Optimizing androgenic media to produce *Sorghum bicolor* embryogenic calli expressing the Green Fluorescent Protein

While the world searches for advanced biotechnology-based solutions for increasing fuel needs, *S. bicolor* has continued to emerge as a solution. Sweet sorghum has stalks that can readily provide sugary water that can be directly fermented into bioethanol but requires a breeding strategy to improve traits. Using Double Haploid (DH) protocols, homozygous lines can be created. While DH protocols are successful in over 200 species, it continues to be a challenge in sorghum. The proper development of a streamlined protocol for plant regeneration towards DH production while allowing for genetic modifications is critical in creating desirable varieties. Using microspores in androgenic media, many of the sorghum recalcitrant barriers can be overcome such as those posed by phenolic exudation and genotype. Microspores are natural protoplasts, immature pollen produced after meiosis present as a mixed population of gametes that can express the entire variation through independent assortment. Therefore, gametophytes contain combinations of genes that may facilitate androgenic response and can be identified after culturing. In this study various nutrient and hormone combinations were screened that allowed for rapid formation of calli on a modified MS based media with proper hormone manipulation. Through protoplast-based methods, Poly Ethyl Glycol (PEG), plasmid DNA containing the reporter gene GFP was used to demonstrate that naked DNA can be introduced into maturing microspores due to their lack of a developed exine wall. Thus, microspores that were genetically modified through PEG based protocols were able to uptake the GFP reporter gene that was expressed in culture derived calli. These calli are being used for further studies to initiate the whole plant regeneration process. These endeavors will create a basis for standardization of genetically modified sorghum plants with enhanced traits towards the increased sugary syrup for biofuel usages.

Max word count: 350 (289)