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DETECTION OF TOTAL RNASE ACTIVITY IN *NICOTIANA BENTHAMIANA* PLANTS TRANSFORMED WITH THE *ZRNASE*II GENE

ВИЯВЛЕННЯ ЗАГАЛЬНОЇ АКТИВНОСТІ РНКАЗИ В РОСЛИНАХ *NICOTIANA BENTHAMIANA*, ТРАНСФОРМОВАНИХ ГЕНОМ *ZRNASE*II

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Plant viral infections are widespread among crops and cause significant yield loss and decrease its quality. Strategies for creating of resistant to viruses plants include both traditional breeding methods and such modern approaches as genetic engineering. Virus related recombinant genes are often used to obtain transgenic virus resistant plants, but heterologous genes of plant origin are also amenable for this purpose. RNases encoded by genes cloned from different organisms including plants were shown to provide antiviral defence against a wide number of viruses. The observed increased resistance to viral infections in transgenic plants expressing heterologous RNases requires a detailed study. The roles of autologous and heterologous ribonucleases in the defence system of higher plants should be investigated to propose RNase based strategies for obtaining of resistant crop varieties. Wounding-induced ribonuclease encoding cDNAs isolated from *Zinnia elegans* were 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. Destruction of viral RNA by RNases retards the development of the symptoms, mitigate their severity or localize the infection in plants.

The goal of our work was to study the total RNase activity in transgenic *N. benthamiana* plants with the *ZRNase*II gene, as *N. benthamiana* is a model plant for virological studies. The transgenic plants of this species with the *ZRNase*II gene were obtained previously. *Agrobacterium*-mediated genetic transformation of leaf discs from *N. benthamiana* have been conducted with *A. tumefaciens* AGL0 strain harboring pbi-RNS vector. T-DNA of pbi-RNS vector included, gene of S-like RNase of *Zinnia elegans* – *ZRNase*II controlled by p35 S CaMV derived from the cauliflower mosaic virus (CaMV) and neomycin phosphotransferase gene *(npt* II*)*, under control of nopaline synthase promoter (pNOS). Plants were regenerated and selected on medium with 100 mg/l kanamycin. PCR was used to analyze the presence of target and selective genes in the kanamycin resistant plants.

After *Agrobacterium*-mediated transformation putative transgenic plants have been obtained and were grown on the MS media supplemented with kanamycin-sulfate. Lines of *N. benthamiana* with T-DNA insertion from pbi-RNS vector were selected. The most vigorously growing kanamycin-resistant lines were selected for PCR. All the selected lines revealed the presence of the amplificated fragment with primers to *ZRNase*II and *npt*II *genes.* Plants were acclimated to the greenhouse condition and grown under 16-h day length with artificial light at 24°C. Activity essay was performed to estimate the total RNase level in nontransgenic plants and to compare it with the RNase activity in transgenic plant.

Total RNase activity in transgenic *N. benthamiana* plants with the *ZRNase*II gene was determined by orcin method. Plant leave samples (400 mg) were triturated in 3 ml of cooled 0.05 M Tris HCl buffer (Ph 7.5) with 0.15 M NaCl and 1 mM N-ethylmaleimide. Aliquots of the obtained extracts were used to determine RNase activity. For this, pairs of extract samples 0.5 ml were incubated for 6 and 66 minutes at +31°C. The samples were mixed with 1 ml of 34% $HC1O_4$ and centrifuged for 20 minutes at 6000 rpm 1 ml of 1% orcin reagent was added to the supernatant. The solution was cooled and measured at 670 nm wavelength. As a result, it was shown that the total RNase activity in extracts from *N. benthamiana* plants with the *ZRNase*II gene significantly exceeded that from wild-type plants. It was found that RNase activity in extracts from transgenic lines RNS1 and RNS2 six times exceeded the control one, while in lines RNS3 and RNS4 it was four times higher than in control. It was shown that the transformation of *N. benthamiana* plants with *ZRNase*II gene increased in the level of total RNase activity compared to wild-type plants. The resistance of established transgenic lines to different plant viruses is to be determined.