

THE ORVILLE A. VOGEL WHEAT RESEARCH FUND

PROGRESS REPORT

Project #: 6448

Title: A genomics approach to understand and manipulate genes controlling composition of wheat straw affecting ethanol production

Researchers: Kulvinder Gill, Bill Pan, and Scot Hulbert

Duration of the project: Three years (2007 – 08 to 2009 - 10)

Reporting Period: January 1 – December 31, 2008

Year Initiated: 2007-08

Terminating Year: 2009-2010

A. Goal and Objectives: The main goal of the proposed project is to identify, characterize and manipulate genes/QTLS controlling wheat straw composition specifically targeting characteristics that influence ethanol yield. Specific objectives are to:

- i: Using association mapping, study variation for the composition of wheat straw of cultivated wheats of the world and identify the composition that results in the highest ethanol yields,
2. Identify genes/QTLs that regulate straw composition especially that controls ethanol yields,
3. Develop user-friendly DNA markers for the genes/QTLs controlling ethanol yields from wheat straw.

ACCOMPLISHMENTS

1. Association mapping: *i. Software development:* The main emphasis this year was to get the association mapping part of the project up and running. Since association mapping is not a routine procedure in any plant system including wheat and no data analysis software for self-pollinated crops is available, we had to spend major effort to develop a user-friendly data analysis software. Working with Dr. Ananth Kalyanaraman, we held weekly discussion meetings among Ananth, his student, and members of my group to devise a good association mapping software. Now we have framework software in place but it will take another 6 months or so before we have a working software to try for actual data analysis. When developed, we expect this software to be the state of the art especially for wheat where no good software is currently available for association mapping. After developing a working software we plan to submit an NSF grant proposal to improve the software further to develop an ultramodern software ideal for association mapping studies in self pollinated crops.

ii. Field evaluation of association mapping population: We collected about 800 wheat cultivars to represent variation among cultivated wheat's of the world. These include 96 PNW varieties, 125 lines of historical wheat collection and 80 lines representing a CIMMYT collection from all major wheat growing regions of the world. The remaining lines are US wheat cultivars that were popular during the last 50 years. To avoid seed mixtures, single plant seed was obtained for each

line by first growing in a greenhouse. The single plant progeny seed was used to plant replicated field trials at the Spillman farm with the objective to estimate plant biomass. Many of the wheat cultivars did not do too well in the field probably because of adaptation to the different parts of the world, different disease packages and maturity times. Therefore, the field experiment was considered unfit for an accurate estimation of biomass. Therefore we decided to estimate shoot biomass in the greenhouse. To test the feasibility of greenhouse-based estimations, the wheat collection was grown in greenhouse using our recently optimized sand culture based method of estimating complex phenotypic traits in the greenhouse. Since the pests and maturity/height differences were not expected to be a factor in the greenhouse, visual evaluation suggested the experiment to be a success therefore two more replications of the spring part of the association mapping collection was grown again in the greenhouse. Single replication straw samples were collected for the entire collection and phenotypic data on plant characteristics were collected to evaluate the extent of variation present among the cultivars. Currently, the data on the two replications are being collected and analyzed.

iii. Straw cellulose/hemicellulose estimation for the association mapping population: For cellulose/hemicellulose estimations, straw from the main tiller about two inches above the soil level was collected and was dried at 40°C for two weeks. The straw was then placed in a pre-weighed two ml screw cap tube. Cellulose/hemicellulose estimations were obtained using the method by Updegraff, D. M. (1969) (Semimicro determination of cellulose in biological materials. Anal. Biochem. 32, 120-124).

The cellulose percentage ranged from 24% to 52% of the dried straw and the range was way more than what was expected. Although detailed analysis of the data is currently underway but the initial indication is that no significant correlations was observed between the cellulose content and wheat market classes or type of wheat. It appears that wheat cultivars with higher cellulose content are present in all market classes and in different regions of the world. As a next step, ten cultivars with the highest cellulose content and ten with the lowest estimates, will be reevaluated for the cellulose, lignin and other straw characteristics to confirm our results, establish any correlations between cellulose and lignin contents, and to study its effect on straw characteristics. Also, the two lines from the contrasting extremes of the cellulose estimates will be used as parents to generate a doubled haploid population to launch a QTL study with an objective to identify genes controlling straw cellulose content. Genes known to influence straw composition (cellulose synthases, for example) will also be used as markers for the QTL study. A pre-proposal will be submitted to the USDA/DOE feedstock genomics grants program (due date Dec. 5, 09). A proposal was submitted last year also to the same program but it was not selected with the main comment of a lack of sufficient preliminary data. Hopefully, the pre-proposal will fair better this year, as additional preliminary data is now available.

