081

Individual microspore isolation from sweet sorghum cultivars towards genetic analysis

In the United States and other countries across the globe, sorghum crop is primarily used for livestock feed and ethanol production but is becoming popular in the consumer food industry and other emerging markets. *Sorghum bicolor* L. plants are high in fiber, as well, help meet dietary needs for forage animals and poultry. Sweet sorghum varieties are bred for syrup production and besides biofuel purposes these can also be used for grain as well as forage. This study proposes to use individually isolated gametophytes of sweet sorghum to facilitate downstream genetic analyses towards improving biofuel relevant traits. Four sweet sorghum cultivars (Dale, Dasht Local, Topper 76-6, and Achi Turi) were grown in a greenhouse for capturing microspores with undeveloped exine. For microspores' isolation, sessile spikelets measuring 3.3 – 3.7 mm in length were dissected from panicles under a microscope to retrieve and macerate anthers in 50 µl PCR tubes while suspended in 6% sorbitol solution. Individual gametophyte isolation will be achieved by using CellTram (Eppendorf North America, Hauppauge, NY) micro-injector mounted on an ultra fine move Marzhauser (HS6) micro-manipulator (ALA Scientific Instrument, Westbury, NY). The individually isolated microspores will be at a developmental level, which is immediately after the tetrad stage when the outer sporopollenin cell wall is not completely formed. Free early microspores genomic DNA will then be extracted and amplified using REPLI-g Single Cell Kit (QIAGEN, Hidden, Germany). Gametophyte DNAs can be sequenced with or without whole genome amplifications for enhanced understanding of this species. Such genetic tools have various applications for plant biodiversity and sustainable use in agricultural production.