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

Project Duration: 1 July 2011 to 30 June 2012

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Reporting Period: May 2011 through July 2011

I. Statement of project objectives and quantifiable progress towards objectives achieved this reporting period

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

One of the criteria that needs to taken under consideration when selecting a testing site for transgenic wheat and other commodities is distance from crops destined to enter the marketplace. We are looking for a location that will maintain a minimum of 0.5 miles. While this buffer exceeds Federal guidelines for testing of transgenic regulated wheat, we feel maintaining an increased buffer zone will aid in our goal of insuring identity preservation of the regulated seed. Given this target buffer zone, establishing a second site at West Central Research and Extension Center presented too many challenges. Following a series of discussions we have decided to carve out a 10-15 acre site in Scottsbluff. We are hoping to begin preparing a site this Fall.

2- Evaluate transgenic wheat events expressing the bacterial *SacB* gene

The goal of this component of the program is to attempt to produce the prebiotic fructan levan in the grain. We re-engineered the construct, however, expression of the *SacB* gene is still rather low. Nonetheless, we are bulking seed from four transgenic events under greenhouse conditions and will monitor for changes in fructan levels in the grain.

3- Develop and characterize transgenic wheat events expressing the maize ramosa 1 (Ra 1) gene

This component of the program is designed to introduce a "global regulator" of gene expression from maize into wheat. In maize this gene is activated during ear formation and governs of the orderly arrangement of kernels on the ear. If this gene mutated in corn the ear is drastically altered in shape and the kernels are arranged in a non-uniform fashion. The goal of this component is to monitor the impact of expression of this maize gene in wheat, as a means to alter the plant's architecture. To this end the hypothesis we are testing is does altering the architecture of wheat influence overall yield?

As communicated earlier strong expression throughout wheat development of the Ra1 gene imparts a rather interesting phenotype, the plants produce more vegetative tissue, and the heads are crowded about the base. However, the heads are only partially filled. We set-up a greenhouse experiment to test if application of the growth regulator GA3 would mitigate the seed filling issue, due to the published work on the impact of GA3 on the signaling relation to Ra1 function in maize. However, the results were the same, regardless of GA3 application. We then carried out additional wheat transformations and swapped out the strong promoter controlling Ra1 gene expression with its native promoter from corn. Under greenhouse conditions we could not observe a change in growth and development of the transgenic wheat plants. We carried out a field release of the transgenic events at our Plant Biotechnology Field Facility at ARDC. The only difference observed was the transgenic events appeared to head earlier than the controls. We will be harvesting the plots by the end of this week, and we will communicate the data in our next report. We also sent seed derived from these transgenic wheat events carrying the Ra1 gene to Iowa State University to the lab that originally characterized the Ra1 gene from maize. They are currently running some deeper characterizations on the transgenic wheat events.

4- Characterization of transgenic wheat events expressing the maize EF-Tu gene for tolerance to both heat and drought

As communicated in our earlier reports we have produced transgenic wheat events that express a corn gene designated EF-Tu. We demonstrated that under growth chamber conditions expression of this gene imparts enhanced tolerance to heat stress. To investigate if this observation will translate to the field we carried out a field release in 2011. The plots will be harvested within the next week and will communicate to the board the results of this field trial in the next report.

5- Strategies for improving nitrogen use efficiency in wheat

As communicated in our earlier report we assembled a set of genetic constructs that carry genes known to be involved with nitrogen metabolism in plants. These include the rice glutamine synthetase (OsGS1), rice glutamate synthase (OsGOGAT), the barley alanine aminotransferase (HvAla-AT), and maize Dof1 transcription (ZmDof1). Wheat transformations have been completed. Three transgenic events from each of the respective constructs are being phenotyped under greenhouse conditions using a full nitrogen treatment, or stress nitrogen treatment. Data is being tabulated on biomass, seed weight, nitrogen level in leaves, and enzyme changes in on proteins involved in nitrogen metabolism. Moreover, we are creating gene stacks with the various transgenes, which will allow us to monitor potential additive effects of the transgenes under consideration in this study. We have completed the first round of study with two of the genes OsGS1, and HvALA-AT. A second experiment is underway with transgenic events derived from ZmDof1 and another construct carrying the HvALA-AT. We will communicate the results of these first two rounds of experiments in our next report. In addition, we continue to prepare plots at our Plant Biotechnology Field Facility whereby we are

planting maize as a means to deplete the soil for N, in expectation of small scale field trials in 2012.

II. Activities planned between now and the next reporting period

We will report to the board the status on building the second site for UNL's Plant Biotechnology Field Facility, currently under consideration to be established in Scottsbluff.

We will characterize T4 generation of wheat seed carrying the latest version of the sacB gene grown under greenhouse conditions for changes in fructan levels.

We will gather data on the seed harvested from our small scale field release of transgenic wheat carrying the maize Ra1 gene, and tabulate the data gathered on the field trial with the transgenic wheat events carrying the putative heat tolerance gene EF-Tu.

We will continue to phenotype the selected transgenic wheat events carrying the respective NUE genetic elements under greenhouse conditions, and tabulate the results we have to date and report these findings to the Board in our next report.

III. Problems, obstacles, new developments for the market/research changes that impact or may impact completion, data, cost or scope of the project

None

IV. Message, questions, comments or requests

I am not sure if the Board wants to have faculty present at their meetings. I would be more than happy to attend to address any questions or concerns the Board has on the program.