1) Title: Analysis and field-testing of multi-stress tolerant and water-use efficient cotton

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3) Summary

Drought, heat, and salt are major environmental factors that limit cotton production in Texas High Plains, which are also the major reasons for the quick depletion of the Ogallala Aquifer. The predicted increase in temperature from climate change will likely make cotton production in this region even more challenging. To sustain cotton production in Texas High Plains, to reduce cotton yield loss caused by drought, heat, and salinity, to elongate the life of Ogallala Aquifer, we must develop drought-, heat-, and salt-tolerant cotton varieties. One way to increase drought-, heat-, and salt-tolerance is to overexpress the rice E3 ligase gene, *OsSIZ1*, that plays important roles in abiotic stress tolerance in plants. Overexpression of *OsSIZ1* dramatically improved drought and heat tolerance in creeping bentagrass and in Arabidopsis. Our preliminary data with *OsSIZ1*-transgenic cotton indicated that overexpression of *OsSIZ1* in cotton significantly increased fiber yields in both laboratory and field conditions. Here we propose to continue analyzing *OsSIZ1*-overexpressing cotton in greenhouse and field conditions. Detailed physiological analysis and field study of *OsSIZ1*-transgenic cotton will yield information on how much water can be saved in the production of cotton fiber for the same yield or even higher yield or how much penalty can be avoided for cotton grown in Texas High Plains during extremely dry and hot seasons. It is predicted that *OsSIZ1*-transgenic cotton will revolutionize cotton production in Texas High Plains, and this project will have a major positive impact on cotton industry in the US and substantially reduce the use of water for cotton irrigation.

4) Project narrative

a) Objectives. Our goal in this project is to develop a multi-stress tolerant cotton variety that will use much less water, yet still maintain high yield, and that will help reduce water withdrawals from Ogallala Aquifer for irrigation by more than 20% in 2020 compared to 2012. The cotton variety developed will enhance the economic viability of the agriculture industry and the vitality of the Southern Ogallala Aquifer Region.

b) Rationale. It was reported that overexpression of the rice E3 ligase gene, *OsSIZ1,* in creeping bentgrass dramatically enhanced tolerance to drought and heat stresses (Li et al., 2013). We also overexpressed *OsSIZ1* in Arabidopsis and found that *OsSIZ1*-transgenic Arabidopsis plants were very salt tolerant (data not shown). These works indicate that *OsSIZ1* is a positive regulator in plant response to abiotic stresses. It is very likely that overexpression of *OsSIZ1* in cotton will increase cotton's drought-, heat-, and salt-tolerance. With the support from OAP in 2013, we introduced *OsSIZ1* into cotton and our preliminary data indicate that *OsSIZ1*-transgenic cotton plants are indeed significantly more stress tolerant than wild-type cotton plants. Achieving greater yield per unit rainfall is one of the most important challenges in dryland and low irrigation agriculture. Enhancing transpiration efficiency or water use efficiency (WUE), the dry biomass produced per unit water transpired may be an important mean of improving yield in semi-arid and arid regions when this improvement is tied to yield. We analyzed *OsSIZ1*-transgenic cotton and wild-type cotton for water use efficiency in greenhouse using a modified method described by Xin et al (2008). Our data indicate that *OsSIZ1*-transgenic plants have higher water use efficiency compared to wild-type plants and took longer time to reach permanent wilt (Fig. 1). The water use efficiency experiments suggested that *OsSIZ1*-transgenic plants should be more drought tolerant. We therefore conducted water-deficit experiments in greenhouse. Our experiments indicated that under normal irrigation conditions, there were no differences in photosynthetic rates, boll numbers and fiber yields between control plants and *OsSIZ1*-transgenic plants (data not shown). However, under reduced irrigation conditions, *OsSIZ1*-transgenic cotton plants maintained 47.5% higher photosynthetic rates, 42.3% higher boll numbers and fiber yields than control plants (data not shown). In 2014, we conducted a small scale field experiment at USDA-ARS Experimental Farm in Lubbock, and our data indicated that *OsSIZ1*-transgenic cotton plants maintained 37.6% higher photosynthetic rates at noon times of July (Fig. 2A), 41.7% higher boll numbers (Fig. 2B) and 38.8% higher fiber yields (Fig. 2C) than control plants at the end of growth season. Because our preliminary data are so promising, we hereby propose to continue analyzing the *OsSIZ1*-transgenic cotton plants under normal, drought stress, heat stress, salt stress, and combined stress conditions and to confirm our preliminary data that *OsSIZ1*-overexpression in cotton substantially improves fiber production in Texas High Plains.

