## *Poster 27. Disruption of circadian clock caused by the earliness per se 3Am locus (Eps-3Am) contributes to early flowering in wheat (Triticum sp. L.).*

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In temperate grasses, such as wheat and barley (*Hordeum vulgare* L.), earliness *per se* is understood as the intrinsic difference in flowering time of fully vernalized plants grown under long day conditions. In the current study, two einkorn wheat lines (*Triticum monococcum* L. x *T. boeoticum* Boiss.), RIL25 (early) and RIL71 (late) were selected from a RILWA1 population to generate a new $F_2$ population for fine mapping of the *Eps3A* locus. About 650 $F_2$ individuals were screened for genetic recombinations, and four new markers were added utilizing the physical map from barley chromosome 3H. This way, the locus could be delimited to approximately 350 kb and contained only two putative genes. Moreover, both genes were found to be deleted in the mutant parent of the RILWA1 population KT3-5 (*T. monococcum*) as well as in RIL25. The deletion of *TmLUX* (*lux arrhythmo*, an evening clock element) caused clock distortion and misexpression of circadian clock-related genes. Both effects were detectable by using delayed fluorescence measurements as well as a time-course qRT-PCR experiment on two key nuclear clock genes *TmTOC1* (*timing of CAB2 expression 1*) and *TmLHY* (*late elongated hypocotyl*). On the other hand, sequences of the *TmPUMILIO* (RNA-binding protein) and *TmLUX* were subjected to screen a barley TILLING population of the cultivar Barke, resulting in 34 confirmed mutations for *HvPUM* (including one nonsense mutation) and 39 putative mutations for *HvLUX*. Future analysis may reveal the phenotypes of the putative TILLING mutants in barley. The better candidate, *TmLUX* also was resequenced in a collection of 96 diploid and tetraploid wheats revealing a single A-genome-specific haplotype with a unique 21nt deletion found in the MYB domain. The Chinese cultivar Yunnan possessing this mutation was heading relatively early, thus supporting *TmLUX* as a sensible candidate for *Eps-3A$^m$*. Time course qRT-PCR on this accession as well as on transgenic putative knock-down lines will be performed to verify the *TmLUX*–circadian clock–earliness *per se* connection.

## *Poster 28. The pleiotropic effects of the master regulator Q and its homoeologous loci in polyploid wheat.*

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The *Q* gene on chromosome 5A is of major importance because it governs the free-threshing character, and also pleiotropically affects many other traits associated with wheat development and domestication. To precisely investigate the function of *5AQ*, we developed three *Q* gene near-isogenic lines (NILs) in the background of the hexaploid wheat cultivar Bobwhite (BW), which consist of the BW wild type *5AQ*, an EMS-induced *5AQ* mutant (*5AQm*) with a premature stop codon, and the recessive *5Aq* allele from *Triticum turgidum* subsp. *dicoccoides*. Phenotypic analyses confirmed the previously identified traits controlled by the *Q* gene, such as spike shape, threshability, glume toughness, plant height, and flowering time. In addition, the grain yield per plant of the *5AQ* NIL was significantly higher than the *5AQm* and *5Aq* NILs due to differences in several yield component traits. Microscopic analysis of spike tissue revealed obvious differences in cell morphology among the NILs, which likely underlies the differences in glume toughness and rachis disarticulation attributed to *Q*. To investigate potential downstream genes of *5AQ* responsible for these traits, we analyzed the expression of wheat genes homologous to known development-associated genes, such as *AGAMOUS*, *FLOWERING LOCUS T* (*FT*), *CONSTANS*, and *SHATTERPROOF*. RT–PCR showed a dramatic change in the expression of

these genes in *5AQ* compared to *5AQm* and *5Aq*, demonstrating the role of *Q* in regulating gene networks associated with wheat development. Homoeologous copies of *Q* exist on chromosomes 5B and 5D, but their functions are less understood. Phenotypic analysis of genetic stocks with various combinations of *Q* homoeoalleles indicated that *5Bq* and *5Dq* also contributed to the suppression of speltoid characters and glume toughness, but to a lesser degree than does the *5AQ*. An RQ-PCR study showed that *5Bq* and *5Dq* are transcriptionally active, but *5Bq* is a pseudogene. Combined phenotypic and expression analysis indicated the presence of complex interactions among the homoeoalleles. The evolution of the *Q*/*q* loci in polyploid wheat resulted in the hyperfunctionalization of the master regulator *5AQ*, pseudogenization of *5Bq*, and subfunctionalization of *5Dq*, but all are finely tuned in a coordinated manner to govern domestication and development in polyploid wheat.

