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OBTAINING OF THE SALT STRESS-TOLERANT TRANSGENIC TOBACCO PLANTS ABLE TO EXPRESS THE RECOMBINANT THAUMATIN II GENE

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Climate change is one of the most pressing issues of our time. Rising temperatures, soil salinization, and infectious diseases are primary factors that might negatively affect plant growth and productivity. Nowadays, the transgenic approaches are successfully used for creation of the crops with over-expression of the genes coding for specific protective proteins that are induced in response to various types of stress and pathogen attacks. A number of recent studies have found that the transgenic plants that contain recombinant genes of thaumatin-like proteins demonstrate the increased resistance to biotic stress (e. g. fungal infection) [2,5,8]. Thaumatin is a sweet-tasting protein isolated from *Thaumatococcus daniellii* fruits and is known for its strong homology to thaumatin-like defensive proteins that are considered to alter the membrane permeability and the cell signal transduction cascades in plants and fungi as a result of stress or pathogen infection [5,9]. Thaumatin-like proteins are a highly complex protein family associated with host defense and developmental processes in plants, animals, and fungi. They are classified as the PR-5 protein family. The expression of pathogen-related proteins is mediated through pathogen-induced signal-transduction pathways.

The aim of our study was to obtain the model transgenic salt stress tolerant tobacco plants able to express the recombinant gene of the thaumatin II protein. The study was carried out in the Laboratory of Experimental Biology of Kyiv Palace of Children and Youth.

Tobacco plants of Virginia variety were introduced in vitro culture by surface sterilization of seeds. We used nopaline GV3101 strain *Agrobacterium tumefaciens* for genetic transformation of the germinated tobacco plants. The plasmid vector was provided by Institute of Cell Biology and Genetic Engineering, National Academy of Sciences of Ukraine. The vector construct was carrying the thaumatin II gene driven by the 35S promoter of cauliflower mosaic virus and the selective phosphinothricinacetyltransferase gene, which enabled the resistance of plants to the herbicide phosphinothricin.

The bacterial suspension culture was precipitated by centrifugation (5000 rpm) and resuspended in liquid MS medium with its further cultivation on rotary shaker. The tobacco leaf explants were incubated in suspension culture for 48 hours. Subsequently, the inoculated explants were transferred to agar MS medium [10] with the addition of 1 mg/l BAP and 0.1 mg/l NAA in order to initiate regeneration of plants, 5 mg/l selective herbicide phosphinothricin and 500 mg/l antibiotic cefotaxime for bacterium elimination.

The transformed plant regeneration was observed in 2–3 weeks. Non-transformed tobacco plants were not able to survive on the media with 5 mg/l selective herbicide phosphinothricin. Here the frequency of the tobacco plant regeneration was about 60% on the selective media.

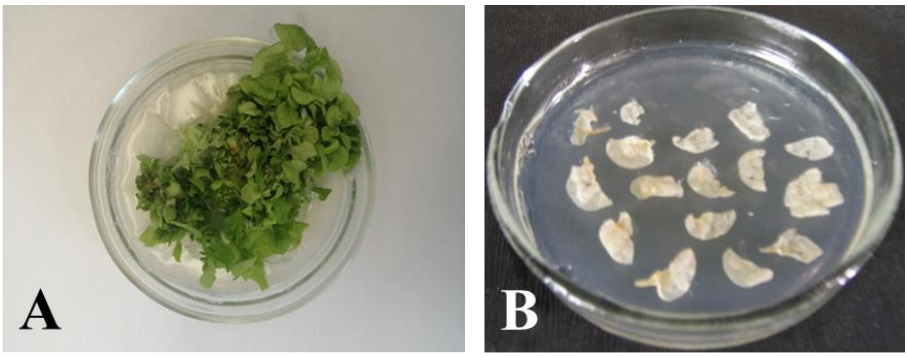

Figure 1. Regeneration of transformed plants on the selective medium (A), the death of untransformed explants on the selective medium (B)

Total plant DNA was isolated by the CTAB method [11]. Molecular genetic analysis of the obtained transformants was performed by PCR in order to prove the presence of the target thaumatin II gene and the selective phosphinothricinacetyltransferase gene. The analysis was carried out using primers for amplification of 642 bp thaumatin gene fragment under such conditions: 3 min at 94 C, then 30 cycles: 30 sec at 94 °C, 30 sec at 56C, 45 sec at 72 C, final synthesis for 5 min at 72 C. PCR analysis confirmed the presence of the target gene for 80% of the studied plants and the presence of the selective gene for all studied transformants.

