ABSTRACT

DOI 10.36074/grail-of-science.29.04.2022.035

OBTAINING OF TRANSGENIC *NICOTIANA RUSTICA* PLANTS WITH HETEROLOGOUS *ZRNASE II* GENE TO PRODUCE PLANT RESISTANCE TO VIRUSES

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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 determinate 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 elegance* 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 kanamicyne. PCR was used to analyze the presence of target and selective genes in the kanamicyne resistant plants. RNase activity was determined by colorimetric method in the presence of orcinol.

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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:

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