

Mapping Resistance to Pre-Harvest Sprouting

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Goal and Objectives:

The goal of this five-year project is to improve common wheat for resistance to pre-harvest sprouting (PHS) by incorporation of exotic seed dormancy genes from wild germplasms into modern varieties. Goat grass (*Aegilops tauschii* Coss., $2n = 2x = 14$, DD), the D genome donor of common wheat (*Triticum aestivum* L., $2n = 6x = 42$, AABBDD), has strong seed dormancy. We selected goat grass-derived synthetic hexaploid wheat ($2n = 6x = 42$, AABBDD) as the bridge to introduce the novel dormancy genes from the wild relative into wheat varieties.

Objectives include:

- 1) to identify gene donors for seed dormancy and develop intermediate breeding materials,
- 2) to map seed dormancy genes as quantitative trait loci (QTLs) and characterize the loci for linkage drag, epistasis, and genotype-by-environmental interactions,
- 3) to evaluate major dormancy genes or gene combinations for resistance to PHS; and
- 4) to introduce novel dormancy genes to elite winter and spring wheat breeding lines to develop varieties (especially white grain-colored varieties) resistant to PHS by marker-assisted selection.

We have screened four (DH1, DH2, DH3, and RL1) segregation populations of more than 600 lines for the seed dormancy trait in 2006 and confirmed the DH1 population as the best one based on the 2006 screening, in field, and greenhouse conditions in 2007. Our research in FY09 will be advanced to a genome-wide scan for dormancy QTLs based on the DH1 population.

In 2009, we will:

1. construct a framework genetic map based on the DH1 population using wheat microsatellite markers screened in FY08,
2. locate QTLs for seed dormancy on the genetic map and estimate their genetic effects, and
3. develop hybrids between dormant lines and the white seed coat-colored varieties of spring wheat to initiate the experiment of dormancy genes introgression in the following years.

Justification:

A majority of wheat varieties have an insufficient degree of seed dormancy to be resistant to PHS. Pre-harvest sprouting (germination on the panicle before harvest) causes a major loss of yield and a reduction of grain quality in wheat production in the US northern central plains area. Incorporation of a moderate degree of seed dormancy into wheat varieties is the most efficient strategy to reduce the PHS damage. To this end, we have identified candidate segregation population and screened wheat microsatellite markers polymorphic for the parental lines. It is the time to advance our research to the stage of detecting dormancy QTLs from the mapping population.

A framework genetic map is prerequisite for genome-wide scanning of QTLs controlling a complex trait such as seed dormancy or resistance to pre-harvest sprouting. We have identified 120 markers polymorphic between the two parental lines of the above-stated mapping population in FY08. Although these markers were selected based on their positions on the reference map, they may vary in linkage strength between mapping populations. Our population was developed using the goat grass-derived synthetic wheat as one of the parent. Mapping the

polymorphic markers based on our population should also provide information for us to improve the map quality, such as selection for or development of additional markers to fill potential gap(s).

Seed dormancy has been associated with the grain color in cultivated wheat, with red colored varieties more resistant to PHS than white colored ones. It remains unknown if the association arises from pleiotropic effects of the red color genes (*R1* to *R3*) on dormancy, or there are dormancy genes tightly linked to the *R* loci. So far, it is impossible to use the dormancy genes in the *R* gene regions to improve white grain-colored wheat varieties for resistance to PHS. QTL mapping is the most powerful strategy to identify dormancy QTLs independent of the *R* genes, ultimately to use the QTL alleles in breeding white grain-colored varieties for resistance to pre-harvest sprouting. Some of the dormant lines displayed strong seed dormancy in the past two years of field experiment. These lines are potential donor parents of dormancy genes. It is necessary to cross these candidate donors with white colored wheat lines to initiate our introgression of useful alleles once the major QTLs are mapped.

Materials and Methods

Marker genotyping and map construction: The mapping population of about 200 plants will be genotyped with markers using the method developed in the Primary Investigator's (PI) Seed Molecular Biology Lab. We will first use the 120 polymorphic markers identified in FY08 to develop a low-resolution genetic map using the MAPMAKER software. It is likely that these markers are insufficient to develop a framework map covering the 21 wheat chromosomes. Thus, we will collaborate with Dr. Chao in USDA-ARS Wheat Genomics Lab in Fargo, ND, to screen for an additional set of microsatellite markers to improve the map coverage.

Field experiment: We will grow the mapping population on the campus farm to repeat the phenotypic identification for seed dormancy. Three replications will be used in the field experiment. Plants will be recorded for flowering time, plant height, and other agronomic traits. Intact panicles and seeds from individual lines in each replication will be harvested at the time of physiological maturation and air-dried in the greenhouse for three days. Air-dried panicles and seeds threshed by hand will be stored in a cold room to maintain the dormancy status.

Germination experiment: The degree of seed dormancy will be evaluated by percentage of germinated seeds on panicles (panicle germination) and the threshed seeds (seed germination). Panicle germination will be done with wetted germination papers at 20°C and 100 percent relative humidity in dark condition for seven days. Five panicles will be counted for germinated seeds. Germination for threshed seeds will be done with nonafterripened (immediately removed from the cold room) seed samples and the samples of seeds partially afterripened [i.e., stored at the room temperature (about 25°C)] for one and two weeks, respectively. A sample of 50 seeds will be distributed in a 9-cm Petri dish lined with a Whatman #1 filter paper and watered with 5 ml of germination buffer (containing 0.12 percent fungicide) and then incubated at 20°C and 100 percent relative humidity in dark condition. The germination experiment will be replicated three times. Germination will be counted daily for a period of 10 days.

