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Plant VLP production system based on bacteriophage MS2 coat protein

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Some types of single-stranded RNAs regulate gene expression. To protect ssRNA from degradation and to deliver it to a target tissue precisely we can incorporate the molecule into a virus-like particle (VLP). Such VLP is a nanocarrier consisting of viral coat protein capsid and nucleic acid filling. Bacteriophage MS2's coat protein (CP) easily forms a stable capsid via interaction with MS2 operator – the 19-nucleotide sequence in the MS2 genomic RNA. Thus, target ssRNA, which mimics the MS2 genome, can be packed into a VLP. There are successful examples of bacteriophage coat protein application for VLP production. We aim to obtain *Nicotiana tabacum* plants steadily expressing MS2 CP and which can evolve into a VLPs producing system for ssRNA delivery. Bacteriophage MS2 (ATCC 15597-B1, United Kingdom) was produced in *E. coli* strain ER2738. Genomic RNA was purified by TRIzol Reagent and MS2 cDNA was synthesized by reverse transcription using iScript, BIO-RAD. CP gene sequence was amplified from MS2 cDNA by PCR with Phusion high-fidelity DNA polymerase (Thermo Scientific) and was transferred first in interim vector pJet1.2 (Thermo Scientific). Then the sequence was subcloned into another vector where the expression cassette harboring CP under control of Cauliflower Mosaic Virus double 35S promoter was assembled. The cassette was then embedded into destination binary vector for *Agrobacterium*-mediated stable plant transformation. The structure of every genetic construct was confirmed by restriction analysis and Sanger sequencing. *Agrobacterium* (strain AGL1)-mediated *N. tabacum* SR1 leaf explants transformation by created plasmid was conducted and primary regenerants T_0 plants were obtained after regeneration with hygromycin selection. PCR with genomic DNA template and RT-PCR with total RNA revealed 7 plants carrying CP gene which is being transcribed. We aim to investigate whether VLPs are able to form in the transgenic plants in the presence of RNA molecule containing MS2 operator.
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