## ABSTRACT

# 049-AGR

**Developing a Four-In-One Gene Editing System to Improve Production and Sustainability of Bioenergy in Sweet Sorghum**

The increasing global demand for renewable energy has intensified research on lignocellulosic bioethanol production as a sustainable alternative to fossil fuels. However, the development of lignocellulosic biofuels is hindered by the recalcitrance of plant cell walls, which are primarily composed of cellulose, hemicellulose, and lignin. This structural complexity poses significant challenges to efficient bioethanol production. Sweet sorghum (*Sorghum bicolor* L.), a C4 crop, holds great promise as a bioenergy resource due to its high biomass yield and adaptability to marginal lands. Nevertheless, its lignocellulosic biomass content, particularly lignin that contributes to its recalcitrance. Therefore, producing sorghum varieties with reduced lignin content would be advantageous. Given the importance of sweet sorghum as a renewable energy source, decreasing lignin content through mutagenesis has become crucial in sorghum breeding programs. Our study explores the use of biotechnology to establish an efficient embryogenic cell culture system in sweet sorghum capable of: (i) modifying lignin biosynthetic genes, (ii) achieving stable genetic transformation, and (iii) enabling regeneration from cells to whole plants. These advancements are expected to expedite genetic modifications of targeting genes and enhance sweet sorghum's utility for biofuel production. The specific objectives of our research are: 1) To develop and establish an efficient *in-vitro* cell suspension culture system in sweet sorghum. 2) To mutate sorghum peroxidases (PRX) genes using CRISPR-Cas9 technology. 3) To develop genetic transformation and regeneration methods for sorghum cell culture systems. 4) To estimate the potential of CRISPR-Cas9 mutagenesis on modified sorghum lines. Currently, we produced embryonic callus lines of two varieties of sweet sorghum, Topper 76-6 and Dale that are being tested for transformation and regeneration. We found that cell lines of both varieties display good cell density up to 1.59 mg/ml - 1.78 mg/ml for density and up to 82-88% for the viability respectively. Both established embryonic cell lines will be first tested for transformation using *Agrobacterium tumefaciens* carrying a binary vector CRISPR-Cas9, and then for the regeneration step to produce transgenic plants from sorghum embryonic cell lines. These promising preliminary results demonstrate the potential for advancing four-in-one tool for genetic manipulation of sweet sorghum, to enhance bioenergy production.

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