QTL analysis: Both the panicle and seed germination will be treated as different parameters for QTL analysis. The analysis will be conducted using the software QTL Cartography.

Hybridization: We introduced 16 dormant lines from four institutions and also identified strongly dormant lines from our populations in 2006 and 2007. These lines will be compared for degree of seed dormancy. The most dormant lines will be selected to cross with the four white grain-

colored lines of spring wheat 00s0264-4w, slw.comp.w, MTHW0471, and NDSW0612 to develop hybrid F₁ generation.

Progress Report

Field experiment: We propagated four segregation populations in the 2006 experiment. One (DH1) of the populations was identified to be an ideal candidate for QTL analysis, because it displayed the widest variation in seed dormancy as shown in our 2007 report. We planted the DH1 population of about 200 lines and 70 dormant lines selected from the remaining three (DH2, DH3 and RIL1) populations on the SDSU campus farm in 2007 to confirm their genetic variation in seed dormancy. These 270 lines were replicated three times in the field experiment. Individual lines, more than 800 ($270 \times 3 = 810$ plus parental lines) in total, were evaluated for seed dormancy with hand-threshed seeds and intact panicles. The seed germination was replicated three times and the panicle germination was conducted using five dried intact panicle from each line.

The seed germination confirmed that there is genetic variation in degree of dormancy in the DH1 population, but the population mean (79 percent) was higher than that (60 percent) in 2006. The panicle germination rate, which was measured by percentage of germinated seeds to the total seeds on a panicle, was much lower than the seed germination. About 100 lines displayed a panicle germination rate lower than 30 percent in the conditions (20°C and 100 percent relative humidity) favorable for seed germination.

These results suggest: 1) there was a large variation in dormancy with threshed seeds between years and therefore it is necessary to repeat the field experiments, 2) seed covering tissues (glumes and palea, and other structures) must contain some factors inhibiting germination and these inhibitors are likely derived from the wild wheat, and 3) germination with intact panicles could be a better method to evaluate degree of dormancy as compared with the germination with threshed seeds.



Images of panicles from two wheat lines showing the genetic difference in sprouting rate in the mapping population. Panicles were incubated at 20°C, 100 percent relative humidity in dark condition for 10 days.

Greenhouse experiment: We also planted the DH1 population of 160 lines in the greenhouse from winter 2006 to spring 2007. Seed germination for the greenhouse plants were poorly correlated with the germination for the field plants, with the correlation coefficients lower than 0.27. It is noticed that our plants in greenhouse (> 5 months) had a longer duration from sowing to maturation than those in the field conditions (~ 4 months). We will repeat the greenhouse experiment to find out key environmental factors influencing the phenotypic variation in seed dormancy in the next year.



Image of wheat plant grown in greenhouse showing a large variation in flowering time.

Phenotypic correlation between environmental conditions for seed dormancy measured by germination percentage (germination index) in the DH1 population.

	2006 field	2007 greenhouse	2007 field-seed
2007 greenhouse	0.2009*(0.2654*)		
2007 field (seed)	0.4992*(0.5031*)	-0.0155 _{ns} (0.1189 _{ns})	
2007 field (panicle)	0.4104*(0.4209*)	0.0159 _{ns} (0.0457 _{ns})	0.6549*(0.7282*)

Note: * or _{ns} indicate the correlation coefficient significant at the probability level of $P < 0.01$ or not significant.

Extraction of genomic DNA: Genomic DNAs were extracted from the mapping population of 213 lines. These DNA samples have been quantified and are stored in freezers for marker genotyping.

Screening for polymorphic markers: About 300 wheat microsatellite markers were screened for polymorphism between the dormant parent ND60 and the non-dormant parent ND495. About 120 polymorphic markers were obtained. We have confirmed 24 polymorphic markers with the plants selected from the mapping population and are working with the remaining polymorphic markers.

Introduction of gene resources for resistance to pre-harvest sprouting: We collected 16 varieties/lines from four institutions in 2007. These materials were introduced because they were reported to be resistant to pre-harvest sprouting. We are growing these varieties/lines in the greenhouse to increase seeds for the summer experiment. A majority of the lines are white grain colored and some of them (e.g., Cayuga, Clark's cream, and Totoumai A) were reported to have the QTLs with a relatively large effect on seed dormancy. These introduced materials will be compared with the lines selected from our populations for degree of seed dormancy in the local conditions.

Wheat varieties/lines introduced in 2007

Name	Seed coat color	Source
Clark's cream*	White	USDA National Small Grains Collection
SUNELG	White	USDA National Small Grains Collection
MW10	White	USDA National Small Grains Collection
MW16	White	USDA National Small Grains Collection
MW18	White	USDA National Small Grains Collection
MW28	White	USDA National Small Grains Collection
MW33	White	USDA National Small Grains Collection
NuSky	White	USDA National Small Grains Collection
Totoumai A*	White, landrace	Kansas State University
Rioblanco*	White, landrace	Kansas State University
SC8019-R1	White, improved	SPARC, Manitoba University, Canada
SC8021-V2	White, improved	SPARC, Manitoba University, Canada
RL4137*	Red, improved	SPARC, Manitoba University, Canada
Genesis	White	SPARC, Manitoba University, Canada
Sonora 64	Red	SPARC, Manitoba University, Canada
Cayuga*	White, improved	Cornell University