

Wheat Research Progress Report - Final

Project #: 3019-5194

Title: Biotechnology for Wheat Improvement

Researchers: SS Jones, TD Murray, K. Garland Campbell, B. Baik, S. Lyon, K. Balow, M. Gollnick, K. Murphy, J. Moran, L. Hoagland, J. Dawson, J. Matanguihan, L. Patzek, J. Keach

Progress Report Year: 2008

Accomplishments:

Over 6000 soft and hard winter wheat crosses were made in the greenhouse during the 2005-2008 crop year and approximately 1200 were planted in the field in the fall of 2006, 2007 and 2008 (Table 1). We continue to rapidly advance the highest priority F₂'s in the greenhouse using single-seed descent. This allows us to plant in the field the most promising crosses in their fifth generation after just two years.

Table 1: Early generation breeding lines developed/selected in 2006-08.

Generation	Number of entries	Location planted
F ₂	2932	Pullman
F ₂	2274	Lind
F ₂ long coleoptile	1320	Lind
F ₃	7044	Pullman, Lind
F ₄	8737	Pullman
F ₅	808	Pullman, Lind

Approximately 2700 crosses were made in 2005-2008 specifically for the introgression of traits associated with superior emergence into our locally adapted cultivars.

The length of the coleoptile for each of the breeding lines in advanced yield trials (**2005:** 343; **2006:** 659; **2007:** 1279 for a total of 2,281) were measured every year of the granting period. This gives us a general idea of their emergence capabilities from deep plantings. The length is determined as the average of 10 samples from 2 replications, so this year over 25,000 measurements were taken.

One facet of the breeding program involves the use of backcross breeding, with and without marker-assisted selection, to develop new breeding lines. A feature of the backcross procedure we utilize is that a backcross derived cultivar will be adapted in the same general environment as the recurrent parent, reducing the testing normally required to confirm adaptation of a backcross derived cultivar. We use genetic markers, whenever possible, in order to identify individuals within backcrossed families that carry the desirable genes for the trait in question. This procedure, used in conjunction with stringent field and laboratory selection criteria, is very effective for incorporating desired traits into an already adapted cultivar and insures rapid progress in varietal improvement.

Quantitative, colorimetric assays, which measure the level of phenol oxidase activity, were conducted on early generation lines in 2006-2007 that had white seed. Using this tyrosinase procedure, all early generation lines with white seeds (2000-4000/yr) were screened for noodle color potential and those with low activity levels were selected for advancement. We continue to use this assay when screening for noodle color.

High molecular weight glutenin seed storage protein analysis is conducted annually on over 1,500 F₄ through F₆ early generation lines. This helps us to identify individual lines with superior end-use quality potential within different market classes for advancement in the breeding program. These lines are also screened for kernel hardness and color and compared with their storage proteins. This allows us to eliminate lines with undesirable quality parameters for a particular market class at a very early generation.

Preliminary quality screening of our F₄ and F₅ breeding lines allows us to historically cull over 50% that do not meet minimum quality standards. This saves an incredible amount of time, land, and money, and greatly improves the efficiency of the breeding program.

The biotechnology component of the Winter Wheat Breeding Program at WSU is focused on the genetic and cytogenetic analysis of species and cultivars with the potential to improve winter wheat. Our current studies are focused on the identification of chromosomes, chromatin and specific genes that confer competitive traits to wheat. We have for several years focused on the wheatgrass genus *Thinopyrum* among others. This genus is commonly mined for useful traits, and yet detailed genetic analysis of the chromosomes of these species has not been achieved. Our current focus is on homeology group 4 chromosomes in this group, and our present objective is to complete mapping of chromosome 4E from *Thinopyrum elongatum* to aid in the isolation of competitive traits. This can be a long process but progress is being made by having several graduate students, a visiting scientist, and an undergraduate intern working on this project.

Cytogenetic introgression of alien chromatin.

We are continuing our efforts to transfer chromatin from the 4E chromosome of tall wheatgrass (*Thinopyrum elongatum*) into wheat. Using the *in situ* hybridization technique, we identified another line which has the short arm of this chromosome translocated onto one of the chromosomes of the base genome of wheat. These sequences should provide genes for salt tolerance, wheat streak mosaic virus resistance and other competitive factors. An ongoing investigation into a new source of resistance to *Rhizoctonia* disease on this chromosomal piece is being conducted in collaboration with the WSU Plant Pathology lab of Dr. Pat Okubara.

