SPEAKER AND POSTER ABSTRACTS

2010 U.S. Wheat Genomics Workshop

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SESSION I: MARKER-BASED BREEDING STRATEGIES

Marker-assisted breeding and the harsh reality of cultivar development.

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The promise envisioned decades ago for marker-assisted selection (MAS) in plant breeding has been only partially realized. First proposed as a means to improve complex traits with low heritability, MAS has been primarily employed for improving traits with simple inheritance and high heritability. This talk will address using MAS in the present for simple traits and the potential utilization for complex traits in the near future. The Ohio State University wheat breeding program currently uses MAS for improving some key traits where single genes or major QTL are available and effective. The program primarily uses an aggressive backcrossing (BC) scheme where backcrossing is initiated early in the variety-development phase using many recurrent parents. The annual fate of any BC population depends on the performance of the recurrent parent in ongoing cultivar-development evaluations. This scheme and the reasons for using it will be presented.

Most key traits for Ohio, such as yield, are truly quantitative and there are few, if any, useful QTL such that traditional MAS has had little impact. A recent analysis of yield gain in the U.S. indicates that the rate of yield improvement needs to be increased by 25 to 50% to increase yield by 35% in 25 years. A plan to utilize historical trends, association analysis, and genomic selection to attain that rate of gain will be presented.

Implementing marker-assisted selection in wheat variety development.

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Marker-assisted selection (MAS) has become a routine part of wheat breeding, enabling efficient backcross breeding, pyramiding of genes, and creation of isolines that allow for the genetic dissection of traits. The wheat genetics group at Montana State University has developed markers and implemented MAS for traits of importance to our state. One successful example of marker development and implementation is with markers for white seed. Resources for breeding programs in much of the Great Plains largely have been directed to hard red wheat development and, as a result, agronomic performance of hard white wheat varieties has tended to be inferior. Based on demands from the Montana wheat industry, we developed markers for white kernel color and used these to convert our best hard red wheat varieties into hard white. Development of markers for white seed required analysis of three mapping populations, each segregating for one of the three controlling loci. Once identified, the markers linked to white seed color pose several challenges in application to variety development. Because white is recessive and controlled by three genes, red-seeded plants have unknown genotypes, so that the breeder does not know how many genes need to be converted. Also, a certain sized band is not diagnostic of all white-seeded lines. Despite these caveats, we were able to use the markers in a backcrossing program to covert the best hard red wheat varieties into hard white isolines. These hard white wheat lines are currently being tested in breeding trials, with the expectation that agronomic performance will equal that of the hard red varieties. In addition, the ability to develop isolines with red versus white kernel color provides the opportunity for experiments that are otherwise difficult to conduct. Our experience with the markers for white kernel color, and with markers for other traits, has provided insights that have helped us refine marker implementation in variety development.

Breeding wheat somewhere between the poverty level and the 99% confidence level.

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The Oklahoma State University (OSU) wheat breeding program has operated continually for the past 60 years but not without significant shifts in financial base support and breeding strategy in only the last decade. The next shift, already in motion, will invoke gene-targeted selection among inbred lines as a means to reduce costs and/or maximize selection gains for adaptation traits relevant to the southern Great Plains. The USDA–CSREES–CAP population, 'Jagger/2174', provided OSU's cornerstone for QTL discovery and mapping of critical traits for reproductive development patterns (stem elongation, heading, and physiological maturity) and disease reaction (leaf rust, stripe rust, and powdery mildew). As a result of their alignment with phenotypic-based selection in our program, most informative among these markers are *VRNA1*, *PPD-D1*, *VRN-D3*, *Lr34*, *Pm3*, and a novel gene on chromosome 2A that confers adult-plant resistance to stripe rust. Little variation was found among elite lines in the OSU wheat breeding program at *VRNA1*, a major locus that regulates the timing of stem elongation, apparently is consequential to intense selection pressure in prior generations against precocious winter dormancy release. All of the gene markers enable selection pressure for traits that may not be consistently measurable from year to year or traits that have very low heritability due to human error. Their high diagnostic capability provides a healthy balance between costs and confidence.

MAS and the future of cereal breeding: how should the genotyping centers fit in ?

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The concept of regional genotyping centers providing marker-assisted selection (MAS) analysis for genetic selection has become ingrained in the U.S. wheat and barley breeding strategies. To remain relevant to the breeding community, the genotyping centers must move past the current paradigm one-gene MAS and toward efficient holistic selection strategies. A chip-based, single nucleotide polymorphism (SNP) marker system applied to genomic breeding is the obvious next step. The transition away from single-gene selection and towards genomic breeding is conceptually easy to understand. The realization of genomic breeding is much more difficult to achieve. To make this leap, the SNPs linked to adaptation, agronomic, and quality traits must be identified for each breeding program. The public U.S. cereal breeding community currently is lacking in genomic information essential to implement the next generation MAS platforms. The USDA genotyping labs, in association with university partners, are currently identifying new SNPs useful in wheat. Identification of SNPs beneficial in specific germplasm also is being developed for PNW programs. Future work will require integrated efforts between genotyping centers and cereal geneticists to discover useful SNPs, identify and implement appropriate marker platforms, and elucidate the association between markers and haplotypes essential in the breeding programs. With the current rate of technological development, now is the time to establish a concerted effort to develop the markers, detection platforms, and the bioinformatics.

SESSION II: APPLICATION OF PHYSICAL MAPS/GENOME SEQUENCE TO BREEDING

The future impact of genomics assisted approaches in maize breeding.

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The U.S. maize community initiated a huge three-step project in 1998, including the sequencing of the maize genome, to obtain the complete sequence and structure of all maize genes and their locations on both the genetic and physical maps of maize. All the information generated is made available to the community, via the maizegenome.org website, and from the EBI database in Europe. Now the maize genome is almost fully sequenced, which is great news for genomics, and so for maize breeding!