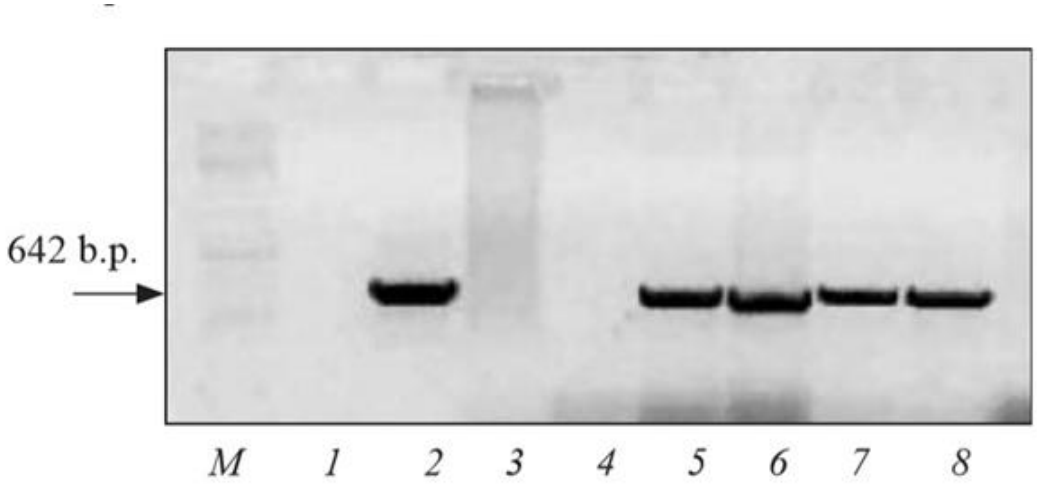

Figure 2. PCR analysis (thaumatin gene fragment) of the transformed plants.
M – DNA marker ladder, 1 – negative control (no template DNA), 2 – positive control (plasmid DNA), 3 – negative control (untransformed plant DNA), 4–10 – DNA of the studied plants.

The expression of the thaumatin gene was resulted in sweet taste properties for such transgenic crops as tomato [1], potato [12], cucumber [13] etc. We were also able to feel the changes in taste, of the obtained transgenic tobacco plants.

The analysis of the adaptive potential of the obtained transgenic plants to salt stress was carried out by the cultivation of the transgenic tobacco plants and untransformed (control) ones on MS media containing 100mM NaCl, 200 mM NaCl, 300 mM NaCl for 14 days. In two weeks we measured the relative growth rate (RGR) of the studied plants. RGR was calculated as RGR = (lnM2 – lnM1) / (t2 – t1), where M1 and M2 – plant biomass, t1 and t2 – harvest times [14,15].

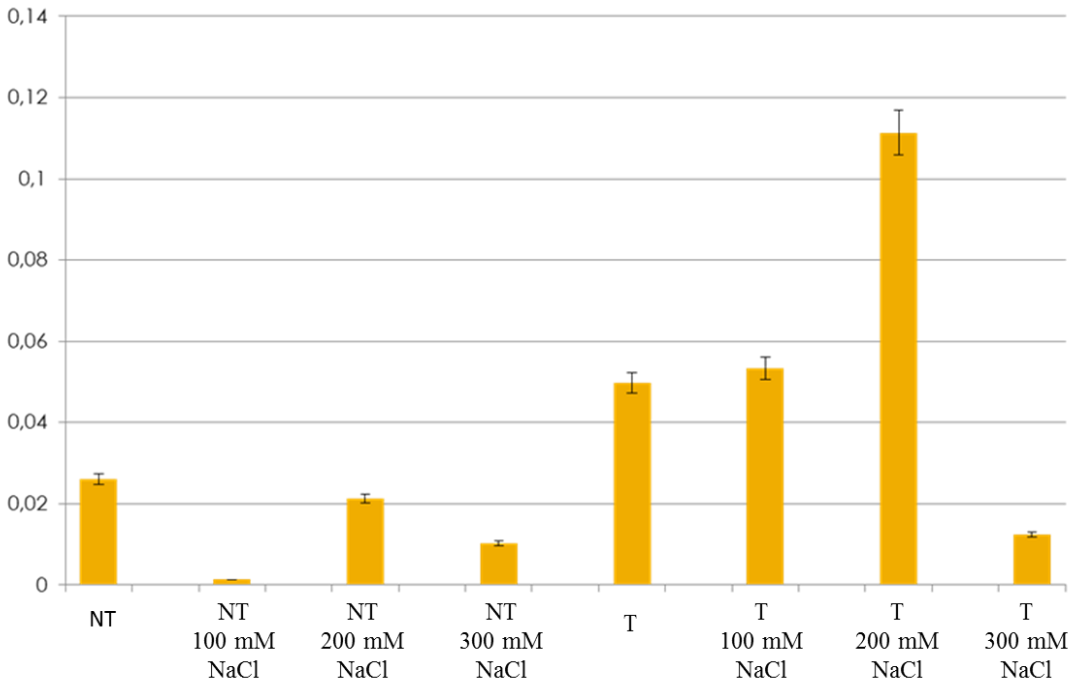

Figure 3. RGR-test of the studied tobacco plants

NT – nontransgenic plants; T– transgenic tobacco plants expressing the thaumatin II gene

The RGR-test proved a significant increase of the resistance of transgenic tobacco plants to salt stress comparing to non-transgenic ones. The graph shows the higher adaptive potential of the transgenic tobacco plants to salt stress comparing to non-transgenic ones. As the concentration of 200 mM corresponds to the average salinity of the soils of the steppe zone of Ukraine, the obtained plants seem the promising models for production of salt-stress tolerant crops.

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