094

Standardizing current research protocols towards genetic modification for androgenic responsive microspores of recalcitrant *Sorghum bicolor*

Sorghum bicolor has continued to be elusive while pursuing androgenic response from its microspores. Such pathway has been established in over 200 agronomic important crops, creating genetically modified plants with enhanced traits. The use of microspores, which act as natural protoplasts, also allows animal cell protocols when creating healthier and improved plant types while avoiding dependence on agrobacterium-based methods. Microspores thus offer a great pathway for genetic modifications that will remain in homozygous form after androgenesis success into plantlets. Therefore, microspores due to the lack of an outer layer of exine, are expected to be genetically modified as natural protoplasts for ultimately producing double haploid homozygous plants through androgenesis. However, such immature microspores can only be harvested during short sorghum flowering window, which closes within two weeks, necessitating a cryopreservation protocol that was developed allowing for persistent research applications. Two protocols were compared to find the best method to isolate large numbers of microspores while addressing the contamination issues during subsequent tissue culture conditions. Firstly, spikelets were surface sterilized to be crushed in 0.1M mannitol and microspores were isolated through using cheese cloth and 40µm cell strainers. This method provided large numbers of microspores while being fast and easy but caused substantial contamination on culture media. Through gradient fractionalization it is possible to remove contaminants, but such extensive modifications are cumbersome, and alternates are more useful. In the second method, surface sterilized spikelets were dissected to retrieve anthers into 1.5ml tubes containing 0.1M mannitol. This method was more laborious and provided fewer microspores but later proved to significantly reduce the contamination levels during subsequent tissue culture conditions. Previous media formulations that worked on explants from *Sorghum bicolor*, or microspores of related species, are being modified to create further protocols. Androgenetic media MS-5501 containing various concentrations of plant growth regulators along with the use of cold stress treatments of microspores was successful in producing initial embryogenic tissues from sorghum genotypes. Through protoplast-based methods, plasmid DNA containing the reporter gene GFP was also successfully inserted into microspores and results were confirmed under microscope. Further standardization of androgenic media along with correct pre-treatments of microspores is continued to finalize the regeneration process to create stable, genetically modified, sorghum plants.