Having access to the genome contributes to crop improvement because comparative genomic approaches make links using the gene information already available on model species, to understand and identify more easily the function of key genes and complex biological mechanisms involved in the agronomic traits of crops. The assembled genome sequence provides a good basis for developing a large number of markers (wet lab or *in silico*) in candidate genes or within gene-rich regions that can be used in genetic studies (QTL and association mapping). Such tools facilitate and stimulate germplasm and allelic diversity characterization and increase breeding efficiency by marker-assisted selection. Traits can then be bred directly (selection of the favorable alleles only). Bioinformatics is an essential component of such studies, because it connects data from very diverse origins (genetic, transcriptomic, phenotypic, and mutants) to the genome sequences, to generate valuable information to be used in applied programs. All this, associated with the new high-throughput and low-cost technologies now available (in sequencing and genotyping), already is speeding up the identification of the most interesting genetic factors involved in agronomic traits, thus improving marker-assisted selection. Breeders need to encourage all the initiatives that aim at sequencing our genomes of interest.

Using genetic diversity to understand phenotypic variation in maize.

Michael McMullen [1] and the Maize Diversity Project [2].

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One of the goals of the Maize Diversity Project is the development of genetic resources for conducting joint linkage-association analysis in maize. We have designated our main genetic resource as nested association mapping (NAM). NAM is constructed from 26 inbred lines chosen to maximize genetic diversity. NAM has a reference design with B73 as the common parent and consists of 25 families of 200 RILs each from B73 crossed by 25 diverse lines (25DL). The broad sampling of allelic diversity and the 136,000 recombination events captured in NAM gives the population extensive power to describe the genetic architecture of agronomic traits for maize. For example, flowering time in maize is controlled by numerous, small-effect QTL that are shared among families, with multiple allelic effects segregating among the founder lines. The true power of NAM is based on ability to project polymorphism from the founder lines onto the RILs allowing genome wide association analysis for maize.

SESSION III: WHEAT TRANSFORMATION

Transgenic solutions to wheat biotic stresses.

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Although conventional breeding approaches will always play a major role in varietal development, transgenic technologies will become an increasingly valuable tool for our wheat breeders in the near future. The current climate for accepting biotech wheat is changing slowly to a more favorable position. Although it is unlikely that transgenic wheat will be released in the next few years, it is important to proceed with transgenic wheat research so that products can be readily deployed after the biotech wheat issue has been resolved. Providing resistance to biotic stresses is one area where transgenic wheat can make a significant impact. Highlighted in this presentation are collaborative efforts providing possible solutions for various biotic stresses including wheat streak mosaic virus, *Triticum* mosaic virus, Fusarium head blight, and Hessian fly resistance.

SESSION IV: BIOINFORMATICS

GrainGenes, the Triticeae Genome Database.

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The GrainGenes database has been serving genomic and genetic data for the Triticeae since 1993. It includes genetic and physical maps, probes used for mapping, nucleotide sequences, bibliographic references, and an address book of colleagues. The GrainGenes website includes additional information and publications, such as the Catalogue of Gene Symbols for Wheat, the *Barley Genetics Newsletter*, and the *Annual Wheat Newsletter*. Recent additions to the database are the physical/genetic map of wheat chromosome 3B, the OPA barley consensus map, and the OPA/DArT map of the Oregon Wolfe Barley population. A new GrainGenes service is TAWG, the Triticeae Annotation Working Group, a public repository for annotated genomic sequences of wheat and barley. Soon, GrainGenes will host The Hordeum Toolbox (THT), a database for genotyping and phenotyping data from the U.S. Barley CAP project.

Brachypodium distachyon: a new model to study Triticeae genomes.

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Brachypodium distachyon (*Brachypodium*) is being developed as a new model organism for structural and functional genomics of temperate grasses because of its several desirable attributes for plant biology research, such as easy growth requirements, small stature, short generation time, and small genome size. With the recent completion of the *Brachypodium* genome sequence, along with the established *Agrobacterium*-mediated, high-efficiency transformation system for T-DNA insertional mutagenesis and other genomics resources, tools are now available for exploiting the utility of *Brachypodium* in facilitating wheat research. Comparative mapping of several disease resistance genes indicated that wheat retains colinearity of disease gene orthologs with *Brachypodium*. Such colinearity is not present between wheat and rice, suggesting that *Brachypodium* will be more useful in map-based cloning of rapid or recent evolving genes such as wheat resistance genes. Higher colinearity between wheat and *Brachypodium* also is observed in genomic regions harboring wheat prolamin genes. Expression of wheat promoters and genes in *Brachypodium* provides direct evidence supporting the usefulness of *Brachypodium* in functional characterization of important genes or traits of wheat. A collection of over 6,000 T-DNA insertional mutant lines is now available for public access at website http://brachypodium.pw.usda.gov/TDNA/. These lines are indexed through flanking sequence tags that facilitate mapping of the T-DNA insertions within the *Brachypodium* genome. Several other useful websites for comparative and functional *Brachypodium* genomics will also be discussed.

SESSION V: EARLY CAREER SCIENTISTS

Identification of a novel QTL for Fusarium head blight resistance on wheat chromosome 7A.

