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Genetic engineering of turfgrass for enhanced multi-stress resistance

Hong Luo
Department of Genetics and Biochemistry, Clemson University

Objectives:

The major objective of this research is to genetically engineer enhanced tolerance to various adverse environmental conditions, such as drought, salt, heat and nutrient deficiency in turfgrass plants using transgenic technologies. We proposed to develop methodology to evaluate and demonstrate the feasibility of genetically engineering multi-stress tolerance in transgenic turfgrass through simultaneous overexpression of three genes encoding an *Arabidopsis* vacuolar H^+-pyrophosphatase, *AVP1*, a rice SUMOylation E3 ligase, *OsSIZ1*, and a cyanobacterial flavodoxin, *Fld*. Specifically,

1. We will prepare a chimeric gene construct, p35S-*AVP1*/Ubi-*OsSIZ1*/Ubi-*FNR:Fld*/ p35S-*bar* constitutively expressing *AVP1*, *OsSIZ1* and *Fld* genes together with a selectable marker gene, *bar*, for herbicide resistance.
2. We will conduct *Agrobacterium*-mediated turfgrass transformation to produce transgenic lines harboring p35S-*AVP1*/Ubi-*OsSIZ1*/Ubi-*FNR:Fld*/p35S-*bar*.
3. We will analyze putative transgenic plants for transgene insertion and expression.
4. We will Examine plant growth and development in transgenics, and evaluate plant performance under various stressful conditions including drought, heat, salt, P and N starvation in comparison with wild type controls.

Start Date: 2016
Project Duration: 3 years
Total Funding: $60,000

In the face of a global scarcity of water resources and the increased salinization of soil and water, abiotic stress is the big challenge of modern agriculture practice. This project aimed to genetically engineer turfgrass with multiple genes involved in plant stress response for enhanced plant performance under adverse environmental conditions. In our previous report, we presented data obtained in 2016, reporting the successful construction and introduction of a chimeric gene construct, p35S-*AVP1*/Ubi-*OsSIZ1*/Ubi-*FNR:Fld*/ p35S-*bar* containing expressing cassettes overexpressing *AVP1*, *OsSIZ1* and *Fld* genes together with a selectable marker gene in transgenic creeping bentgrass plants. This year, we have conducted transgenic analysis to evaluate transgene insertion and expression in primary T_0 transgenic plants using PCR and Northern hybridization (see examples in **Figure 1**). Our data showed that all the 20 putative transgenic lines contain the three stress-related genes, *AVP1, OsSIZ1* and *Fld* as well as the selectable marker gene, *bar*.

All the transgenic lines were then transferred into soil and grown in greenhouse for further analysis. Morphologically, they did not show significant difference from the non-transgenic controls. Based on transgene expression levels, we selected three

representative transgenic lines and they have been vegetatively propagated for further analysis. So far, we have accumulated enough materials of these three representative transgenic lines and are in the process of conducting different experiments evaluating their performance under various abiotic stresses.

Summary Points:

- Conducted molecular analysis of primary putative transgenic plants for transgene insertion and expression, and demonstrated that all the 20 transgenic lines harbor the chimeric gene expression construct, p35S-*AVP1*/Ubi-*OsSIZ1*/Ubi-*FNR:Fld*/ p35S-*bar*, and express three stress-related genes, *AVP1*, *OsSIZ1* and *Fld*.
- Transferred regenerated transgenic plants into soil and grown in greenhouse for further analysis.
- Selected three representative transgenic lines for vegetative propagation. Enough plant materials have been accumulated and are being used for performance evaluation under various environmental stresses.

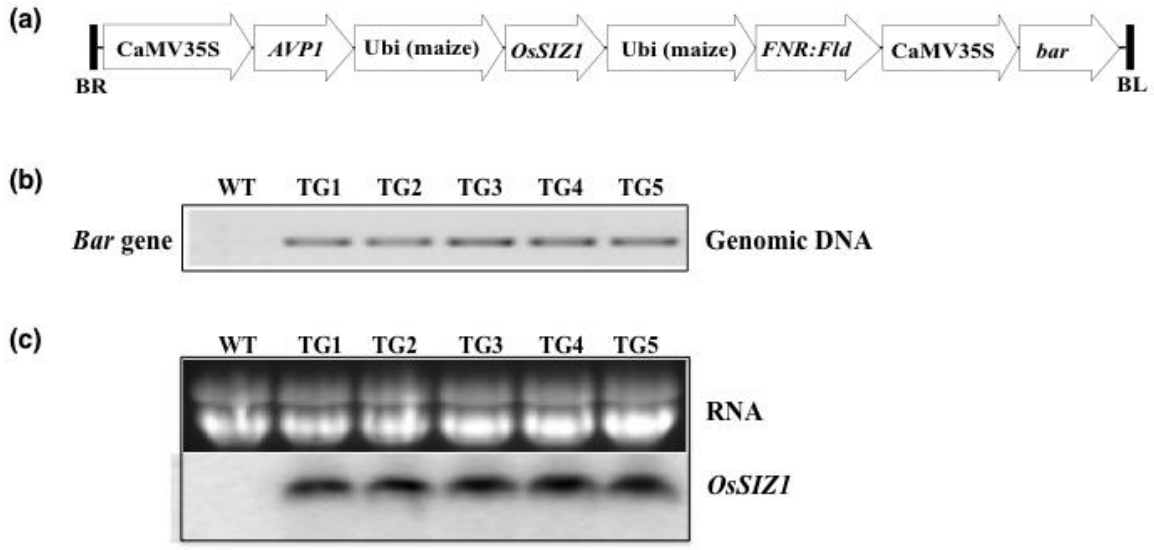

Figure 1. Generation and molecular analysis of transgenic lines expressing *AVP1*, *OsSIZ1* and *Fld* genes . **(a)** Schematic diagram of the chimeric gene expression construct, p35S-*AVP1*/Ubi-*OsSIZ1*/Ubi-*FNR:Fld*/p35S-*bar* p35S-*AVP1*/Ubi-*OsSIZ1*/Ubi-*FNR:Fld*/p35S-*bar*, in which the *AVP1* gene driven by the cauliflower mosaic virus 35S (CaMV35S) promoter, *OsSIZ1* gene driven by the corn ubiqintin (Ubi) promoter, and *FNR:Fld* gene (the Fld gene translationally fused to the pea FNR chloroplast-targeting transit signal peptide) driven by the Ubi promoter were linked to the herbicide glufosinate (*phosphinothricin*) resistance gene, *bar*, driven by the cauliflower mosaic virus 35S (CaMV35S) promoter. The right border (BR) and the left border (BL) of the T-DNA in the binary vector were labeled. **(b)** PCR analysis of the *bar* gene using genomic DNA of wild type (WT) and five representative transgenic plants (TG1-5) to detect transgene insertion into the host genome. **(c)** Northern blot analysis demonstrating transgene expression in transgenic plants. *OsSIZ1* gene expression in five representative transgenic lines (TG1-5) in comparison to wild type (WT) control was shown as example. *OsSIZ1* DNA fragment was used as probe for hybridization. EtBr-stained gel shows the amount of RNA from each sample loaded for Northern analysis.