Obtaining of Transgenic *Nicotiana rustica* Plants with Heterologous *ZRNase II* Gene to Produce Plant Resistance to Viruses

Potrokhov A.O.
the corresponding author

Institute of Cell Biology and Genetic Engineering, 148, Zabolotnoho str., Kyiv, Ukraine

Nesterenko O.G.

Institute of Cell Biology and Genetic Engineering, 148, Zabolotnoho str., Kyiv, Ukraine

Biotechnology approaches allow the production of transgenic plants with increased resistance to viruses. The main transgenic strategies for virus resistance include: RNA interference, coat protein mediated resistance, replicase mediated resistance, RNA dependent RNA polymerase mediated resistance, RNA satellites, antisense RNAs, ribosome-inactivating proteins (RIP), ribonucleases, enhancement of HR/SAR, hammerhead ribozyme, microRNAs, plantibodies [1].

Ribonucleases (RNases) are supposed to be engaged to antiviral response in plants. Heterologous RNase gene expression can be a tool for production of cultivars with multiple resistance to viruses and viroids. *Nicotiana rustica* plants are known to be a model used in plant virology as experimental host in the studies of plant – virus interactions.

The aim of our research was the production of transgenic *N. rustica* plants expressing heterologous *ZRNase II* gene as a future model to determine the susceptibility of obtained plants to a number of plant viruses and their systemic movement in plants. Wounding-induced ribonuclease encoding cDNAs isolated from *Zinnia elegans* are predominantly induced in response to wounding. Isolated *ZRNase II* gene from *Zinnia elegans* encodes extracellular ribonuclease, which hydrolyze virus genomic RNA at some stages of their penetration into the plant cell [2].

Methods: *Agrobacterium*-mediated genetic transformation of leaf discs from *N. rustica* have been conducted with *A. tumefaciens* AGL0 strain harboring pbi-RNS vector. T-DNA of pbi-RNS vector included *ZRNase II*, S-like RNase gene of *Zinnia elegans* controlled by p35 S CaMV derived from the cauliflower mosaic virus (CaMV) and *npt II*, neomycin phosphotransferase gene, under control of nopaline synthase promoter (pNOS). Neomycin phosphotransferase gene was used as selective marker. Plants were regenerated and selected on medium with 100 mg/l kanamycine. PCR was used to analyze the presence of target and selective genes in the kanamycine resistant plants. RNase activity was determined by colorimetric method in the presence of orcinol.
Results: We have obtained the transgenic *N. rustica* plants with ZRNase gene via Agrobacterium-mediated. The plants have been obtained and were grown on the MS media supplemented with kanamycin-sulfate with pbi-RNS vector were selected. Resistant shoots were regenerated and rooted under the selective pressure of 100 mg/l of kanamycin in the cultural medium. The stable integration of ZRNase II gene was confirmed by PCR-analysis.

Thus, the transgenic plant material was created for future investigation of extracellular ribonuclease influence on plant resistance to virus movement.

References:
