Poster 75. Androgenic response of Nebraskan winter wheat (Triticum aestivum L.) varieties to isolated microspore culture for doubled haploid plant production.

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Isolated microspore culture based androgenesis is being used for production of haploid (H) and doubled haploid (DH) plants in wheat. This technique can facilitate the efficiency of wheat breeding and genetic mapping as well as research in functional genomics and gene expression. Until now there was no report of microspore culture in Nebraskan winter wheat varieties. The objectives of the present report were to (1) study the androgenic response of Nebraskan winter wheat varieties and (2) establish efficient procedure for green DH plant regeneration. Three Nebraskan winter varieties (Anton, Antelope, and Camelot) were used. The spikes were collected from greenhouse-grown plants when microspores were at mid-late to late-uninucleate stage. For each batch of pretreatment, anthers from 16 spikes were pretreated in solution B at 25°C for 4–5 days followed by microspores isolation (no cold pretreatment). For cold pretreatment, anthers were incubated at 4°C for additional five days. The numbers of embryogenic microspores, multicellular, and embryo-like structures were recorded and analyzed. Compared to no cold pretreatment, a cold pretreatment increased the number of embryogenic microspores significantly in Anton by two fold, but no significant differences between the two pretreatments were observed in Camelot and Antelope. *In vitro* development of microspores into multicellular and embryo-like structures were quicker in Camelot than Anton and Antelope. The green plants were regenerated in all three varieties following both cold and no cold pretreatment. The number of regenerated green plants per batch of pretreatment was four (no cold) and eight (cold) in case of Antelope. However, for Anton and Camelot, there was one green plant per batch in both the cold and no cold treatments. It seems that higher number of embryogenic microspores due to cold pretreatment in Anton was not regenerated into proportionate number of green plants. An experiment is under progress to determine a similar response in Camelot and Antelope. This is the first report of androgenic response of Nebraskan winter wheat varieties. We believe that this method will be a beneficial tool in our wheat breeding efforts. However green plant regeneration frequency needs to be increased for cost-effective use.

Poster 76. Chromatin state affects the DNA breakage/repair mechanism in wheat.

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Meiotic recombination, the basis of genetic mapping is not uniformly distributed across the genome. The regions of high and low recombination that result in uneven map resolution across the chromosomes are evident across many grass genomes including wheat (*Triticum aestivum* L.). Recombination is believed to be linked to the double-stranded DNA break and repair, a phenomenon highly dependent on the chromatin state. Radiation hybrid (RH) maps have been proposed to provide i) higher, ii) be more uniform resolution than genetic maps, and iii) to be independent from recombination constraints. We generated an RH panel for mapping of wheat chromosome 3B and used it to test these three assumptions. Our RH map contains 541 markers anchored to chromosome 3B BAC contigs. Detailed comparisons with a genetic map of similar quality confirmed that i) the resolution of the RH map was 10.5X higher and ii) six times more uniform. We identified a strong interaction ($r = 0.879$ at $p = 0.01$) between the DNA repair mechanism in mitotic cells and the distribution of crossing-over events in meiotic cells. We could explain this finding only by admitting the possibility that the DNA repair mechanism is affected by the chromatin state in a way similar to the effect that chromatin state

has on recombination frequencies. Our RH data support for the first time *in vivo* the hypothesis of noncasual interaction between recombination hot-spots and DNA break/repair. This means that since the initial RH application 37 years ago, we were able to show now for the first time that the third hypothesis of RH mapping (iii) might not be entirely correct.

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_2H_2, whereas group III contains a C_2HC 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. The optimized peptide and DNA models were able to form a complex that showed interaction between the W-box and the conserved WRKY domain. The protein–DNA binding was validated by observing shifts in electrophoretic mobility shift assay using heterologously produced recombinant WRKY proteins. Temporal and spatial gene expression profiling was done by quantitative real-time PCR using universal probe library based, WRKY-specific probe and primers with RNA isolated at different time points from resistant and susceptible plants that were either mock inoculated or infected with virulent leaf-rust pathogen. During pathogenesis, maximum expression of *WRKY* gene was observed at 24-hours post-inoculation in both compatible and incompatible interaction.

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