APPLICATION OF AIRLIFT BIOREACTORS FOR HIGHLY EFFICIENT GENETIC TRANSFORMATION OF AMERICAN CHESTNUT

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Airlift bioreactors were constructed and applied for proliferation of chestnut embryogenic tissue. More than ten genotypes of American chestnut (*Castanea dentata*) and backcross hybrids of American chestnut and Chinese chestnut (*C. dentata x C. mollissima*) were cultured in bioreactors, of which eight have been used for *Agrobacterium*-mediated genetic transformation. The basal culture medium for bioreactors was woody plant medium (WPM). Medium for tissue proliferation was WPM supplemented with 3 g/l sucrose, 0.5 g/l glutamine and 2 mg/l 2, 4-D. Medium for embryo maturation was the proliferation medium minus 2, 4-D and gelled with 5 g/l Phytagel. In most genotypes, the optimum culture conditions for a one-liter bioreactor included 2% (w/v) tissue density for initial inoculation, 200 ml/min airflow rate, weekly fresh medium feeding (85% fresh medium/15% spent medium, v/v) and monthly fractionation through nested sieves of 1 mm pore size to remove large, old cell clumps. Compared with flasks, bioreactors generated higher yields of tissue mass and larger fractions of tissue consisting of small cell clumps (< 1 mm in diameter) that were suitable targets for transformation. Bioreactor-generated tissue demonstrated high mature embryo yields and high amenability to transformation via *Agrobacterium* co-cultivation. Using bioreactor-grown embryogenic chestnut target material, two reporter genes (*GUSi*, and *YFPGUSi*) and ten candidate genes (CGs) for chestnut blight resistance have been transformed into chestnut cells, resulting in thousands of geneticin-resistant cell clumps (transclones). Transformation rates varied with genotype and construct. In one genotype, the number of transclones peaked at approximately 70% of the total cell clumps of target material. Transclones were further selected on the basis of morphological characteristics for embryogenicity and screened by reporter gene expression and/or molecular markers to assure stable transformation. Airlift bioreactors have enabled a great acceleration of chestnut transformation by producing high-quality embryogenic tissue in larger quantities and with lower labor and operating expense than previously used approaches.