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Carbon nanotubes as DNA-nanocarriers for genetic transformation of plants

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Carbon nanotubes (CNTs) are viewed to be promising for the design of novel gene delivery methods in plant biotechnology. The major technical barrier for application of CNTs in biological objects is high hydrophobicity of their surface. In this study single-walled CNTs (SWNTs) and multi-walled CNTs (MWNTs) non-covalently functionalized with biomolecules for subsequent dispersing in water were used for the delivery of genes into plant protoplasts and walled cells of tobacco *Nicotiana tabacum* L. Properties of functionalized CNTs were characterized using transmission electron microscopy, atomic-force microscopy and Raman spectroscopy. Genetic transformation was carried out using the binary vector pGreen 0029 containing the gene sequence of membrane protein of protein-storage vacuole, fused to the reporter gene of yellow fluorescent protein (YFP) under control of 35S promoter and NOS terminator, as well as the kanamycin resistance selective marker gene *nptII*. Using SWNTs at a concentration of 20 μg/ml and MWNTs at a concentration of 15 μg/ml genetic transformation of *N. tabacum* L. mesophyll protoplasts with plasmid pGreen 0029 was conducted and transient expression of the reporter *yfp* gene in 16% and 13% protoplasts, respectively, was observed. Using SWNTs at a concentration of 40 μg/ml and MWNTs at a concentration of 30 μg/ml genetic transformation of *N. tabacum* callus and leaf explants with *nptII* gene was carried out and regeneration of plants on selective medium containing 50 mg/l kanamycin was shown. The frequency of stable transformation of *N. tabacum* callus using SWNTs as nanocarriers reached 8%, using MWNTs – only 3%. The frequency of stable transformation of leaf explants was 6% using SWNTs and 2% using MWNTs. SWNTs-based nanocarriers demonstrated their applicability to transformation of protoplasts as well as walled plant cells. Whereas, MWNTs-based nanocarriers were suitable only for transformation of protoplasts due to the limiting role of cellulose walls in cell penetration.

1. *Rafsanjani M.S.O., Alvari A., Samim M., Hejazi M.A., Abdin M.Z.* Application of novel nanotechnology strategies in plant biotransformation: a

contemporary overview // Recent Pat. Biotechnol. – 2012. – **6**. – P. 69–79.