2. Phenotypic evaluation under controlled conditions: With the objective to optimize a reliable and reproducible method to measure biomass and other agronomic traits under controlled conditions, cultivar Wichita (WI), Cheyenne (CNN), and chromosome 3A substitution lines WI(CNN3A) and CNN(WI3A) were grown in hydroponics, sand culture and normal greenhouse conditions. Root and shoot biomass and grain yield traits were measured over 10 randomized plants. Biomass estimates were very reliable under hydroponics conditions with less than 8% CV however plants took very long time to mature and it became increasingly

difficult to maintain the required pH especially around the flowering state. Estimates under sand culture were equally reliable when watered with Hoagland's solution as the CV was about the same as that with hydroponics. With normal greenhouse conditions however, the CV was slightly higher (9.5%) than that for sand culture. Therefore, sand culture was selected as the method of choice considering reliability and ease.

3. **DarT marker analysis:** In order to increase marker density of our chromosome 3A map, we had 96 chromosome 3A RICLS along with their parents, analyzed by DarT markers (<http://www.triticarte.com.au/>). The Diversity Arrays Technology (DArT®) uses an array of individualized fragments of genomic representations of wheat and barley, prepared from pools of genotypes that cover the genetic diversity of each species. Representations of varieties to be genotyped are labeled and hybridized to the array. The polymorphisms scored are the presence versus absence of hybridization to individual array elements. The detailed data analysis using the DarT markers is currently underway but we have already identified 12 new markers for chromosome 3A of wheat (Figure 3, markers with 'Pt' numbers). The QTL analysis using the newly developed map is currently underway and its current version is given in figure 2.

4. Genes/QTLs controlling root and shoot biomass: Chromosome 3A of wheat is known to harbor many hormone signaling genes and grain yield related traits, therefore we tested if any of the genes controlling plant biomass are present on chromosome 3A. Ten randomized plants of each of the chromosome 3A substitution lines involving WI and CNN along with the parents were grown in sand culture. To ensure uniformity, weighted amount of sand was used for each pot and measured amount of Hoagland's solution was added to each pot at a regular interval. At maturity, plants were uniformly dried and both root and shoot biomass was measured. Both root as well as shoot biomass in CNN(WI3A) was significantly higher than CNN (Figure 1). Similar estimates for WI(3ACNN) were significantly lower than WI. These results suggested that chromosome 3A of WI carry gene(s) controlling plant biomass in wheat.

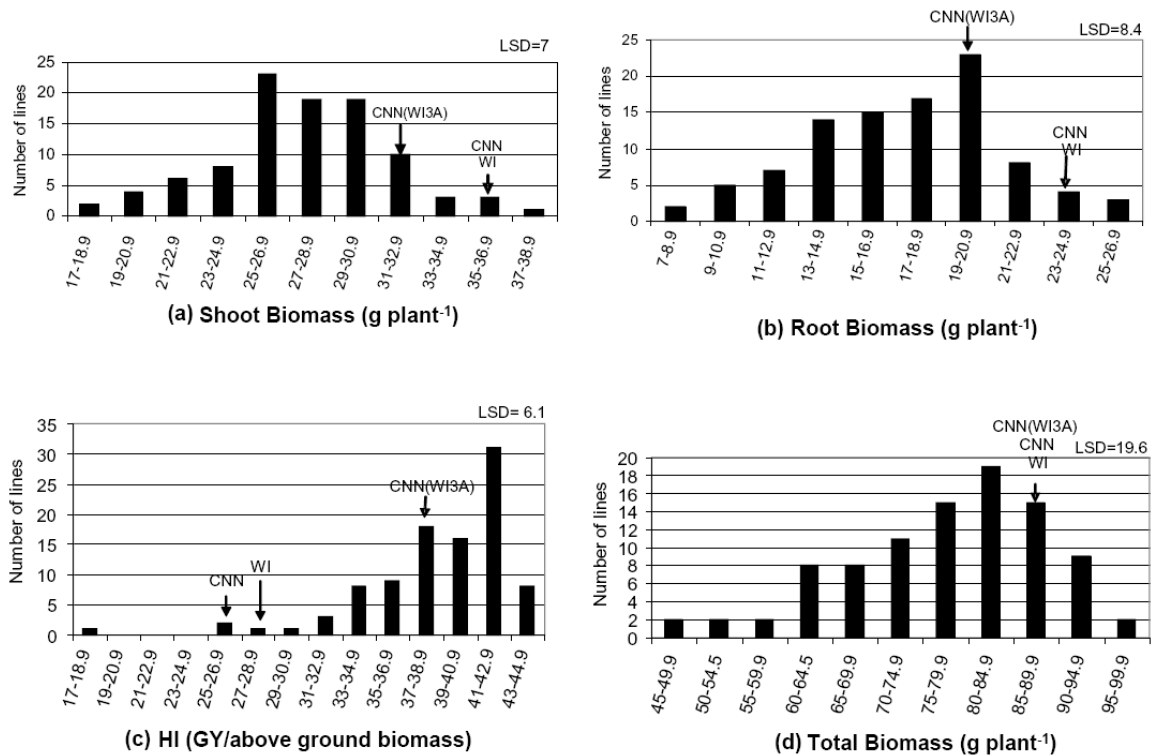


Figure 1. Frequency distribution of nonnormalized data of biomass related traits in the WI x CNN (WI3A) RICLs population.

