BEB
Conference on Biomedical Engineering and Biotechnology

BEB6330

Design and development of the expression cassette for plant production of Dengue virus NS1 protein.

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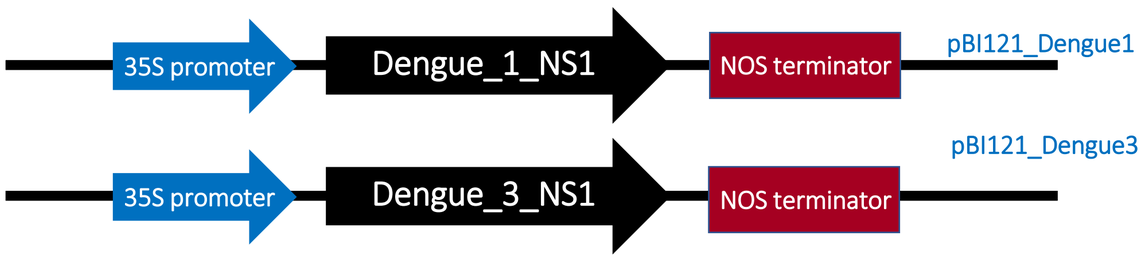
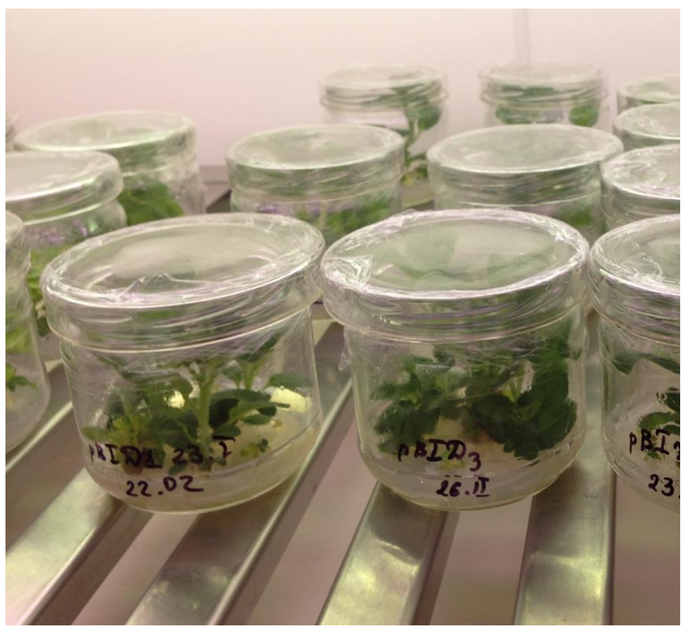
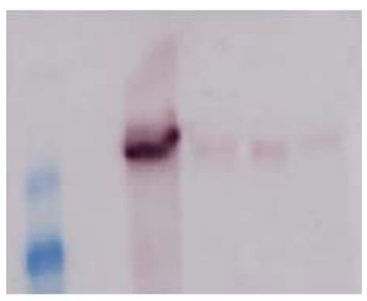
In connection with the increasing frequency of infectious diseases outbreaks caused by arboviruses, the monitoring of the epidemiological situation in the Russian Federation requires development of immunological diagnostic kits for differential diagnosis. These kits could be developed using individual recombinant antigen proteins of selected viruses. NS1 non-structural proteins of flaviviruses were described in the literature as reliable serological markers.







Western blot of expressed NS1 proteins in transgenic plants: M-protein ladder; K- - wild tobacco; K+ - commercial protein; 1,5-tobacco lines with Dengue1 NS1; 7-tobacco lines with Dengue3 NS1

Standard eukaryotic systems, for example insect cells, have a number of limitations (productivity and cost). In our work we use plants for the production of flavivirus antigens. Plants are ideal biofactories because of their ability to generate large amounts of proteins with low cost and to produce an appropriate post-translation modifications of recombinant proteins.

NS1 antigens require eukaryotic post-translation modification

The NS1 protein sequences of the two serotypes of Dengue virus (DV1 and DV3) have been optimized for the expression of the target proteins in Nicotiana tabacum plants. The sequences were assembled de novo from previously synthesized oligonucleotides by the enzymatic method "Two step PCR" and cloned into expression cassettes of plasmid pBI121, on the basis of which four binary vector systems for expression of NS1 proteins in plants were created. Transformation of leaf explants of Nicotiana tabacum was carried out using Ti-plasmids of bacteria Agrobacterium tumefaciens strain AGL0 and further regeneration of tobacco plants. Recombinant NS1 proteins of the two Dengue fever viruses (types 1 and 3) were extracted and pre-purified.

Western blotting and enzyme-linked immunosorbent assay of recombinant NS1 has shown that the obtained antigen could bind to anti-NS1 antibodies Mab to Dengue virus NS1 (Meridian LifeScience, USA).

Problem- low protein yield
Solution-construction of the new expression cassette

Rb7 MAR (matrix attachment region) – avoid negative regulation by plant immunity
UBQ10 intron – increase the expression level through intron-mediated amplification
35S – constitutive virus promoter for high expression level
5 'and 3' UTRs non-coding regions of RuBisCO (UTRs) – have tissue-specific and enhancer motifs
Signal peptide of alpha-amylase and HDEL – signal peptides for endoplasmic reticulum localization of expression
6H – histidine tag for purification of the NS1 protein

Evaluation of the effectiveness of the developed design was confirmed by a number of bioinformatics tools (WoLF PSORT / NetGene2 / NetOGlyc / (http://www.kazusa.or.jp/).

This work was carried out within the framework of the grant of the President of the Russian Federation No. MK-569.2020.4.