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Fusarium head blight (FHB), caused by *Fusarium graminearum,* is an important cereal disease worldwide. Resistance to disease spread within a spike (type II) is the more stable type of resistance. A previous study identified a Chinese Spring (CS)–Sumai3 chromosome 7A substitution line having a high level of type-II resistance, but an associated quantitative trait locus (QTL) on 7A has never been reported. In this study, we developed CS–Sumai3 7A chromosome recombinant inbred lines (CRIL) from a cross between CS and CS–Sumai3 7A disomic substitution lines. A genome-wide marker analysis with 72 chromosome-specific simple sequence repeats (SSR) confirmed that entire 7A chromosome and a small fragment from chromosome 3BS were from Sumai3 and all other chromosomes were from CS. A total of 191 F_5 CRIL were evaluated for type-II, FHB resistance using single-spikelet inoculation in 2009. The proportion of symptomatic spikelets (PSS) for each CRIL was calculated to measure FHB resistance. The frequency distribution of PSS was bimodal, ranging from 6% to 84%. Out of 75 SSR markers screened from chromosome 7A, 33 were polymorphic and only 7 of 30 SSR markers and 30 sequence tagged sites from 3BS chromosome were polymorphic. The linkage maps for chromosome 7A spans a distance of 181.7 cM and for 3BS, over 2 cM. Composite interval mapping feature of Qgene software was used for QTL mapping with a LOD score threshold of 2.0 ($P < 0.005$) to claim a significant QTL based on 1,000 simulations. A new, major QTL for type-II FHB resistance was detected on the short arm of chromosome 7A with a LOD score of 11, flanked by markers *Xwmc17* and *Xwmc9*. FHB1, a previously reported major QTL on 3BS, was also detected in this study. Both QTL explained 24% (7A) and 45% (FHB1) of the phenotypic variation. An additive effect was observed between the two QTL. Replacement of both alleles of CS with these of Sumai3 resulted a 66 % reduction in disease severity. Therefore, the QTL from CS 7A is a new major QTL for FHB resistance and can be used for enhancing FHB resistance in breeding.

Wheat–rye T2BS·2BL-2RL recombinants conferring resistance to Hessian fly (H21).

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The Hessian fly, *Mayetiola destructor* (Say), is a destructive insect pest of bread wheat *Triticum aestivum* L. worldwide. Although 32 genes conferring resistance to Hessian fly have been identified, only a few of them are still effective. One such highly effective gene is *H21*, which was transferred to wheat from Chaupon rye via a compensating T2BS·2R#2L Robertsonian, whole-arm, wheat–rye translocation. This translocation also has a locus for field resistance to powdery mildew. To broaden the use of T2BS·2R#2L in wheat improvement, we attempted to transfer both resistance loci, via homologous recombination, to a T2BS·2BL-2R#2L chromosome. The *H21* locus was linked closely to the telomere; the powdery mildew locus was distal, but closely linked, to the translocation breakpoint in T2BS·2BL-2R#2L. Recovered short-segment, rye translocation chromosomes confer resistance to Hessian fly; no crossover event in the desirable configuration was recovered to produce a short-segment, wheat–rye translocation with both *H21* and the powdery mildew resistance gene present. The T2BS·2BL-2R#2L recombinant chromosome has been transferred to adapted winter and spring wheat cultivars.

An adult-plant resistance gene to stripe rust is located on chromosome 2AS in the hexaploid wheat cultivar Jagger.

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Stripe rust is one of the most common and persistent wheat diseases worldwide. With the continuous evolution of different pathogen races, new resistance genes are needed to defend against the mutated pathogens. In this study, we report that a major quantitative trait locus (QTL) for stripe rust resistance was located on the short arm of chromosome 2A (*QYr.osu.2A*) in a population of recombinant inbred lines (RILs) generated from a cross between Jagger and 2174, two prominent winter wheat cultivars in the southern Great Plains, USA. *QYr.osu.2A* was mapped when this population was tested at two sites in Washington where stripe rust frequently occurs and in Beijing, PR China, where CYR32 was inoculated on adult plants. *QYr.osu.2A* explained 81 to 85% of the total phenotypic variation in relative area under the disease progress curve (rAUDPC) value, showing its nearly complete resistance against natural field infection of stripe rust on adult plants in Washington. Stripe rust races included PST-100, PST-114, PST-116, and PST-138, which frequently occur in Washington and other regions of the U.S. such as the Great Plains. *QYr.osu.2A* also accounted for 36% of the total phenotypic variation, showing its partial resistance to CYR32, currently one of the predominant Chinese races and virulent to 80% of commercial cultivars and germplasm in China. In addition, a minor QTL was mapped on the long arm of chromosome 5A (*QYr.osu.5A*), explaining 22 to 30% of the total phenotypic variation across years and locations. Jagger carried a resistant allele at both *QYr.osu.2A* and *QYr.osu.5A*, whereas 2174 carried a susceptible allele at both loci. Our findings suggest that the *de novo* resistance gene at *QYr.osu.2A* in Jagger can provide consistent and broad-spectrum, adult-plant protection to stripe rust. Resistance in Jagger has remained effective during the 15 years since its release, and we recommend this source of resistance be used in breeding applications in conjunction with the molecular markers. This study also demonstrated that many resistance genes present in local cultivars and available mapping populations can be identified and characterized when they are tested in diverse geographical areas of wheat worldwide.

Toward cloning of a major QTL for preharvest sprouting resistance in white wheat.

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Preharvest sprouting (PHS) is a major constraint to white wheat production. Previously, we mapped a major quantitative trait loci (QTL) for preharvest sprouting resistance in the U.S. white wheat Rio Blanco and located the QTL in the distal end of 3AS using a recombinant inbred line (RIL) population derived from the cross 'Rio Blanco/NW97S186'. To validate and fine map the QTL, a new segregation population consisting of 1,874 F_2 lines was developed by selfing the progenies of a RIL (RIL25) that was heterozygous for the three SSR markers in the QTL region. The segregation ratio of PHS resistance in the population fits monogenic inheritance. Plants with all Rio Blanco marker alleles at the three marker loci were resistant to PHS, whereas those with all NW97S186 alleles were susceptible. The additive effect of the QTL played major role on PHS resistance with dominant effect was also observed. Fifty-six recombinants among the three SSR markers were identified in the population to produce homozygous recombinants. Fine mapping delimited the QTL in the region close to *Xbarc57* flanked by *Xbarc321* and *Xbarc12*. The QTL region was further saturated by 11 AFLP and seven wheat EST-derived markers. Microcolinearity was established between the QTL region and the corresponding region on rice chromosome 1 according to the EST information. The QTL was narrowed down to a region about 0.4 cM after analyzing the PHS resistance of the homozygous recombinants. A physical map of the QTL region was constructed by screening a Chinese Spring chromosome 3AS arm-specific BAC library with markers flanking the QTL. Two contigs were identified to span the QTL region. Sequence analysis of these contigs is underway.