To identify genes/QTLs influencing root and shoot biomass we utilized a set of 95 recombinant inbred chromosomal lines (RICLs) derived from a cross between CNN(WI3A) × CNN; phenotyped them in greenhouse (for dry root biomass and dry shoot biomass) and genotyped them using 41 molecular markers (including RFLPs and SSRs). This has led us with the identification of three major QTLs (all QTLs were detected at >3.0 LOD), one for shoot biomass in the proximal region of 3AL (*Xcdo1523*) and two for root biomass one each on 3AS (*Xgwm218*) and 3AL (*Xmwig802*) (see Figure 2). We are currently utilizing rice-wheat synteny to increase number of markers in these regions and to identify potential candidate genes for these traits.

QTL analyses conducted for root/shoot biomass and a number of other agronomically important traits revealed QTLs for plant height, grain yield and grain weight coinciding with the QTL for root biomass on 3AS. The above observation can be explained as pleiotropic effect of a single gene/QTL or as a cluster of QTLs influencing correlated traits (see Figures 2 and 3).

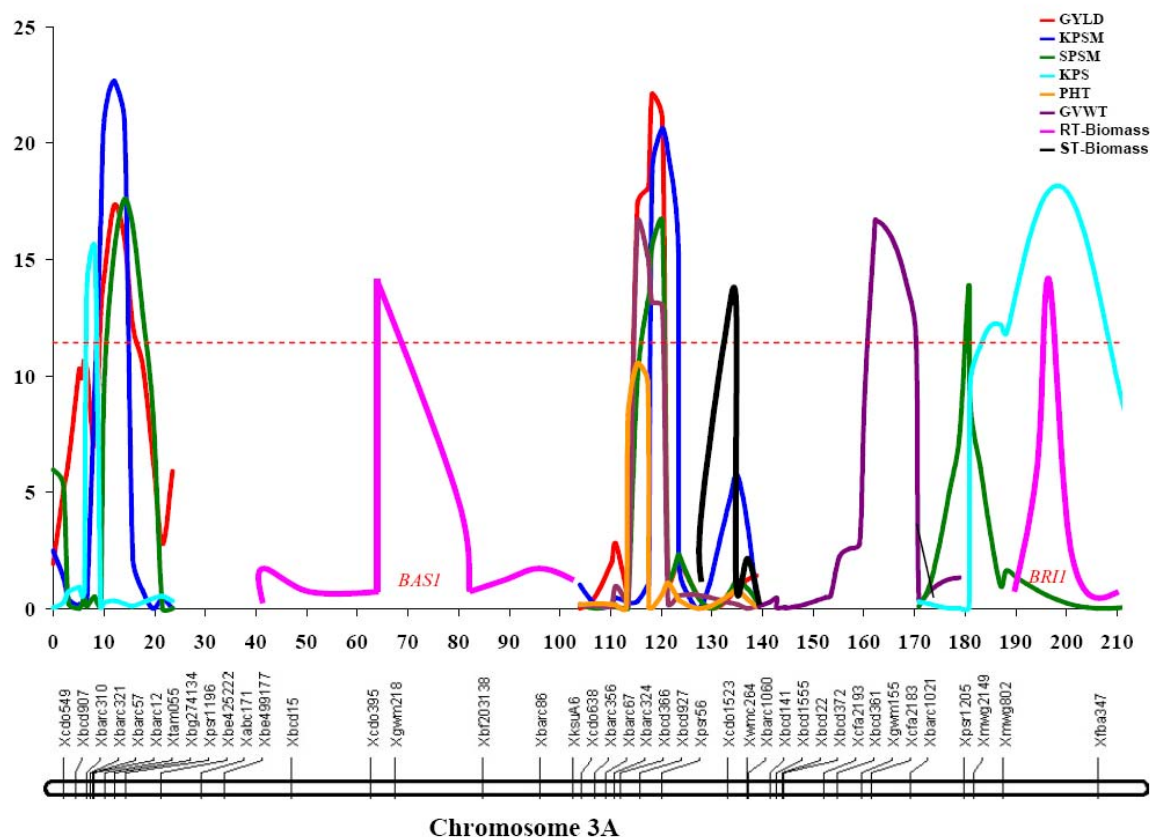


Figure 2. QTL Cartographer plots for chromosomes 3A obtained following composite interval mapping (CIM) for a number of agronomically important traits including grain yield (GYLD), kernels/square meter (KPSM), spikes/square meter (SPSM), kernels/spike (KPS), plant height (PHT), grain volume weight (GVWT), root (RT-biomass), and shoot (ST-biomass) biomass; marker designations and genetic distances (cM) are given below the horizontal line (data recorded over 95 RICLs; candidate genes for biomass QTLs are indicated in red – both of these candidate genes are known to be involved in brassinosteroid signaling/metabolism).

