Project Report
Nebraska Wheat Board

Project Title: Establishing a pipeline for the evaluation of novel input and output traits in wheat from the tools of biotechnology

Reporting Period: June 2009 through June 2010

Principal Investigator: Tom Clemente (Dept of Agronomy & Horticulture/Center for Plant Science Innovation)

Progress towards meeting the objectives

Objective 1: Strengthen the capacity of UNL's Plant Biotechnology Field Facility to allow for handling of regulated wheat seed under strict identity preservation.

We purchased a trailer to allow us to move dedicated equipment between Lincoln, and North Platte. While we did not conduct a transgenic wheat field trial this year at North Platte, we did run some plantings of control wheat at the site we will use in 2011. We are working with Greg Kruger, who will assist in the oversight of the North Platte location.

Objective 2: Evaluate transgenic wheat events expressing the bacterial *SacB* gene

We conducted a third field trial in 2010, with material we identified to be homozygous lineages carry the *SacB* gene. Expression of this gene in wheat should alter fructan composition of the grain. It appears that with the current construct expression of the gene was very low. We have gone back, and remade the construct altering the promoter element size. Wheat transformations with this new *SacB* construct have been initiated.

Objective 3: Develop and characterize transgenic wheat events expressing the maize ramose 1 (Ra 1) gene.

The goal of this study is to evaluate expression of the Ra 1 gene, a global regulator of gene expression that is involved in the orderly array of seeds on a corn cob. The goal here is to monitor impact on wheat head architecture upon expression of Ra 1 under control of its native promoter from corn. A set of 15 independent transgenic wheat events have been generated that carry the Ra 1 gene, along with a set of 8 transgenic wheat events that carry the same promoter, but with the visual marker gene GUS, as a set of controls. Progeny from these respective transgenic wheat events are currently growing in the greenhouse, we will be conducting phenotypic and molecular characterization of these biologicals over the next few months.

Objective 4: Characterize transgenic wheat events expressing defensin constructs

The goal of this component of the program is to monitor for enhanced tolerance to biotic stress, namely fungi. T2 populations have been generated from these transgenic events. We will ship the seed to the Donald Danforth Center in St. Louis for evaluations.

Objective 5: Characterization of transgenic wheat events expressing the maize EF-Tu gene for tolerance to both heat and drought.

The maize EF-Tu gene was initially characterized in a collaborative effort with researchers from South Dakota, Nebraska and Pioneer. This gene has been demonstrated under growth chamber conditions to enhance heat tolerance in wheat. We conducted a small scale field release with this material in 2010. We have sufficient seed to begin larger field trials in 2011, that should allow us to gain insight on potential agronomics attributes of this transgene (note: currently in a spring wheat background).

Objective 6: Strategies for improving nitrogen use efficiency in wheat

We have assembled a series of plant expression constructs for wheat with a series of genes previously reported to play a role in nitrogen metabolism in plants. These genes are, the rice glutamine synthetase (OsGS1), the rice glutamate synthase (OsGOGAT), the barley alanine amino transferase (HvALA-AT), and the maize Dof1 transcription factor. Wheat transformations with these various genetic constructs are complete, with the exception of the OsGOGAT vector. This reason for the delay in the GOGAT transformations was it was very difficult for us to get the vector assembled. Nonetheless, we have in hand over 90 transgenic wheat events derived from transformations with these genes. A new Ph.D. student joined the laboratory this past September (2010), her project will be to characterized these events, and select lead events per gene, and begin monitoring for nitrogen use efficiency first under greenhouse conditions, then follow up in the field. Moreover, we will design crossing strategies to create gene stacks of the various genes, to allow us to gain insight on the potential additive effect of various gene combinations on N use.