POSTER SESSION ABSTRACTS

Poster 1. Saturation and comparative mapping of the Tsc2 region in hexaploid wheat.

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Ptr ToxB is a proteinanceous, host-selective toxin produced by the tan spot fungus, *Pyrenophora tritici-repentis*, capable of causing chlorosis in susceptible wheat (*Triticum aestivum* L.) cultivars. Sensitivity to Ptr ToxB is governed by the *Tsc2* gene located at the distal end of wheat chromosome arm 2BS. *Tsc2* was initially mapped in the International Triticeae Mapping Initiative (ITMI) mapping population, which was derived from the synthetic hexaploid wheat W-7984 and the hexaploid variety Opata 85. The main objectives of this study were to validate the chromosomal location of *Tsc2* and its effects in an intervarietal hexaploid wheat population, develop or identify user-friendly PCR-based markers suitable for marker-assisted selection (MAS) against toxin sensitivity conferred by the *Tsc2* locus, and determine the utility of rice and *Brachypodium* genomic sequences for fine-mapping of the *Tsc2* region. A population consisting of 121 $F_{2:7}$ recombinant inbred lines derived from a cross between the Ptr ToxB-sensitive hexaploid wheat cultivar Katepwa and the Ptr ToxB-insensitive hexaploid landrace Salamouni was used for mapping and phenotypic analysis. SSR markers known to map to 2BS and sequence tagged site (STS) primers developed for 2BS-bin mapped ESTs were mapped in the 'Salamouni/Katepwa' (SK) population. Monomorphic EST–STSs were further mapped as RFLPs. To date, the 2BS map developed in the SK population consists of 32 SSR, 9 EST–STS, and 3 RFLP markers. The SSR marker *Xmag681* and RFLP marker *XBE444541* flanked the *Tsc2* locus at distances of 2.8 cM and 2.6 cM, respectively. *Xmag681* will be suitable in MAS schemes and efforts are underway to convert *XBE444541* to a PCR-based marker as well. Results regarding the effects of the *Tsc2* locus on conferring tan spot susceptibility, comparative analysis of the *Tsc2* region with rice and *Brachypodium*, and discussion regarding the usefulness of using the genomic information from rice and *Brachypodium* for developing additional markers, genomic analysis, and map-based cloning of *Tsc2* will be presented.

Poster 2. Development, mapping, and haplotype analysis of EST-based SNPs for wheat Fusarium head blight resistance QTL Fhb1.

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Fusarium head blight (FHB) is a destructive disease that reduces wheat grain yield and quality. The Chinese variety Sumai3 and its derivatives such as Ning 7840 have a high level of resistance to FHB symptom spread within a spike (type-II resistance) and have been widely used as resistant parents in breeding programs worldwide. The quantitative trait locus (QTL) in chromosome 3BS (*Fhb1*) from Sumai3 has been identified to have the largest effect on FHB resistance to date. This QTL has been linked to restriction fragment length polymorphism, simple sequence repeat (SSR), amplified fragment length polymorphism, and sequence tagged site (STS) markers. Single nucleotide polymorphism (SNP) is the most common form of genetic variation, and SNP may be the next generation marker system for mapping and marker-assisted selection (MAS). In this study, we developed SNP markers based on wheat expressed sequence tags (ESTs) associated with the 3BS QTL region. A total of 131 SNPs were identified between Ning 7840 (FHB resistant) and Clark (susceptible) based on the sequences of ten ESTs. SNPs were analyzed in 71 'Ning 7840/Clark' BC_7F_7 populations using the single-base extension method. Seven SNP markers mapped between *Xgwm533* and *Xgwm493*; SSR markers flanking *Fhb1* in 3BS. Five of these SNP markers clustered with four other SSR/STS markers and covered a 7.4-cM interval, 12.9 cM from *Xgwm533*. This marker-dense region gave the highest R^2 (40–54%) and LOD values (9.16–11.80) and is the most likely location of *Fhb1*. Haplotype analysis of 63 lines from eight countries based on EST sequence (SNP), SSR, and STS markers associated with *Fhb1* identified four major groups: (1) Clark, (2) Asian, (3) Ernie, and (4) Chinese Spring. The Asian group consisted of Chinese and Japanese lines that carry the *Fhb1* resistance QTL and one *Xsnp-11* marker haplotype could differentiate these lines from lines in other groups. All Sumai3-related lines formed a subcluster within the Asian group, and an *Xsnp3BS-8* marker haplotype is specific for these lines. The SNP markers identified in this study should be useful for fine-mapping and MAS of *Fhb1*.

Poster 3. The International Wheat Genome Sequencing Consortium: a genome sequence-based platform to accelerate wheat improvement.

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Bread wheat is grown on over 95% of the wheat-growing area, and its sequence holds the key to genetic improvements necessary to meet the increasing demands for high-quality food and feed produced in an environmentally sensitive, sustainable, and profitable manner. Furthermore, because of its recent history, hexaploid wheat is a very good model to study polyploidy, a driving force for plant genome evolution. The International Wheat Genome Sequencing Consortium (IWGSC) was established by plant scientists, breeders, and growers who are dedicated to sequencing the wheat genome to enhance our knowledge of its structure and function and deploy state-of-the-art molecular tools to accelerate wheat improvement and meet the challenges of the 21st century. The Consortium is committed to ensuring that the wheat genome sequence, and the resulting DNA-based tools are available for all to use without restriction. To achieve the vision of a sequenced wheat genome, the IWGSC develops strategic plans with short- and mid-term goals; defines areas of coordination; facilitates and coordinates research projects and funding efforts at the national and international levels; develops and supports the design of research proposals; provides a framework for the establishment of common guidelines, protocols, and resources; and organizes scientific meetings and workshops. The IWGSC is governed by six co-chairs, a Coordinating Committee, and an executive director. General membership is open to any individual, laboratory, or entity with an active interest in meeting IWGSC objectives. The mission, goals, organizational structure, projects, and online membership registration are available at http://www.wheatgenome.org.