Generation of a 4E deletion series.

This approach employs gametocidal chromosome 2C^c from *Aegilops cylindrica* L., a wild relative of wheat, which can induce terminal chromosome deletions when it is present in wheat in a monosomic condition. This genetic system can also be used to produce deletions of alien chromosomes that are added to a wheat genetic background. The deletions induced by *Ae. cylindrica* chromosome 2C^c result almost exclusively from single breakage events with concomitant loss of distal segments. The use of the gametocidal properties of chromosome 2C^c to induce deletions is well characterized and efficient, with up to 8% of progeny having deletions for the critical chromosome.

Disomic CS-*Th. elongatum* addition line 4E was used as a female parent to cross with disomic CS-*Ae. cylindrica* addition line 2C^c. The F₁ hybrid was then backcrossed to disomic CS-*Th. elongatum* addition line 4E. The plants carrying 21 pairs of wheat chromosomes, one

chromosome 2C^c, and one pair of chromosome 4E were selected from the BC₁F₁ generation using chromosome C-banding and genomic *in situ* hybridization techniques. The BC₁F₁ plants carrying 21 pairs of wheat chromosomes, one chromosome 2C^c, and one pair of chromosome 4E (21''+1'+1'') were then selfed to produce BC₁F₂; deletions caused by 2C^c occurred in the gametes of this generation. The subsequent BC₁F₃ will contain individuals homozygous for terminal deletions on the critical 4E chromosomes. Over 2000 individuals from these generations were grown in the greenhouse and seed was harvested from each individual plant.

The frequency of deletions on the critical chromosome using this system can be high. In previous research utilizing the same gametocidal chromosome and foreign chromosomes in a CS background, deletion frequencies of the foreign chromosome ranged from 3.7% - 8% of individuals for metacentric chromosomes and 1.8% for a foreign telocentric chromosome. With an expected deletion rate of a conservative 2% for the metacentric 4E chromosome, we expected about 40 individuals in our BC₂F₁ and BC₁F₂ generations to be heterozygous for deletions. The BC₁F₃ and BC₂F₂ progeny from this generation were screened visually for plants that fail to initiate PSCR, in order to identify those individual segregants homozygous for critical deletions. Initially, approximately 1,522 individuals representing 254 families were screened, and tissue was taken for DNA extraction. DNA was extracted from 407 individuals. We have developed a series of ten PCR-driven, cDNA-based markers that cover intervals along the entire length of 4E. These markers are referred to as Cleaved Amplified Polymorphic Sequence (CAPS) markers, as they are based on differential cleavage of coding and intron sequences under treatment with restriction enzymes. The 407 samples included both non-regrowing lines and a random sample of regrowing lines. Plants that have retained *Thinopyrum* DNA were screened for the presence of the 4E-specific alleles of the ten CAPS markers. Those lines whose marker profiles indicated 4E deletions were subjected to analysis with 46 AFLP markers to determine the exact nature and location of the deletion events. Genomic *in situ* hybridization (GISH) was then used to visually ascertain the nature of the deletion. This cytogenetic data, coupled with data obtained from assignment of Amplified Fragment Length Polymorphism (AFLP) based markers provides an accurate picture of each chromosomal aberration. Translocations, as well as deletions, were recovered. Progeny of confirmed deletion lines were screened to determine their PSCR phenotype. Based on a comparison of short arm deletions found in non regrowing plants compared to deletions found within regrowing plants, we have identified the proximal quarter of the 4E short arm as being necessary for the PSCR phenotype.

A key question we have been attempting to solve is whether the centromeric region of 4ES is, by itself, sufficient for initiating PSCR. Our results have revealed a propensity for deletion events on the short arm rather than the long arm. This result coincides with the earlier statement that 4ES telosomes are difficult to isolate, most likely due to stability issues or genetic effects. Our isolation of translocations bearing large portions of the 4E short arm indicate that these regions are insufficient to initiate PSCR, but we are still unable to determine if this is because loci on 4EL are critical or because the PSCR gene is highly centromeric on 4ES.

