEYS of Czech Republic (Operational Programme Research and Development for Innovations No. ED0007/01/01), Internal Grant Agency PrF-2012-001, and by Estonian Ministry of Agriculture.

Cleistogamy in the Triticeae: genetic variation and its regulation.

Ning Wang, Shunzong Ning, and Takao Komatsuda. National Institute of Agrobiological Sciences, Tsukuba, Ibaraki, Japan.

Cereals are requested for human energy and protein needs. Efforts in particular brought attention to concerns of seed-borne disease contamination as well as enhance seed production. In the typical grass flower, the florets gape as a result of the swelling of the lodicules (non-cleistogamous), but non-gaping (cleistogamous) variants are known in many species. The possibility of manipulating flower type is of particular relevance for the prevention of pollen flow from transgenic types and for the control of Fusarium head blight, a disease to which non-cleistogamous barley and wheat cultivars are especially vulnerable. In barley, the development of lodicule is genetically determined by allelic variation at the *cly1* (syn. *HvAP2*) locus. In the non-cleistogamous type flower (*Cly1.a*), *HvAP2* activity is suppressed by miR172-directed cleavage. A single nucleotide substitution at the microRNA target site generates the recessive allele *cly1.b*, in which no cleavage occurs, converting the flower into a cleistogamous type. *HvAP2* homologues among various diploid and polyploid wheats are regulated in the same fashion as in barley; in non-cleistogamous wheats, miR172-directed cleavage can be detected at each AP2 homoeologue. As the cleistogamous type is produced by a recessive *AP2* allele, to convert hexaploid wheat into a cleistogamous type, we would need to identify the recessive allele at each of the A, B, and D homoeologi and then combine them into a single individual.

Variations in patterns of gene expression are central to evolution. A large-scale screen of barley germplasm has identified an alternative de-repressor of lodicule development SV235. The relevant HvAP2 coding sequence is identical to that of clyl.b, so that HvAP2 down-regulation is not associated with miR172-directed degradation; instead, transcriptional repression appears to be induced by the maintenance of the HvAP2 promoter in a hyper-methylated state during the period when the vascular tissue in the lodicule would normally develop. The cleistogamous type can also arise in the presence of non-cleistogamous type lodicule development. In certain Clyl.a x clyl.b hybrids, the spikes remain within the boot during anthesis, and the lodicules remain shrunken even after the emergence of the spike from the boot.

Genetics and molecular evolution of a 3.1-Mb genomic region harboring both wheat prolamin and disease resistance gene families.

Lingli Dong ^{1,2}, Naxin Huo ^{1,2}, Yi Wang ^{1,2}, Karin Deal ², Frank You ³, Jan Dvorak ², Olin D. Anderson ¹, Ming-Cheng Luo ², and **Yong Q. Gu** ¹.

¹ United States Department of Agriculture-Agricultural Research Service, Western Regional Research Center, Albany, CA 94710, USA; ² Department of Plant Sciences, University of California, Davis, CA 95616, USA; and ³ Cereal Research Center, Agriculture and Agri-Food Canada, 195 Dafoe Road, Winnipeg, Manitoba, Canada, R3T 2M9.

Several important wheat prolamin and disease resistance gene loci have been mapped at similar locations in the short arm of the group-1 chromosomes in wheat. However, genetics and structural organization of genomic regions harboring these important traits have not been well characterized. We therefore sequenced a 3.1-Mb region harboring prolamin multigene families in the genome of Ae. tauschii, the D-genome donor of hexaploid bread wheat. The sequence revealed a much higher gene density (98 genes; one gene/31 kb) in the sequenced region than the average value (ca. one gene/112 kb) expected for the wheat D genome. The high gene density is primarily due to the large number of duplicated genes, present either in tandem or interspersed with other genes. In addition to different types of prolamin gene families (γ -gliadin, ω -gliadin, and LMW-glutenin), multiple NBS-LRR disease resistance gene homologues of Lr21 (resistance to leaf rust) and Pm3 (resistance to powdery mildew) were identified. Furthermore, leucine-rich receptor protein kinase (LRK) genes, representing a different class of resistance genes, also were highly duplicated in this region. Comparative analyses indicated that the orthologous regions in the rice, Brachypodium, and sorghum genomes are highly conserved, whereas in Ae. tauschii, only 16 out of 98 genes are syntenic. Most of these highly duplicated genes are unique in the Ae. tauschii genome, suggesting rapid evolution in this region. Genetic analysis revealed the sequenced region spanned over 10-cM genetic distance with 13 markers mapped to this region. Recombination rates ranged from ca. 200 to 1,000 kb/cM between two marker intervals. Both co-evolution and independent evolution in different gene families were observed. Mechanisms underlying the molecular evolution of prolamin and resistance gene families in this complex region will be presented.

SESSION V: YOUNG TRITICEAE RESEARCHERS

Genetic provenance and genetic providence in a diverse crop.

Ana M. Gonzales and Peter L. Morrell. Department of Agronomy and Plant Genetics, University of Minnesota, St. Paul, MN, USA.

Multiple factors converge to contribute to high levels of genetic and phenotypic diversity in barley. The progenitor species is ecotopically and genetically diverse, with a large geographic range and large effective population size. Cultivated barley arose from multiple source populations and became adapted to diverse agronomic conditions across much of Eurasia and North Africa over several millennia of human prehistory. Exploiting this diverse adaptive history for barley improvement is challenging if methods are limited to top-down approaches based on genotype-phenotype associations. A new generation of bottom-up approaches provides the opportunity to investigate the genetic differentiation and genetic provenance of adaptive allelic variation and associations between SNPs and ecogeographic variables. Both sets of approaches provide new opportunities for associating genetic variation with phenotypic traits of agronomic importance, including disease resistance and climate adaptation. We make use of genotyping data from 2,417 worldwide barley accessions from the USDA National Small Grains Collection (landraces and cultivars) genotyped with 7,800 SNPs.