Poster 4. RNAi-mediated viral resistance in transgenic wheat.

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Wheat streak mosaic virus (WSMV) and *Triticum* mosaic virus (TriMV), are two of the major viruses in the Great Plains of the United States. Cultural practices, mite vector control, and deployment of resistant varieties are the primary methods of disease management; however, they are not fully effective. We evaluated the use of interference RNA, recognized as a natural defense mechanism, as a biotech approach to generate resistance to these wheat viruses. RNAi expression vectors were independently created from the sequences of the coat proteins (CP) of both WSMV and TriMV. Immature embryos of the wheat cultivar Bobwhite were independently co-transformed by biolistic particle-delivery system with these RNAi expression vectors and pAHC20, which contains the bar gene for glufosinate selection. After tissue culture, putative transformed plants were analyzed through PCR for the presence of the appropriate RNAi CP gene. Transgenic T_1 seeds were collected and each line was tested for transgene expression via RT-PCR. To determine viral resistance, T_1 progeny were mechanically inoculated with the corresponding virus. Viral presence was established by ELISA. In the T_1 generation, resistance was seen in up to 60% of the plants evaluated for both constructs, although some events that showed transgene presence did not exhibited resistant phenotype. Analyses of transgene presence and expression in the T_2 generation evidenced events of transgene silencing and deletion. Regardless of these phenomena, consistent resistance response in two lines of WSMV CP construct and one TriMV CP transgenic line was found.

Poster 5. QTL detection and factor analysis of yield and adaptive traits in winter wheat.

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The relationships between yield components, adaptive traits, and molecular markers were investigated in two populations of recombinant inbred lines (RILs) using QTL analysis and factor analysis. The RIL populations were derived from single crosses with Tubbs, an Oregon soft white winter wheat variety, with two western European hard red winter wheat varieties. The populations were grown in two replications at two locations in Oregon. Each plot was evaluated for total grain yield, yield components, measures of maturity, and other important traits. The values of all traits were broadly distributed and transgressive segregates were observed in both populations. The yield components most highly correlated with yield were fertile spikelets/spike and seeds/spike in both populations. In one population, these correlations ranged from 0.4 to 0.47. They ranged from 0.21 to 0.25 in the other population. Flowering time was inversely correlated with yield in both populations, ranging from -0.17 to -0.32. Heritabilities were determined for each population across locations. The heritabilities for yield components ranged from 0.44 to 0.84. Heritability for grain-fill duration varied the most between populations, ranging from 0.33 to 0.54. The interrelatedness of the traits was examined by factor analysis of each population at each location using the principle-component method. Five factors, accounting for 69.7% to 73.4% of the total variance, were selected. Important factors were observed for head fertility, tillering, and maturity. Genetic linkage maps composed of DArT and SSR markers were used to detect putative QTL, and their locations are presented. The extension of QTL analysis to factor scores was investigated.

Poster 6. Establishment of a double-haploid production technique using microspore culture for Midwestern U.S. wheat varieties.

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Double haploids (DH) are genetically 100% homozygous plants. Microspore culture is the method of production of DHs from microspores (immature pollen) by androgenesis in a single step. Success of DH plant production is determined by the genotype and health of the donor plant, environmental conditions under which it is grown, staging of the microspore, pretreatment methods, and composition of induction and regeneration media. The objective of this study is to establish a DH-production technique using microspore culture for the Midwestern U.S. wheat varieties. We used Macon, a Washington spring wheat variety, as a check and three Nebraska winter wheat varieties, Anton, Millennium, and Pronghorn, as representatives of the U.S. Midwest. Plants were grown in the greenhouse with 16 hrs of light at 21–25°C and an 8-hr dark period at 16±2°C. Two pretreatment methods (0.4 M mannitol at 4ºC and solution B containing 0.3 M mannitol with inorganic components at room temperature) and one regeneration media was used. We have standardized the staging of microspores and established the steps of the technique (pretreatment, induction, and regeneration) in our laboratory conditions. We were able to produce DH plants for Macon. The microspore culture technique for the winter wheat varieties is in progress. The DH-production technique will be useful in wheat-breeding programs throughout the U.S. Midwest.

Poster 7. Towards a sequence-ready, physical map of chromosomes 1D, 4D, and 6D of hexaploid wheat.

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A physical map is a prerequisite for sequence assembly of large genomes such as that of wheat (*Triticum aestivum* L). Because of the high repeat content and genome triplication, assembly of a high-quality, whole-genome sequence may be difficult. Fortunately, flow cytometry allows the division of wheat chromosomes into three fractions based on size (fraction I: 1D, 4D, 6D; fraction II: 1A, 3A, 6A, 2D, 3D, 5D, 7D; and fraction III: 2A, 4A, 5A, 7A, 1B, 2B, 4B, 5B, 6B, 7B) in addition to an individual chromosome 3B. We have developed fraction-I physical maps of chromosomes 1D, 4D, and 6D and three BAC libraries (312,576 clones) from Chinese Spring fraction-I, with a total coverage of 15.3x of the chromosome length. The BAC clones are being fingerprinted with SNaPshot HICF technology. Fingerprinting of first BAC library (*Eco*RI) with 26,112 clones has been completed and fingerprinting of the *Hind*III BAC libraries is in progress. In total, 70,112 clones (3.5x chromosome coverage) have been fingerprinted with a success rate of 96% and are being used for the initial assembly with FPCv9.3. Progress on the assemblies will be discussed. Mapping populations have been developed to anchor, order, and orient the FPC contigs.