## *Poster 29. Molecular mapping of Brittle rachis (Br-A1) gene in Triticum timopheevii.*

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Free shattering at maturity is an important strategy in plants for seed dispersal in nature. Domestication of wheat in the Bronze Age occurred mainly due to two important evolutionary changes, namely the loss of rachis fragility at maturity and evolution of free threshability. The *Brittle rachis* (*Br1*) gene located on homoeologous group-3 chromosomes controls rachis fragility in wheat and barley. In *Triticum timopheevii* subsp. *armeniacum* ($A^tA^tGG$ genomes, 2n=4x=28), characteristic wedge-type disarticulation occurs due to the formation of abscission zone at the base of the spikelets making them spear shaped dispersal units at maturity. Mapping of the $A^t$-genome, brittle rachis gene (*Br-A$^t$1*) was done in a recombinant inbred line (RIL) population of tetraploid *T. timopheevii* subsp. *armeniacum* and *T. timopheevii* subsp. *timopheevii*. The RIL population segregated 62 brittle and 73 tough rachis, fitting the monogenic segregation ratio of 1:1. Nine out of a total of 72 BAC-end derived markers from wheat 3AS BAC library and 8/24 SSRs from the publically available databases were found to be polymorphic between the parents. The gene was mapped to a 7.4-cM region on short arm of chromosome $3A^t$ flanked by microsatellite markers *Xgwm2* and *Xbem44*. Previously this gene was located in a 35-cM region on chromosome $3A^tS$ between RFLP markers *XksuA6* and *Xpsr1196*. The *T. timopheevii* subsp. *armeniacum Br-A$^t$1* gene may be orthologous to the hexaploid wheat *Br1* locus. Five recombinants were found in the RIL population for these two markers, and 30 recombinants for the closest markers were found in a set of 355 individuals of $F_{2:3}$ families segregating for *Br-A$^t$1*. Heterogenous inbred families from the recombinants for the closest flanking markers are being used for fine mapping. Primers are being designed, tested, and mapped from syntenous regions of rice chromosome 1 and *Brachypodium* chromosome 2. Resources available for chromosome 3A sequencing will be used for the fine mapping and cloning.

## *Poster 30. Metabolic, physiological, and molecular characterization of cuticular variation in wheat.*

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All the aerial organs of land plants are covered with a layer of hydrophobic lipids, termed the cuticle. It plays important roles in development, growth, and defense against biotic and abiotic stresses. Structurally, the cuticle consists of a cutin frame, with intracuticular wax embodied in and an epicuticular wax overlaid on them. A field of densely distributed, epicuticular wax crystals is visible as bloom or glaucousness. In wheat, variation of glaucousness is mainly controlled by wax-production genes *W1* and *W2*, and wax-inhibition genes *Iw1* and *Iw2*. *W1* and *Iw1* are located on the short arm of chromosome 2B (2BS), and *W2* and *Iw2* are on 2DS. Although early field studies showed glaucousness played an important role in drought and heat tolerance, little is known about the molecular mechanisms and metabolic products of these wax genes. We are characterizing a set of six wax near-isogenic lines (NILs) in an S-615 background by combining the genetic, metabolic, physiological, and molecular approaches. Wax profiling by GC-MS indicated that the wax load and composition vary greatly among the NILs. The *Iw2*-NIL has highest wax load, followed by *Iw1*-NIL, S-615, *W2*-NIL, *W1*-NIL, and *w*-NIL. Wax profiles suggested that (1) gene *W2* plays an important role in decarbonylation pathway for n-