# Poster 10. Association of seed dormancy with red pericarp color in weedy rice arises from pleiotropy of a predicted transcription factor.

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Seed dormancy has been associated with grain color in wheat and rice, with the red-colored genotypes being more dormant than white-colored. However, whether the association arises from pleiotropy or linkage remains uncertain. We introduced a segment of chromosome harboring a cluster of QTL for seed dormancy (qSD7-1) and pericarp color (qPC7) from weedy into cultivated rice to clone and characterize their underlying gene(s). High-resolution mapping narrowed the QTL to the same locus of Os07g11020 (a predicted transcription factor) and obtained a rare recombinant intragenic to the transcription factor. Sequence comparison for the 6,445-bp region identified 33 point mutations between alleles from the weedy and cultivated lines and that intragenic recombinant retains a segment of 2,000 bp from the weedy rice. A pair of dormant and nondormant isogenic lines was developed from the recombinant. These lines differed in seed dormancy, pericarp color (red vs. white), grain weight, and abscisic acid (ABA) content at about 10 days of seed development. The transcripts of the dormancy gene were detected in both seed and leaf tissues from the isogenic lines. Sequence comparison between the genomic DNA and the full-length cDNA identified eight exons and the 14-bp deletion in exon 7 that accounts for the molecular lesion for the aforementioned natural variation. We conclude that the above association in rice is a pleiotropic effect of the predicted transcription factor, and the dormancy allele cannot be used to improve white pericarp-colored varieties for resistance to preharvest sprouting. This research also suggests that the qSD7-1 underlying gene may regulate the natural variation in seed dormancy and pericarp color by ABA- and pigment-related physiological pathways, respectively, and may have other effects on the traits expressed in the vegetative tissues.

### Poster 11. GrainGenes: Serving the wheat community for 15 years, over a billion served.

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GrainGenes (graingenes.org) is a comprehensive database for molecular and genetic information on wheat, barley, rye, and oats. In addition, the GrainGenes project helps coördinate wheat community research projects, such as the International Triticeae EST Cooperative (ITEC), the mapping of ESTs in Chinese Spring deletion lines, development of the D-genome physical map, development of genome-specific SNPs, and analysis of Triticeae repeat sequences (TREP). The GrainGenes map collection comprises 165 mapping studies including genetic, consensus, and physical maps, viewable using the CMap comparative map display. Additional database tools are in development to build genotype, trait and QTL relationships for germ plasm to assist in wheat marker-assisted selection (Wheat CAP project, maswheat.ucdavis. edu). Other tools, such as preformatted Quick Queries, advanced SQL, and Batch Queries have been updated to aid user access to the database. The web log shows that 30,000 different people use GrainGenes per month. Most of you have not told us what you do not like about GrainGenes, either what data should be there that is not or questions about how to find what is there. Please speak up.

## Poster 12. Influence of flanking sequences on transgene expression levels in the endosperm of transformed wheat.

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There is often no relationship between transgene copy number and expression levels in different transgenic plants transformed with the same DNA construct. We hypothesized that expression of the integrated DNA can be influenced

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 highmolecular-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 1Dy10 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 1Dx5 HMW-glutenin gene. The second construct contains a 425-bp version of the 5' flanking sequence that comprises the IDyI0 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 *1Dx5* 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<sub>7.8</sub> 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 5BL 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*,