Poster 8. Threading the line between GrainGenes and other genome resources.

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GrainGenes (graingenes.org) has long used molecular markers for the comparison of genomes between its related grass species, mainly those of the Triticeae and *Avena*. Available tools have mainly focused on the CMap and GBrowse (gmod.org) displays. New genome sequence data from other grass species adds to the utility of comparative mapping between the grass species.

With a growing interest in the study of temperate cereals and forage grasses, a new model for the grasses, *Brachypodium distachyon*, has evolved within the research community, some of it leveraged toward bioenergy research. Resources for *Brachypodium* to date include a whole-genome sequence, ESTs, SNPs, a high-density genetic linkage map, and germplasm resources. The high-efficiency transformation system of *Brachypodium* using *Agrobacterium tumefaciens* has yielded a resource of T-DNA insertional mutant lines. Greater than 4,300 T_0 lines have been generated to date, and from these, flanking sequence tag (FSTs) data yielded 1,601 (46.2%) shown to contain *Brachypodium* genomic sequences. As work continues to visually and physically screen these for mutant phenotypes, protocols for working with *Brachypodium*, information about the T-DNA project, and links to seed resources is now available from a website (brachypodium.pw.usda.gov).

Sequencing the wheat genome is now underway in one form or another. There is a need to develop molecular markers for this complex genome. The complexity of the wheat genome has been leveraged against the abundance of randomly mobilized repetitive sequences, many represented by transposable elements (TE). Software, TEPrimers (wheat.pw.usda.gov/demos), was built to recognize this complexity and define candidate repeat junction-junction markers (RJJM) sites, which could predictively be used as unique marker sites. The software has been tested using BAC end sequences and 'next-generation' sequences (Roche 454) of wheat. These and other resources that help bind the genomes together will be presented.

Poster 9. Development and characterization of wheat–alien translocation lines conferring stem rust resistance from Aegilops searsii and Ae. geniculata.

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Stem rust, caused by *Puccinia graminis* f. sp. *tritici*, is potentially one of the most damaging wheat diseases. Although crop loss to stem rust has been infrequent in the past several decades, the emergence of the Ug99 lineage of stem rust races now threatens a large proportion of the wheat acres in the world. Exploiting novel genes effective against Ug99 from wild relatives of wheat is one of the most promising strategies for the protection of wheat production. In this study, resistance to Ug99 was identified on the short arm of $3S^s$ of *Ae. searsii* (2n=2x=14, S^sS^s) and long arm of $5M^g$ of *Ae. geniculata* (2n=2x=28, U^gM^g) by testing of a disomic and ditelosomic addition lines with the chromosomes. To transfer the gene(s) into common wheat, we produced three double-monosomic chromosome populations (3A/$3S^s$, 3B/$3S^s$, and 3D/$3S^s$) of wheat–*Ae. searsii* and and two populations of T550 (T5M^gS·5M^gL-5DL) crossed with *ph1b* mutant and Lakin, and then applied integrated molecular and cytogenetic approaches to develop wheat–alien recombinants conferring stem resistance. Three wheat–*Ae. searsii* compensating, Robertsonian translocations (T3S^sS·3AL, T3S^sS·3BL and T3S^sS·3DL) and three wheat–*Ae. geniculata* translocation lines with shortened 5M^gL were selected and confirmed on the basis of genomic in situ hybridization and analysis of $3S^s$S and $5M^g$ using homoelogous wheat chromosome-specific SSR/STS–PCR markers. These translocation lines were highly or moderately resistant to stem rust race RKQQ. Evaluation of Ug99 resistance and agronomic characterization of the recombinants are currently in progress; efforts to reduce potential linkage drag associated with $3S^s$S of *Ae. searsii* also is underway.

Poster 10. Reactive oxygen species are involved in plant defense against a gall midge.

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Reactive oxygen species (ROS) play a major role in plant defense against pathogens, but evidence for their role in defense against insects is still preliminary and inconsistent. In this study, we examined the potential role of ROS in defense of wheat and rice against Hessian fly (*Mayetiola destructor*) larvae. Rapid and prolonged accumulation of H_2O_2 was detected in wheat plants at the attack site during incompatible interactions. Increased accumulation of both H_2O_2 and superoxide was detected in rice plants during nonhost interactions with the larvae. No increase in accumulation of either H_2O_2 or superoxide was observed in wheat plants during compatible interactions. A global analysis revealed changes in the abundances of 250 wheat transcripts and 320 rice transcripts encoding proteins potentially involved in ROS homeostasis. A large number of transcripts encoded class-III peroxidases that increased in abundance during both incompatible and nonhost interactions, whereas the levels of these transcripts decreased in susceptible wheat during compatible interactions. The higher levels of class-III peroxidase transcripts were associated with elevated enzymatic activity of peroxidases at the attack site in plants during incompatible and nonhost interactions. Overall, our data indicate that class-III peroxidases may play a role in ROS generation in resistant wheat and nonhost rice plants during response to Hessian fly attacks.

Poster 11. Use of barley stripe mosaic virus for virus-induced gene silencing and gene expression in various wheat tissues.

