Field Performance of Drought Tolerant Spring Wheat Transformed with the *mtlD* Gene

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Abstract

Drought is one of the major abiotic factors affecting plant growth and crop production around the world. Consequently the need to develop new varieties that are more drought tolerant is of great importance. Previous studies have shown that the accumulation of mannitol can help alleviate the effects of moisture stress by acting as a osmoprotectant and/or antioxidant. Transgenic wheat lines with the mannitol 1-phosphate dehydrogenase (*mtlD*) gene derived from *Escherichia coli* accumulated mannitol and had previously showed drought tolerance under greenhouse conditions. We conducted a two-year field trial under moderate moisture stress and well watered conditions in Colorado. Results from both years showed no significant improvement of the transgenic lines over the parental cultivar under either treatment. However, two lines were nearly equal to the parent and were not further research.

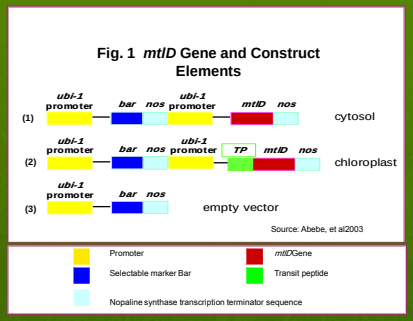
Objectives

- To evaluate *mtlD* transgenic spring wheat for drought tolerance in field trials.
- To compare lines in which the *mtlD* protein accumulate in the cytosol and chloroplast

Materials and Methods

- The *mtlD* gene was introduced into immature embryos of the spring wheat 'Bobwhite' by microprojectile bombardment at Oklahoma State University
- Three construct were used in the transformations and shown below:

Fig. 1 *mtlD* Gene and Construct Elements



- The empty vector (3) was used as a positive control and only contains the bar gene.
- The main difference in gene construct (2) is the transit peptide sequence that directs the transport of mannitol 1-phosphate dehyhrogenase to the chloroplast so mannitol can accumulate in this organelle
- The 7 transgenic lines, parent and checks evaluated were:

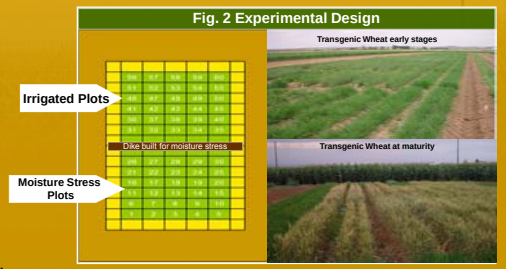
Table 1 Lines evaluated in Field Trials

Cytosol Lines	Chloroplast Lines	Empty Vector	Parent	Checks
pTA2-109 pTA2-110 pTA2-115 pTA2-118	pTA5-104 pTA5-105	pAH20	Bobwhite	RTO1-12 Reeder

Field Work and Experimental Design

- Field evaluations were conducted in Fort Collins, Colorado in 2006 and 2007 to assess the performance of the *mtlD* transgenic wheat under stress moisture conditions.
- In each year of the study, two adjacent trials were planted. One for full irrigation and the other for imposing moisture stress. (Fig.2)
- Each trial was randomized complete block design with three replications and 10 entries. The plots consisted of four rows planted on two raised beds of about 4.88 m long by 1.52 m wide. (Fig.2)
- All plots were furrow irrigated until anthesis. Then, no additional water was given to the stress trial, while the well watered trial continued to be irrigated at regular intervals.

Fig. 2 Experimental Design

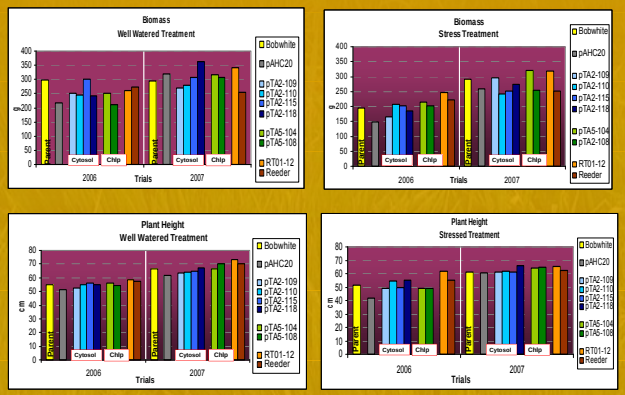


Results

Table 2 P-values for effects of entries under stress and well watered treatments and entry by treatment interaction

	2006			2007		
	Grain Weight	Biomass	Plant Height	Grain Weight	Biomass	Plant Height
Entry (Stress)	< 0.001	0.049	0.007	< 0.001	N.S	N.S
Entry (Well Watered)	< 0.001	N.S	N.S	< 0.001	N.S	< 0.001
Entry * Treatment	N.S	N.S	N.S	N.S	N.S	N.S

Fig. 3 Selected Agronomical Traits Measured in the Field in 2006 & 2007



Results (cont.)

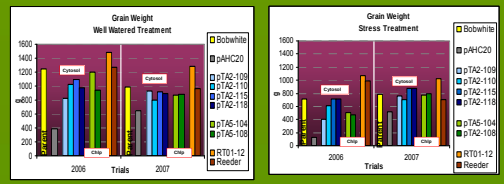
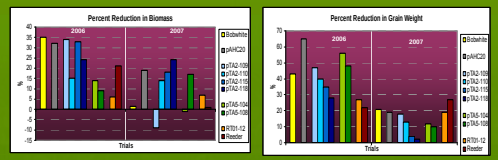


Fig. 4 Reduction Biomass and Grain Weight Between Well Watered and Stress Treatments



Discussion and Conclusions

- Moderate stress levels were observed, with a grain weight reduction of 39% in 2006 and 15% in 2007.
- In general the transgenic lines did not differ significantly from the parental line 'Bobwhite' for any of the traits
- Among the transgenic lines pTA2-115 and pTA2-118 were the best performers. In 2007, their grain weight under stress conditions was reduced only 4 and 2% respectively from the well watered trial, compared to a reduction of 21% for Bobwhite.
- The two chloroplast lines pTA5-104 and pTA5-108 performed relatively better for biomass, especially in the more stressful year, 2006.
- Based on the Colorado results pTA2-115 and pTA2-118 were not further research to understand the improved performance.
- These results will be integrated together with field results from North Dakota and physiological data from Oklahoma to determine the future use of these lines.

REFERENCES:

Abebe, T., A.C. Guenzi, B. Martin, and J.C. Cushman. 2003. Tolerance of mannitol-accumulating transgenic wheat to water stress and salinity. Plant Physiol. 131:1748-1755.

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