The genetic system used to induce deletions of *Th. elongatum* chromosome 4E in disomic CS-*Th. elongatum* addition line 4E also induces wheat-*Th. elongatum* chromosome translocations as well as deletions of CS chromosomes. CS chromosome deletions and the translocations are reduced in the population by crossing the 4E deletion stocks to wild type CS. CS chromosomes with deletions and translocated chromosomes can then be replaced, by the normal corresponding CS chromosomes, through screening F₂ populations of the crosses using C-banding and GISH. This ensures that the PSCR phenotypes observed in our lines are not

caused by mutations in the CS genome unlinked to the 4E deletions, and will also help to stabilize and preserve these stocks.

Use of deletion mutants to integrate physical and genetic maps of *Th. elongatum* chromosome 4E.

The genus *Thinopyrum* continues to be an important source of disease and stress resistance for wheat improvement because of its crossability with wheat and genetic diversity, but little work has been done on genome organization in this genus. We have developed mapping populations by crossing amphiploids of closely related *Thinopyrum* species such as *Th. scirpeum* and *Th. elongatum*. The E genomes in such crosses are sufficiently related that meiotic pairing occurs in the presence of the wheat Ph locus.

We have identified polymorphisms between E genomes using PCR based markers previously mapped to chromosome homoeology group 4 of the Triticeae (Arterburn and Jones, submitted), and have developed 10 PCR based markers to known coding regions that span the Triticeae Group 4 consensus map. In addition, we have identified 46 new 4E -specific AFLP markers (Arterburn and Jones, submitted), 26 of which are polymorphic between our amphiploid parents and have been integrated into a genetic map of 4E. The mapping populations are being used to confirm the degree of synteny between *Th. elongatum* group 4 and group 4 of other members of the Triticeae. The positioning of CAPS markers on both the genetic and physical map of 4E agrees with existing genetic maps of barley chromosome 4H.

The deletion lines produced through this study have been analyzed with all 56 molecular markers to form a physical map. The bias for proximal, short arm deletions observed in this study may indicate that these areas are gene-rich; the concept of chromosome hotspots for aberration, recombination and gene density is well established. By using cDNAs and ESTs previously mapped in other species to define breakpoints in our deletion series, we have been able to assess the co-linearity of gene rich islands between *Thinopyrum spp.* and other more intensively studied members of the Triticeae. These gene rich islands where gene densities are in the order of one gene/ 5-20 kb, are interspersed with large regions of noncoding, repetitive DNA and appear to be preferential sites of both recombination and gametocidal induced chromosome breakage.

We also used the AFLP technique to genotype selected samples from advanced breeding populations. The goal of this analysis was to determine if selection criteria used were successful at generating haplotypes conferring increased competitiveness for specific geographic locations. The analysis revealed a number of unique sequence polymorphisms, indicating emerging genotypes.

Inter-Genomic recombination breeding.

We are also experimenting with the use of mutant lines deficient for the meiotic pairing regulator *Ph1*, to promote recombination between wheat lines and their wheatgrass relatives. The goal is a high level of inter-genomic recombination to transfer competitive traits from these perennial grasses. These techniques have been successful before in introgressing small targeted regions into wheat, usually via translocations or small interstitial recombination events. We are attempting an aggressive form of recombinatorial “mutagenesis” to introgress large amounts of alien chromatin which can be crossed between competitive lines and selected using marker assisted plant breeding techniques. We have initiated this experiment and are eager to see if this bold technique will be feasible.

Carbohydrate Allocation.

Total nonstructural carbohydrates (TNC) are thought to be an important energy source for plant maintenance. Among grass species, TNC levels have been found to differ significantly both in quantity and in allocation to specific storage organs (roots, rhizomes, and crown tissue).

Carbohydrate allocation to the crown of the plant may be under separate genetic control from the master-regulation of PSCR, and may contribute to the penetrance of the PSCR trait.

Spectrophotometry can be used to evaluate TNC's and serves as a common method to analyze the vigor of plant roots. This experiment is currently being replicated by postdoctoral research associate Jennifer Moran. We have completed one full cycle of growth for these lines and are currently analyzing the carbohydrate content of the crowns. A second replicate experiment is in the harvest stage and will also be analyzed for levels of crown carbohydrate, harvest index (ratio of grain yield to biomass). Results are expected this winter.

Publications and Presentations:

See project # 6194.