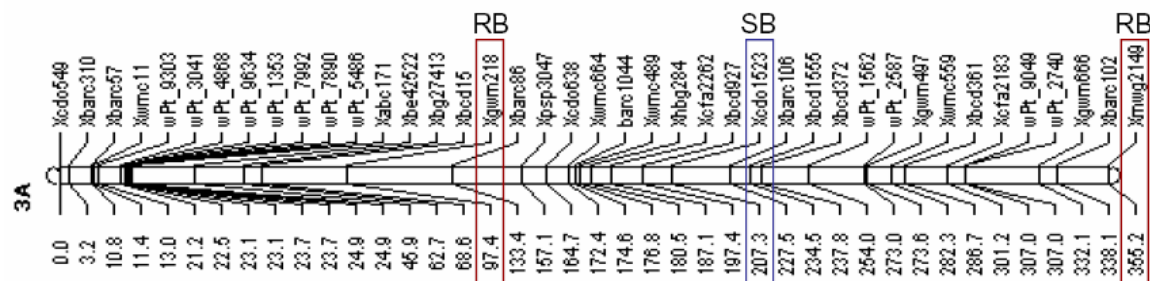


Figure 3. Schematic representation of three major QTLs detected for root and shoot biomass on a very high-density (total 81 markers and 357.8 cM) genetic linkage map prepared recently for chromosome 3A using RFLP, SSR and DArT markers. **SB = shoot biomass and RB = root biomass.**

Publications:

Ali, M., J. Rajewski, P. S. Baenziger, K. S. Gill, K. M. Eskridge and I. Dweikat. 2008. Assessment of genetic diversity and relationship among a collection of US germplasm by SSR markers. **Molecular Breeding** (Accepted).

Dhungana P, KM Eskridge, PS Baenziger, BT Campbell, KS Gill, and I Dweikat 2007 Analysis of gene-by-environment interaction in wheat using a structural equational model and chromosome substitution lines. *Crop Science* 47: 477-484.

Shafqat, Mustafa and Kulvinder S. Gill 200- . Accurate estimation and mapping of root and shoot biomass, and other agronomic traits under controlled conditions of hydroponics and sand culture. *Plant Physiology* (submitted).

Randhawa, HS, Mutti, JS, and Gill, KS. 2008. Optimizing marker-assisted background selection for rapid introgression of desirable genes. Proceedings of the 11th International Wheat Genetics Symposium, Brisbane 24-29 August 2008 / edited by Rudi Appels, Russell Eastwood, Evans Lagudah, Peter Langridge, Michael Mackay, Lynne McIntyre, and Peter Sharp. Sydney, Sydney University Press, 2008. 4 pages.

Morris, CF, Li, S. Bettge, AD, King, GE, Garland-Campbell, K., Gill, KS. 2008. Arabinoxylan content of hard winter and spring wheat's of the US pacific northwest. In: Proceedings of the 11th International Wheat Genetics Symposium, Brisbane 24-29 August 2008 / edited by Rudi Appels, Russell Eastwood, Evans Lagudah, Peter Langridge, Michael Mackay, Lynne McIntyre, and Peter Sharp. Sydney, Sydney University Press, 2008. 3 pages.

Grant proposals submitted:

1. Gill, KS and C. Steber. 2007. Functional analysis of wheat *DELLA* protein genes to study their role in regulating preharvest sprouting, height, emergence, and grain number and weight. USDA-NRI.

2. Gill, KS, et al. 2007. Cultivate strong interaction between Punjab Agricultural University, Ludhiana, India and Washington State University to facilitate cellulosic ethanol production from plant residue. USAID-AKI knowledge initiative.

3. Kulvinder S Gill, Kanwarpal Dhugga, Renata Bura. 2007. A genomics approach to understand and manipulate genes controlling composition of wheat straw affecting ethanol production. USDA-DOE feedstock genomics grant program.

4. Gill, Kulvinder 2008. Genes controlling cellulose and lignin content of wheat straw and their characterization using various genomics approaches. USDA-DOE feedstock genomics grant program (Due Dec 5, 2008).