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Barley stripe mosaic virus (BSMV)-induced gene silencing (VIGS) has shown to be an effective strategy for rapid functional analysis of genes in the leaf tissues of barley and wheat. To extend the potential of VIGS in wheat, we investigated gene silencing in roots and reproductive tissues, compared severity of VIGS in 12 bread wheat cultivars, and demonstrated the transmission of silencing in three selfed generations of inoculated plants. Out of the 12 bread wheat cultivars, Zak and Eltan were most responsive to the silencing of *phytoene desaturase* (*PDS*), and a range from 53–85% suppression of *PDS* transcripts was observed in various wheat cultivars. Incidence of *PDS* gene silencing ranged from 8–11% in the progeny of pγ.PDSas-inoculated plants, from 53 to 72% in the first selfed generation, and 90–100% in the second selfed generation. Spread of the VIGS vector, monitored using green fluorescent protein, was observed in inoculated leaf tissues, phloem, and root cortex at 10 and 17 days-post-inoculation but was absent in apical meristems and reproductive tissues. An antisense construct of the wheat *coronatine insensitive1* (*TaCOI1*) showed suppression of *TaCOI1* transcripts by 50–70% in the roots and 63–68% in the foliage. Similarly, successful silencing of *seed-specific granule bound starch synthase* (*GBSS*) with antisense and hairpin constructs resulted in up to 81% reduction in amylose content, and silencing of the wheat homologue of disrupted meiosis cDNA1 (*TaDMC1*) resulted in 75–80% suppression of the *TaDMC1* transcripts in pollen mother cells.

Poster 12. Characterizing the lignocellulose pathway in wheat by TILLING Triticum monococcum subsp. monococcum.

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Cellulosic biofuel crops are poised to become a major source of energy in the United States, necessitating the understanding the basic biology underlying the traits that control the utility of wheat biomass as an energy source. Mutagenesis is an important tool in crop improvement and is free of the regulatory restrictions imposed on genetically modified organisms. We are developing 'TILL monococcum' a resource for discovery of chemically induced mutants in the diploid wheat ancestor (*T. monococcum* subsp. *monococcum*) to better understand basic wheat biology. A TILLING population of 2,700 single M_2s was developed in *T. monococcum* subsp. *monococcum* using EMS mutagenesis (0.24% EMS). Pools of four M_2 plants were used to screen for lignocellulose pathway mutants in the TILLING population using Cel-I endonuclease. In our preliminary experiments with RT- PCR, 16 ESTs (homologous with annotated genes for lignin precursors in rice) showed a significant developmental regulatory pattern and were in close agreement with total lignin content. Primers were designed from all 16 ESTs for screening the TILLING population. One mutant each was identified for the *PAL 6* and *HCT* locus from the first 716 M_2s screened. The genomic constitution of the selected mutants was determined by Cel-I digestion of the M_3 progeny. Phenotypic validation for the total lignin content of the mutants will be done at maturity.

Poster 13. Unraveling a meiotic gene complex on wheat chromosome arm 5BL.

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The *Ph1* (*pairing homoeologous 1*) locus has been at the heart of wheat research since its discovery in early 1950s. From that time, much speculation and hypotheses have been proposed to explain its mode of action but the identify of the underlying gene(s) remained illusive. Building upon its localization to chromosome arm 5BL, we first localized the locus to a ~3-Mb segment bracketed by the deletion breakpoints of 5BL-1 (FL 0.55) and *ph1c* on chromosome 5B (*Ph1* gene region). Additional mapping of 238 wheat group-5 specific markers assigned nine loci to the *Ph1* gene region. A consensus genetic-linkage map of the whole region also was constructed to determine the order of markers within the region. Extensive blastn/tblastn comparisons of the *Ph1* gene region marker sequences with the rice genomic DNA sequences allowed identification of a 450-kb orthologous region on the rice chromosome 9. This wheat–rice comparison not only allowed alignment of the *Ph1* gene region to the BAC scaffold of rice R9 but also with the BAC scaffold of wheat chromosome 5B. To determine the location of the deletion break points of 5BL-1 and *ph1c* (delimiting the *Ph1* gene regions) on the BAC scaffold of bread wheat, we designed primers from six selected genes and used additional deletions spanning the region. The analysis allowed demarcation of the *Ph1* gene region to a very small fraction of the 2.5-Mb wheat BAC scaffold, carrying only 12 genes. To identify gene responsible for the *Ph*-like phenotype, we undertook virus-induced gene silencing (VIGS) of three candidate genes and nine other genes flanking the region of interest (including *TaDMC1*, *TaASY1*, and *TaCDC2-4*). The candidate genes were short-listed on the basis of domain/motif searches. Silencing of the *TaDMC1* via VIGS showed univalents, whereas *TaASY1* showed multivalents. When VIGS was performed on the *Ph1* gene candidates mapping in the *Ph1* gene region, one of the candidates (*TaWSU-1*) showed formation of quadrivalents/higher order pairing upon silencing, which is a characteristic phenotype of the *ph1* gene mutants. Another candidate (*TaH51L*) showed an average of four univalents and 19 bivalents. These findings suggest that one of the candidate genes, *TaWSU-1*, represents a novel meiotic gene that influences diploid like pairing behavior of hexaploid wheat and also suggests the role of other genes in chromosome just apposition and synapsis at meiotic prophase I.

Poster 14. Virus-induced gene silencing for durable Russian wheat aphid resistance in wheat.

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Russian wheat aphid (RWA), *Diuraphis noxia* (Kurdjumov), is an important insect pest of wheat (*Triticum aestivum* L.) and barley (*Hordeum vulgare* L.) in the western United States. The most effective control strategy is the development of resistant cultivars, but a gene-for-gene relationship between RWA virulence effectors and R genes results in biotype-specific resistance. Therefore, identifying more durable resistance, effective against all RWA biotypes, would be a significant advantage. Our objective was to test whether silencing a candidate gene, with suspected involvement in compatible interactions between the aphid and wheat, would confer resistance to a susceptible wheat cultivar. Several genes have been identified as being up-regulated in the susceptible cultivar 'Gamtoos-S' (GS) or down-regulated in the near-isogenic resistant line 'Gamtoos-R' (GR; carrying *Dn7*), in a transcript profiling study. Virus-induced gene silencing (VIGS), using the barley stripe mosaic virus, was used to test whether a candidate gene identified from the microarray experiment is involved in the susceptible reaction of GS. Controlled infestation with RWA2, the most virulent biotype to date, was used to estimate aphid fecundity and aphid prenymphopositional period (PNP) and to assess symptom development. No variation in PNP was observed among the treatments. However, silenced plants did show significantly lower aphid fecundity compared to GS and the viral control and similar fecundity to GR. At 14 days-post-infestation, chlorosis scores for the silenced treatment were not significantly different from GR. There also was a significant correlation between the average aphid counts and expression of the candidate gene across treatments. These results indicate that this gene may play an important role in susceptibility and could be exploited for breeding broad-spectrum resistance.

