Poster 77. Molecular characterization and protein-DNA interaction analysis of a WRKY transcription factor in wheat (Triticum aestivum L.) showing differential spatiotemporal expression during leaf-rust pathogenesis.

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The plant WRKY proteins are one of the largest families of transcription factors involved in physiological processes including biotic and abiotic stresses. These transcription factors have a recognition sequence (T)(T)TGAC(C/T), known as the W-box, found in the promoter region of WRKY and other defense-related genes. These transcription factors have a strictly conserved 60 amino acid region containing the WRKYGQK peptide sequence and zinc finger-like motifs. The WRKY gene family is subdivided into three different groups (I, II, and III) based on the number of WRKY domains and features associated with the zinc finger like motifs, group I and group II have finger motif C$_x$H$_2$, whereas group III contains a C$_x$HC motif. Very little information on WRKY transcription factors of wheat are available and, hence, the present study was undertaken to characterize a wheat WRKY transcription factor and decipher its role during leaf-rust infection. WRKY-specific, wheat consensus sequences were obtained from NCBI to design primers to amplify genomic and cDNA sequences from leaf-rust resistant and susceptible near isogenic wheat lines. The sequences were analyzed using multiple sequence alignment and a conscientious phylogenetic tree was constructed to study the relationship with WRKY transcription factors of other plants. Various catalytic domains were identified using ScanProsite and other bioinformatic softwares. In silico docking between a 26-bp oligonucleotide containing the W-box sequence and a 74 amino acid sequence having the conserved 60 amino acids including the core WRKYGQK domain was performed at Haddock server. Different maize lines harboring Activator (Ac) and Mutator (Mu) transposable elements were crossed with bread wheat lines and the haploid embryos were rescued. Regenerated wheat plants were treated with colchicine for chromosome

Poster 78. Transferring maize transposable elements to bread wheat through ‘wheat x maize’ crossing.

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Transposon tagging and insertional mutagenesis are important reverse genetic tools in eukaryotes. These are widely used for understanding gene function and targeted manipulation of genes in crop improvement. Although no characterized transposable element system is available in wheat, the maize systems have proven to be very successful for gene tagging not only in maize but also in other plant species including tobacco, Arabidopsis, peas, and barley. Normally, genetic transformation is used to introduce these active transposable elements (TE) into nonhost plants. In the case of wheat, however, genetic transformation is technically challenging, variety specific, and the frequency is usually very low. We attempted a simpler method based on direct ‘wheat x maize’ crossing to transfer well-characterized maize transposable elements into wheat. Wheat doubled haploids are routinely produced by ‘wheat x maize’ crossing where the resulting zygotes undergo uniparental chromosome elimination giving rise to haploid wheat plants. The maize chromosomes are present during the few initial cell cycles during zygote differentiation, giving enough time for the transposition of TEs. Different maize lines harboring Activator (Ac) and Mutator (Mu) transposable elements were crossed with bread wheat lines and the haploid embryos were rescued. Regenerated wheat plants were treated with colchicine for chromosome
doubling. These plants were screened by PCR amplification using multiple maize TE specific primers. Three of the 64 plants from the Louise/Maize Activator crosses showed a maize-specific, PCR fragment amplified using Ac-specific, PCR primers. Similarly, four of the 40 plants derived from PBW 621/Maize Mutator crosses showed a maize-specific, PCR fragment amplified using Mu-specific PCR primers. Amplified fragments were sequenced to confirm their specificity. All the fragments were matching up with their corresponding maize transposable element sequences. DNA gel blot analysis and inverse PCR are underway to confirm the integration and subsequent transposition activities. The most current status of the project will be presented at the meeting.

Poster 79. The wheat meiotic cohesin gene TtRec8 and its role in haploidy-dependent, unreductional meiotic cell division.

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Meiosis is a specialized cell division that halves chromosomes and generates haploid gametes in eukaryotes. It ensures genomic integrity and generates genetic variability. Variation of the meiotic process leads to aneuploidy and polyploidy. Unreductional meiotic cell division (UMCD) was observed in the polyhaploid of the tetraploid wheat cultivar Langdon (Triticum turgidum L.) and its interspecific hybrid with Aegilops tauschii. This haploidy-dependent UMCD gives rise to unreduced gametes, leading to polyploidy. It has been considered a major mechanism of polyploidization in wheat. The meiotic cohesin gene Rec8 has been proven to play a significant role in kinetochore orientation and chromosome segregation at meiosis I in model species. In the present study, we attempted to understand the function of the Rec8-like gene in the meiotic cell division of wheat and determine the role of this gene in the onset of the haploidy-dependent UMCD. We cloned the Rec8 homologue in LDN, designated TtRec8, and developed the polyclonal antibody against the TtRec8 protein in rabbit. TtRec8 exhibited an expression pattern similar to Rec8 in model species as revealed by real-time PCR and Western blotting throughout the meiotic process in anthers. In addition, the TtRec8 protein exhibited analogous kinetics of the meiotic cohesin Rec8 as revealed by the immunolocalization of the cohesion protein on chromosomes over the meiotic stages. Two homoeoloci of TtRec8 were identified on chromosome 1A and 1B. We have been determining the map location of this gene on the chromosome in a doubled haploid population of tetraploid wheat. Meanwhile, we have been investigating the expression profiles of TtRec8 and the kinetics of the TtRec8 protein in an LDN haploid as well as the interspecific hybrid of LDN with Ae. tauschii. This will provide new insights into the role of this meiotic cohesin gene in the onset of the haploidy-dependent UMCD in wheat.