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Regeneration of Miscanthus sp. for Agrobacterium-mediated transformation purposes. Preliminary studies

Joanna Cerazy, Karolina Malec, Aurelia Ślusarkiewicz-Jarzina, Aleksandra Ponitka, Stanisław Jeżowski and Tomasz Pniewski
Institute of Plant Genetics Polish Academy of Sciences, Poland

Miscanthus is a genus perennial giant grass with great potential for biomass production, which can be used as a renewable feedstock for bioenergy or bioethanol. Biotechnological methods, including in vitro cultures and plant transformation can substantially complement projects aimed to improve miscanthus biomass yield or quality. Several genotypes of *M. sinensis* (lines MS1, MS16 and MS17), *M. x giganteus* (line D-116 and MG3) and *M. sacchariflorus* ('Robustus') were subjects of studies on plant regeneration. Among young spikelets, spike axles, inflorescence receptacles and nodes, the first appeared appropriate initial explants for all genotypes. Callus was induced 2-3 weeks on MS medium with 5.0 mg/l 2,4-D and 0.5 mg/l BAP, while plants were regenerated within 2 months on MS medium + 2.0 mg/l BAP. The efficiency of obtaining callus and plants depended on the genotype and the highest regeneration rate was noted for *M. sinensis* line 17 and *M. x giganteus* D-116, where 659.0 and 517.9 calluses and 2454.4 and 1811.9 plants per 100 explants were obtained, respectively, but only 192.0 calluses and 78.4 plants for *M. sacchariflorus.* Callus induction and plant regeneration were approx. 1.3 and 2.0 times improved by using respectively, C17 medium with 5.0 mg/l 2,4-D and 0.5 mg/l BAP and 190-2 medium with NAA and KIN, each 0.5 mg/l. Developed regeneration procedure was partially adopted for Agrobacterium-mediated miscanthus transformation trials. All used *Agrobacterium tumefaciens* strains carried pCAMBIA1201 vector with T-DNA containing hygromycin phosphotransferase marker gene (hpt) and GUS reporter gene, both under control of 35S RNA CaMV promoter. Embryogenic 10-week-old calli (50 per variant) were inoculated with Agrobacterium hypervirulent strains: EHA105, AgL0 and AgL1. Callus was induced on MS medium with L-proline (0.5 mg/l), L-glutamine (0.5 mg/l), casein (1 mg/l) and 2,4-D (2 mg/l) but plants were regenerated as described above. All media were supplemented with 2.5 mg/l of hygromycin as a selection agent. Finally 12 putative transgenic plants, 5 for MS17 and 7 for MG, were obtained, then micropropagated up to 5 clones, rooted and transferred to soil. Probable T-DNA genomic integration was confirmed by PCR in all clones for 5 transformants, hence the transformation efficiency was about 1 to 2%. Further plant analyses as assay of GUS or hpt activity, etc. are in progress.

kmal@igr.poznan.pl

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