Poster 15. Association analysis of wheat resistance to stem rust in U.S. winter wheat.

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Stem rust, caused by *Puccinia graminis* f. sp. *tritici*, has become a new threat for wheat production in the U.S. after the emergence and quick spread of race TTKSK from East Africa to other countries. To evaluate the current status of U.S. winter wheat resistance to stem rust, validate markers associated with known genes, and identify new loci effective against the disease, an association mapping population was assembled with 174 U.S. winter wheat cultivars and breeding lines from the 2008 regional nurseries. A total of 267 genome-wide simple sequence repeat (SSR) markers, including those linked to reported major stem rust resistance gene/QTL, were used to genotype this population. The population was evaluated for seedling resistance to race TTKSK and both seedling and adult-plant resistance to a bulk of U.S. races. About 40% of accessions showed resistance or moderate resistance to the U.S. races in the seedling or adult stage, but only 11.5% of seedlings were resistant to the race TTKSK with an infection type (IT) of 2 or lower. The accessions carrying *Sr36* showed a high level of resistance to both U.S. races and TTKSK in the seedling stage and appeared to confer the best resistance to TTKSK in the population. *Sr38* and *Sr24* conferred a high level of resistance to the U.S. races at the adult stage, with severities lower than 10% and at least moderate resistance. *Sr24* also showed seedling resistance to TTKSK with ITs of ;2 to 2. Accessions with *Sr31* or the new SSR allele, *Xgwm334-123* on chromosome 6A, showed resistance to U.S. races in seedling and adult stages but not to TTKSK. Three additional marker alleles were associated with a low IT (2 or lower) to TTKSK and *Sr* genes linked to these alleles need further investigation. However, the frequency of all these resistance alleles for TTKSK was low in the population studied. Introducing new *Sr* genes and increasing the frequency of known effective resistance genes should be the focus of research to improve wheat resistance to stem rust.

Poster 16. A gut transcriptome of the Hessian fly (Mayetiola destructor), a member of the gall midges.

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Hessian fly, *Mayetiola destructor*, is a serious pest of wheat and an experimental organism for the study of gall midge-plant interactions. In addition to food digestion and detoxification, the gut of Hessian fly larvae also is an important interface for insect–host interactions. Analysis of the genes expressed in the Hessian fly larval gut will enhance our understanding of the overall gut physiology and may also lead to the identification of critical molecules for Hessian fly–host-plant interactions. Over 10,000 expressed sequence tags (ESTs) were generated and assembled into 2,007 clusters. The most striking feature of the Hessian fly larval transcriptome is the existence of a large number of transcripts coding for so-called small secretory proteins (SSP) with amino acids less than 250. Eleven of the 30 largest clusters were SSP transcripts with the largest cluster containing 11.3% of total ESTs. Micorarray and qPCR analyses of representative SSP transcripts revealed that most of them were predominantly present in the gut tissue and the transcript levels of many SSP were affected by plant genotypes on which larvae feed. Transcripts coding for diverse digestive enzymes and detoxification and metabolic proteins also were identified. The putative digestive enzymes included serine proteinases (trypsins and chymotrypsins), cysteine proteases, aspartic protease, endo-oligopeptidase, aminopeptidases, carboxypeptidases, and α-amylases. Putative detoxification proteins included cytochrome P450s, glutathione S-transferases, peroxidases, ferritins, a catalase, and peroxiredoxins. This study represents the first global analysis of gut transcripts from a gall midge. The identification of a large number of SSP transcripts in the Hessian fly larval gut provides a foundation for future study on the functions of these genes.

Poster 17. Two homoeologous wheat genes confer sensitivity to a single, host-selective toxin and susceptibility to Stagonospora nodorum blotch.

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The pathogen *Stagonospora nodorum* produces multiple host-selective toxins that interact with corresponding wheat sensitivity genes in an inverse gene-for-gene manner to cause the disease Stagonospora nodorum blotch (SNB) in wheat. We screened accessions of *Aegilops tauschii*, the D-genome donor of common hexaploid wheat (*Triticum aestivum*), with culture filtrate derived from isolate Sn4. One sensitive (TA2377) and one insensitive (AL8/78) accession were selected to develop an F_2 population. Bulked-segregant analysis and molecular mapping indicated that the new toxin sensitivity gene, temporarily designated *Snn5DS*, mapped to chromosome arm 5DS. Inoculation of the population with spores from Sn4 indicated that a compatible host–toxin interaction explained 100% of the variation in SNB development. In related research, *SnTox3*, which interacts with the *Snn3* gene on wheat chromosome arm 5BS, was isolated. Further evaluation of the F_2 population indicated that the toxin interacting with *Snn5DS* was SnTox3. Comparative mapping revealed that *Snn3* and *Snn5DS* are homoeologous and, thus, derived from a common ancestor. Further characterization indicated that, as opposed to most host–toxin interactions in the wheat–*S. nodorum* pathosystem, the *Snn3*/*Snn5DS*-SnTox3 interaction is not dependent on light, which suggests that a different host metabolic pathway is exploited to cause disease. Saturation and high-resolution mapping delineated the *Snn5DS* locus to a 1.4-cM interval, and analysis of colinearity indicated the *Snn5DS* region is well conserved between wheat, rice and *Brachypodium*, which will aid in the map-based cloning of *Snn5DS*.