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Sweet Sorghum Biofuel Traits: The Journey from Seed to Microspore

Abstract

In the ever-demanding market for increased energy, scientists have sought alternative energy pathways to ease the demand for fossil fuel use are sought. Sweet sorghum has been an emerging crop for biofuel usage due to the ease of extractability of its sugary water from the stalks, that can be directly fermented into ethanol. We have grown several sweet sorghum varieties in the TSU field and collected their microspores as well as sugary water towards pre-breeding genetics research. Sweet sorghum was also grown in the greenhouse to determine proper harvesting time towards capturing microspores, which are the male gametes, in large numbers for further studies. Once plants matured, panicles containing developing microspores were harvested for mass isolation towards cryopreservation. Five varieties were used in this study (Achi Turi, Dale, Dasht Local, RTx430 and Topper 76-6) and through greenhouse data (plant height, flag-stem length, and spikelet maturation characteristics) on correlation with microspore developmental levels, the anthers were found to show strongest regression to gametogenesis stages (unicellular to mature pollen). Mass isolated microspores were frozen for subsequent androgenesis and transformation research. For cryopreservation, 0.9ml of the resuspended cells were aliquoted in tubes with the addition of 5% DMSO and 5% glycerol to facilitate cell survival post-storage. Microspore density was observed before and after the procedure to ascertain viability percentage of cryopreserved cells. Recovered microspores were then transformed using binary vector (pY31) based cassette containing Arabidopsis promoter with GFP gene through using a PEG based protocol. Microspore suspensions were then plated on 2.5% agar with full strength MS media containing 30g/L sucrose along with 5mg/L 2,4-D and 2mg/L Kinetin to induce androgenesis. This procedure is being streamlined to help pave a pathway for genetically enhanced sweet sorghum plants towards addressing global food and energy demands.