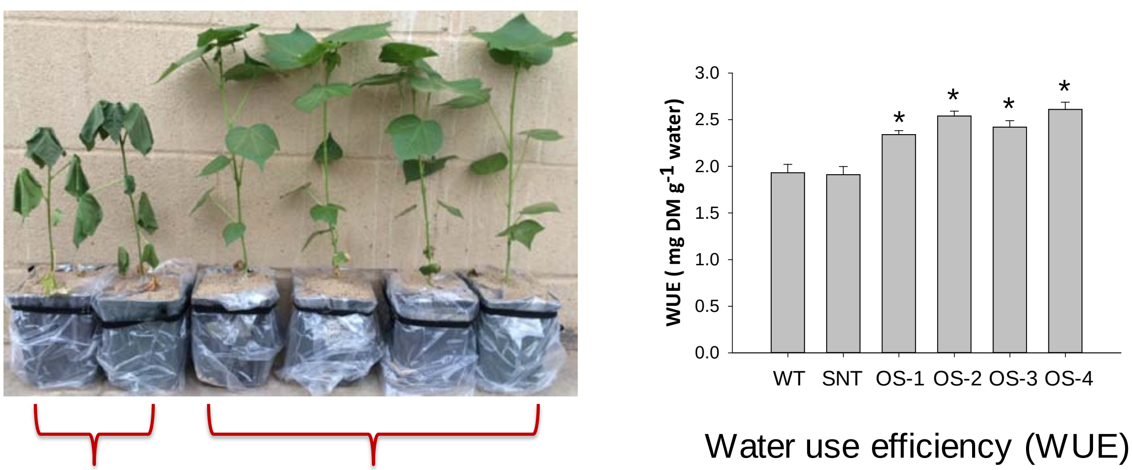

Fig. 1. Phenotypes of wild-type and *OsSIZ1*-transgenic plants 30 days after sowing into saturated soil. Pots were sealed with gas-permeable plastic that is impermeable to water forcing all water to be moved through the transpiration stream. Following permanent wilt, above-ground biomass was measured and final gravimetric pot weights were taken to determine transpiration efficiency or water use efficiency (WUE). N = 30 plants.

