

by the surrounding genome sequences, a phenomenon known as position effects. Matrix attachment elements (MAR) are segments of DNA that anchor chromosomes to the nuclear matrix. Inclusion of a MAR upstream and/or downstream of genes in transformation constructs has sometimes resulted in higher and/or more copy-number dependent transgene expression levels. MAR elements have been identified in the 5' flanking regions of the *Glu-D1* genes that encode high-molecular-weight glutenin subunits Dx5 and Dy10. To test the effects of flanking sequences on transgene expression in wheat endosperm, we transformed Bobwhite wheat with four constructs that express the *uidA* (GUS) marker gene under control of the promoter of the wheat *IDy10* HMW-glutenin gene. One construct consists of the GUS-coding region flanked by about 2,800 bp upstream of the start codon of the native *IDy10* gene and about 2,000 bp downstream of the stop codon of the native *IDx5* HMW-glutenin gene. The second construct contains a 425-bp version of the 5' flanking sequence that comprises the *IDy10* gene promoter but lacks the MAR region upstream. The third construct has the nopaline synthase transcription terminator (Nos 3') in place of the 3' regions from the *IDx5* gene. The fourth construct contains GUS flanked by the 425-bp version of the promoter and the Nos 3' transcription terminator. Fifteen to twenty independent transgenic events for each construct were identified and characterized in detail. Transgene inheritance and homozygous progeny were identified for each event by histochemical staining of endosperm. GUS enzyme activities in homozygous mature seeds of each event were measured using a fluorimetric substrate. GUS transgene copy numbers were measured by quantitative real-time PCR, using *PinB* as a single-copy reference gene. The relationship of copy number to expression level for each of the four plant populations will be discussed. These comparisons will show whether inclusion of large regions of flanking DNA in transformation constructions can buffer position effects in transgenic wheat.

Poster 13. The genetic basis of variation in vernalization requirement duration in winter wheat.

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The transition from vegetative to reproductive development in wheat is mainly determined by the three vernalization genes *VRN1*, *VRN2*, and *VRN3*. These genes have been cloned and characterized in recent studies on allelic variation that occurs between winter wheat, which requires exposure to low temperatures to accelerate the developmental transition (vernalization), and spring wheat, which requires no vernalization. However, little is known about allelic variation in the flowering process among winter wheat cultivars that are practically categorized, based on their various requirements to vernalization, as weak winter, semi-winter, and strong winter types. We developed a mapping population using a cross between the two winter wheat cultivars Jagger (low vernalization requirement) and 2174 (high vernalization requirement) and mapped 96 F_{7:8} recombinant inbred lines using approximately 200 SSR markers. Our preliminary results have shown that the vernalization requirement and flowering date were controlled by a major genetic locus and several minor modifiers. Identification of genes located on this major locus controlling the vernalization requirement in winter wheat is in progress.

Poster 14. A novel source of resistance in wheat to *Pyrenophora tritici-repentis* race 1.

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Tan spot, caused by the fungus *Pyrenophora tritici-repentis*, causes serious yield losses in wheat and many other grasses. Race 1 of the fungus, which produces the necrosis toxin Ptr ToxA and the chlorosis toxin Ptr ToxC, is the most prevalent race in the U.S. Great Plains. Wheat genotypes with useful levels of resistance to race 1 have been deployed, but this resistance only reduces damage by 50-75%. Therefore, new sources of resistance to *P. tritici-repentis* are needed. Recombinant inbred lines developed from a cross between the Indian spring wheats WH542 (resistant) and HD29 (moderately-susceptible) were evaluated for reaction to race 1 of the fungus. Composite interval mapping revealed QTL on the short arm of chromosome 3A explaining 23% of the phenotypic variation and the long arm of chromosome 5B explaining 27% of the variation. Both resistance alleles were contributed by the WH542 parent. The QTL on 5B is probably *tsn1*, which was described previously. The 3AS QTL (*QTs.ksu-3AS*) on 3AS is a novel QTL for resistance to *P. tritici-repentis*,

race 1. The QTL region is located in the most distal bin of chromosome 3AS in a 2.2-cM marker interval. Flanking markers *Xbarc45* and *Xbarc86* are suitable for marker-assisted selection for tan spot resistance.

Poster 15. Computer tools for high-throughput wheat genome data analysis.

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The GrainGenes (graingenes.org) database project has worked alongside many Triticeae research projects over the years, and has developed several computer tools which have assisted in wheat genome research. Initial applications have dealt mainly with sequence processing and archiving (e.g., SQPR), and clone identification and selection (e.g., Hybsweeper). These software were important toward generating EST collections and assisting in clone picking methods for furthering genetic research within the laboratory. Other applications have been applied toward extracting high information content fingerprint traces (e.g., GenoProfiler) used for constructing physical mapping scaffolds of and viewing (e.g., FPC WebViewer) the wheat genome. These tools have been useful in determining the linear order of cloned genes and have helped to build a linear order of clones along a chromosome. Additional applications have been developed for sequence data analysis used for generating genome primers (e.g., BatchPrimer3) and reporting sequence alignments (e.g., SNPReporter) to distinguish germ plasm differences. This collection of tools has been useful in the development of genome-specific primers and new molecular markers. Other applications serve to report analysis in a database format (e.g., WheatDB and SNPdb). These resources have proven useful as a starting point for the design of many genetic experiments and surveys. Other projects under development attempt to assist gene discovery using gene-mining algorithms (e.g., CCV). For these and other examples, please visit the GrainGenes demonstration page at wheat.pw.usda.gov/demos. These and other tools from community resources can assist in the study of the wheat genome.

Poster 16. Structural characterization of the model Brachypodium genome and the observation of synteny conservation with wheat.

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Because of its small genome size (~350 Mb) and several desirable attributes, *Brachypodium distachyon* is emerging as a model system for temperate grasses, including important crops like wheat and barley. Analysis of 10.9% of the *Brachypodium* genome based on 64,696 BAC end sequences (BES) revealed that the genome consists of ~18.4% repetitive elements (TEs), with 11% known TEs and 7.4% unique *Brachypodium* TEs. Sequence analysis indicated that approximately 21.2% of the *Brachypodium* genome represents coding sequence. The BESs were integrated into the BAC-based physical maps of the *Brachypodium* genome, which allows for comparison of gene order and contents with that of another grass model genome, *Oryza sativa* (rice), at a genome-wide level. Large, conserved genomic regions were readily identified between the two small grass genomes. We also analyzed the sequence conservation at the microcolinearity level by comparing sequenced *Brachypodium* BACs with the orthologous regions from rice. Genomic rearrangements, differential gene amplification, and deletion appeared to be the common evolutionary events that caused variations of microcolinearity at different orthologous genomic regions. Our conclusions also were supported by a preliminary analysis of the 4X whole-genome sequence produced by the *Brachypodium* sequencing project at the DOE Joint Genome Institute. In addition, several annotated genes in *Brachypodium* BACs have matches to the wheat deletion bin-mapped ESTs. In some cases, genes in the same BACs matched to wheat ESTs that were mapped to the same wheat deletion bins, suggesting that the *Brachypodium* genome will provide useful information in placing the order of mapped wheat ESTs within the deletion bins and developing specific markers in the targeted regions of wheat chromosomes.