Summary of research progress and results

Title: **Genetic engineering of turfgrass for enhanced multi-stress resistance**

Hong Luo (PI), Department of Genetics and Biochemistry, Clemson University

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. 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. Specifically, we propose 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*. To this end, we have prepared a chimeric gene construct containing expressing cassettes overexpressing *AVP1*, *OsSIZ1* and *Fld* genes together with a selectable marker gene for plant transformation, *bar*, for herbicide resistance (**Fig. 1**). In this chimeric gene construct, p35S-*AVP1*/Ubi-*OsSIZ1*/Ubi-*FNR:Fld*/p35S-*bar*, the *AVP1* gene driven by the cauliflower mosaic virus 35S (CaMV35S) promoter, the *OsSIZ1* gene driven by the corn ubiqintin (Ubi) promoter, and the *FNR:Fld* gene (the Fld gene translationally fused to the pea FNR chloroplast-targeting transit signal peptide for chloroplast targeting of the Fld protein) driven by the Ubi promoter were linked to the herbicide glufosinate (*phosphinothricin*) resistance gene, *bar*, driven by the cauliflower mosaic virus 35S (CaMV35S) promoter. This construct was then introduced into *Agrobacterium* strain, LBA4404 for plant transformation. We have simultaneously prepared creeping bentgrass embryogenic callus from mature seeds and used as targets for gene transfer by *Agrobacterium* infection using the LBA4404 strain harboring the chimeric gene construct, p35S-*AVP1*/Ubi-*OsSIZ1*/Ubi-*FNR:Fld*/p35S-*bar*. Potentially transformed plant cells were selected in the presence of herbicide, glufosinate (*phosphinothricin*). Transformed cells surviving herbicide selection were regenerated into plants and grown in greenhouse for further analysis (**Fig. 2**). So far, we have successfully generated about 20 independent transgenic lines harboring p35S-*AVP1*/Ubi-*OsSIZ1*/Ubi-*FNR:Fld*/p35S-*bar*. Next step would be to conduct experiments analyzing transgene expression in theses transgenic plants. Representative lines will be propagated in greenhouse and evaluated for their performance under various environmental stresses. In summary, we have:

- Prepared chimeric gene expression construct, p35S-*AVP1*/Ubi-*OsSIZ1*/Ubi-*FNR:Fld*/p35S-*bar*, containing expression cassettes to overexpress three stress-related genes, *AVP1*, *OsSIZ1* and *Fld* as well as a selectable marker gene for plant transformation, *bar*, for herbicide resistance.
- Prepared embryogenic callus of creeping bentgrass from mature seeds as targets for gene delivery.
- Conducted creeping bentgrass transformation using *Agrobacterium*-mediated transformation of embryogenic callus with the chimeric gene expression construct, p35S-*AVP1*/Ubi-*OsSIZ1*/Ubi-*FNR:Fld*/p35S-*bar*.
- Produced around 20 independent transgenic lines harboring p35S-*AVP1*/Ubi-*OsSIZ1*/Ubi-*FNR:Fld*/p35S-*bar* for further analysis.

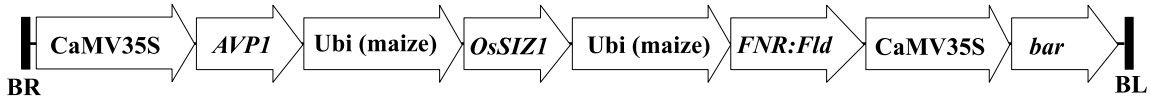

Figure 1. Schematic diagram of the chimeric gene expression construct, p35S-*AVP1*/Ubi-*OsSIZ1*/Ubi-*FNR:Fld*/p35S-*bar*, in which the *AVP1* gene driven by the cauliflower mosaic virus 35S (CaMV35S) promoter, *OsSIZ1* gene driven by the corn ubiqintin (Ubi) promoter, and *FNR:Fld* gene (the Fld gene 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.

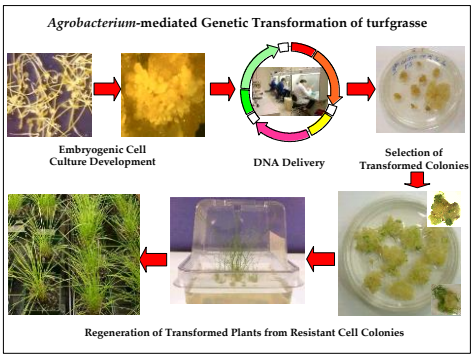

Figure 2. *Agrobacterium*-mediated transformation of turfgrass. Embryogentic callus was induced from mature seeds of the creeping bentgrass and used as target for gene transfer by *Agrobacterium* infection. Potentially transformed plant cells were selected in the presence of herbicide, glufosinate (*phosphinothricin*). Transformed cells surviving herbicide selection were regenerated into plants and grown in greenhouse for further analysis.