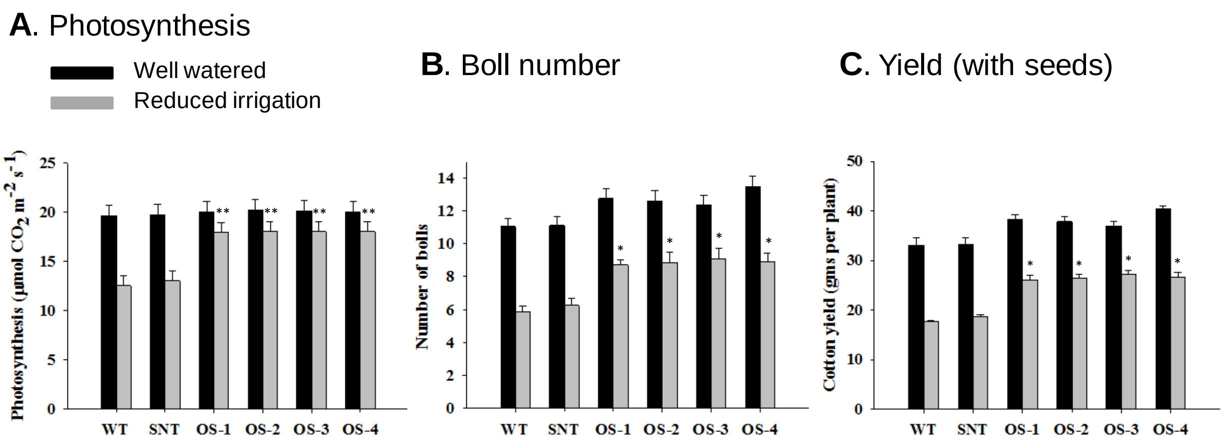

Fig. 2. Photosynthetic performances, boll numbers and fiber yields of wild-type and *OsSIZ1*-transgenic cotton plants under field conditions in 2014. Plants were planted into soil that was well watered, then control plants received 6 inches of water weekly, whereas plants in the reduced irrigation group received only 3 inches of water weekly. N = 30 plants. * and **, significant at 5% and 1%, respectively, based on Student *t*-test.

c) How the objective will be met

We have created 41 independent transgenic cotton plants that express the *OsSIZ1* gene, from which we obtained 31 independent homozygous lines and selected 5 high-expression lines for physiological experiments and field-trial experiments. With the new fund from OAP, we plan to study the *OsSIZ1*-transgenic cotton plants by analyzing how these transgenic cotton plants will respond to drought, heat, salt and combined stresses in greenhouse and growth chambers, and we will conduct large scale field experiments at USDA-ARS Experimental Farm in Lubbock, Texas, using 3 different irrigation conditions: no irrigation (dryland), low irrigation (1/3 to 1/2 of normal irrigation) and normal irrigation (full irrigation). Fiber yields and fiber qualities will be analyzed at the end of experiments, as we have done with other transgenic cotton plants that we created and analyzed before (Yan et al., 2004; He et al., 2005; He et al., 2007; Pasapula et al., 2011; Kuppu et al., 2013; Shen et al., 2015).

d) Expected outcomes including publications, economic assessments and technology transfer activities.

Publications: We will publish two papers from this research, one from analyzing *OsSIZ1*-transgenic cotton plants and one from analyzing the fiber qualities of *OsSIZ1*-transgenic cotton plants.

Economic Assessments: Our goal in this project is to create cotton that can use water more efficiently and increase fiber production in the semi-arid land of Texas High Plains. Cotton industry contributes 4 billion dollars to Texas economy each year. Yet cotton industry in Texas is threatened by the lack of water and the depletion of the Ogallala Aquifer. If cotton is made more drought-, heat-, and salt-tolerant, the cotton yield can be maintained and less water will be used to irrigate cotton, therefore the life of the Ogallala Aquifer will be longer. The economic impact of the drought-, heat-, and salt-tolerant cotton developed in this project will be assessed by comparing the yields produced by these transgenic cotton plants to the non-transgenic cotton in dryland and irrigated conditions, and by how much water that can be saved by using these transgenic plants each year.

Technology Transfer: Transgenic cotton plants developed from this project will be released to cotton industry after extensive testing in laboratory, greenhouse, and field conditions and with the approval from federal regulatory agencies.

5) Relevant publications by PI and Co-PIs

Shen, G., Wei, J., Qiu, X., Kuppu, S., Qin, H., Hu, R., **Auld, D**., Blumwald, E., Gaxiola, R., **Payton, P**., and **Zhang, H**. (2015). Co-overexpression of *AVP1* and *AtNHX1* in cotton further improves drought- and salt-tolerance in transgenic cotton plants. *Plant Mol. Biol. Rep. 33, 167-177.*

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Li, Z., Hu, Q., Zhou, M., Vandenbrink, J., Li, D., Menchyk, N., Reighard, S., Norris, A., Liu, H., Sun, D., and Luo, H. (2013). Heterologous expression of OsSIZ1, a rice SUMO E3 ligase, enhances broad abiotic stress tolerance in transgenic creeping bentgrass. *Plant Biotech J*. 11, 432-445.

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