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Genetic segregation detected through single nucleotide polymorphisms of sweet sorghum microspores

Abstract

A total of five varieties of *Sorghum bicolor* (Achi Turi, Dale, Dasht Local, Topper 76-6, and Tx 430), relevant to biofuel production potentials' were maintained at Tennessee State University greenhouse (Agricultural Research and Education Center, Latitude 36.12′ N, Longitude 86.89′ W). Specific single nucleotide polymorphic (SNP) sequences relevant to sorghum production traits were selected from Generation Challenge Programme Group (ICRISAT, Cornell University), based on compatibility with LGC Genomics (Teddington, Middlesex, UK) genotyping services. Thus, 20 SNP markers where selected which were assessed to provide a high level of reproducibility along with good separation applicability for homozygote and heterozygote genotypes. Sweet sorghum microspores were individually isolated from aqueous solution (27% D-sorbitol) using a pulled (Harvard Apparatus, Holliston, MA) capillary on a

Cell-Tram Manual Micro-injector (Eppendorf North America, Hauppauge, NY) mounted over a UMMJ- 3FC (Narishige International USA, Amityville, NY) micromanipulator. A combined total of 170 free early microspores were lysed and their genomes multiplied through multiple displacement amplifications, each providing spectrophotometer-based UV absorbance (A230/260) readings of 1.8 for single cell DNAs yields of 2800 - 3000 ng/µL. The DNeasy Plant Mini Kit (QIAGEN, Hidden, Germany), was used to isolate high-quality DNA, from young leaves tips of 15 to 20 days old healthy parental plants and sent to LGC genomics for SNP primers' validations. After conducting KASP (LGC Genomics) assays on above *Sorghum bicolor* plants and gamete samples; SNP density, segregation, and genetic variations were recorded. The SNP based segregation data from individual sweet sorghum gametes, which are natural haplotypes, is also being collected. The KASP system was found to be low cost, providing high accuracy, while being a time saver and high throughput for assessing sorghum polymorphism and genetic diversity.