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SENSIBILITY TO PHYTORHABDOVIRUSES OF TRANSGENIC TOBACCO PLANTS

Spivak N. Ya.[1], Kochetov A. V[3-4], Lozova O. I.[1], Levenko B. A[2], Yuzvenko L. V.[1], Nikolaychuk M. V.[5], Levchuk O. B.[1], Didenko L. F.[1], Trifonova E. A.[3], Sangaev S. S.[3]

Sensibility to phytorhabdoviruses of transgenic tobacco plants. — N. Spivak[1], A. Kochetov[3,4], O. Lozova[1], B. Levenko[2], L. Yuzvenko[1], M. Nikolaychuk[5] ,O.Levchuk[1], L.Didenko[1], E.Trifonova[3], S.Sangaev[3]. — *Transgenic tobacco plants, that express a gene of bovine pancreatic ribonuclease, are characterized by increased virus resistance to a virus of a buckwheat burn (VBB). Transgenic plants of tobacco contained a virus in 2-3 times less quantity in comparison with nontransgenic control.*

Keywords: *transgenosis, phytorabdoviruses, virus resistance.*

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У трансгенних рослинах тютюну, які експресують ген панкреатичної рибонуклеази бика визначена підвищена вірусостійкість до вірусу опіку гречки (ВОГ). Вони містили вірус у 2–3 рази меншій кількості в порівнянні з нетрансгенним контролем.

У трансгенных растений табака, экспрессирующих ген панкреатической рибонуклеазы быка отмечается повышенная вирусоустойчивость к вирусу ожога гречки (ВОГ). Содержание вируса у них было в 2–3 раза меньше по сравнению с нетрансгенным контролем

Introduction

Increase of virus resistance of different plant species is coordinated by an expression of the genes turning on the complex mechanism of development of the antiviral response.

It is known, that some nucleases function as antiviral agents. Proofs of protective functions of nucleases and variants of practical use of these functions both for animals, and for plant cells have been received. It was shown earlier that the transgenes of extracellular ribonucleases increased plant tolerance to viruses. It was shown, that tobacco plants, expressing foreign gene of bovine pancreatic ribonuclease, are characterized by the increased resistance to TMV (1).

The purpose of the present investigation was to study a sensibility of transgenic tobacco plants *(Nicotiana tabacum)*, carrying the genes of bovine pancreatic ribonuclease and extracellular ribonuclease of *Zinnia elegans*, in each separate case, to phytorhabdovirus – a virus of a buckwheat burn (VOG). Interest of rhabdovirus use is motivated by the fact, that similar researches with phytorhabdoviruses were not carried out/ The mechanism of their reproduction differs from earlier studied TMV (1), besides that VOG infest valuable agricultural crops including buckwheat, potato, tomato, tobacco (3) VOG according to its morphology, structure of proteins, lipids and carbohydrates belongs to the family Rhabdoviridae (4), which includes pathogenic rhabdoviruses of human and animals – a rhabdovirus of rabies (RV) and a virus of a vesicular stomatitis (VSV) (5).

Therefore experimental data can be considered as potentially possible uniform mechanism of obtaining virus resistance of plants and animals to phyto- and zoorhabdoviruses.

Material and methods

Transgenic *Nicotiana tabacum* plants, that expressed genes of bovine pancreatic ribonuclease (1) and of extracellular ribonuclease of *Zinnia elegans* were studied. A virus of buckwheat burn was isolated from makhorka plants (*Nicotiana rustica)* as it is described earlier (3).

Transgenic and control plants of tobacco were inoculated by the cleared virus preparation in concentration of 100 mkg/ml. Tobacco plants, inoculated by the buffer which did not contain the virus, were as the control. All transgenic and nontransgenic plants, without dependence on a visual estimation of the virus pathology developed on them, have been used for VOG isolation, that finally reflects real accumulation of a virus in investigated samples.

Analysis of a protein spectrum with ribonucleic activity in extracts of tobacco transgenic and control plants was done according to the procedure (6) with some modifications. Ribonuclease activity was measured in each experiment in the plants of the same age, which have been grown up in standard conditions of a green-

house. Extracts of plant proteins were extracted as follows: 1g of leaf tissue was freezed in liquid nitrogen, homogenized and suspended in 1 ml 50мM Tris-HCl (pH 7,5). Homogenate was centrifuged during 10 min. at 12000g at 4°C. In supernatant total concentration of protein was defined according to Bradford (6). Extracts of tobacco leaves, containing 25 mkg of total protein were analyzed for ribonuclease activity by electrophoresis in 10% PAAG (7). Dividing gel contained 200 mkg/ml of bovine fibrinogen and 2 mg/ml of yeast RNA (Sigma). After electrophoresis at a room temperature sodium dodecyl sulfate was washed out from gel twice during 15 min. by 25 % isopropanol solution in 10mM Tris-HCl (pH 7.0). Isopropanol was washed away by washing up of gel thrice by 10mM Tris-HCl (pH 7.0). Then gel was incubated in 100 mM Tris-HCl (pH 7.0) during 3 hours. These conditions provide degradation of RNA in gel composition by renaturated RNA-ases. After incubation gels were rinsed by 10mM Tris-HCl (pH 7.0) and imbued during 10 min. by 0,2 % toluidine blue (Sigma) in 10 mM Tris-HCl (pH 7.0). Gels washed from a die by 10 mM Tris-HCl (pH 7.0) and scanned.

Results and discussion

Transgenic plants that expressed in each separate case a gene of bovine pancreatic ribonuclease and extracellular ribonuclease of *Zinnia elegans* did not differ from control plants by a phenotype and time of their development.

For clarification of virus resistance of transgenic tobacco plants to VOG, transgenic and control plants were inoculated by a virus in 100 mkg/ml concentration.

Isolation of a virus from investigated samples have detected approximately identical accumulation of a virus in transgenic lines of tobacco, that expressed a gene of bovine pancreatic ribonuclease and a gene of extracellular ribonuclease of *Zinnia* in 14 days after infection. At 21-st day in the first case increased concentration of a virus was not revealed, while in transgenic tobacco plants, that expressed gene of extracellular ribonuclease of *Zinnia,* a marked increase of a virus concentration (approximately in 2,5 times) was noted. Some increase of virus contents was noted at 28-th day.

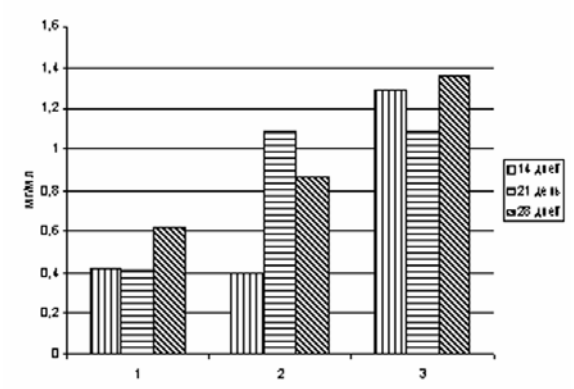
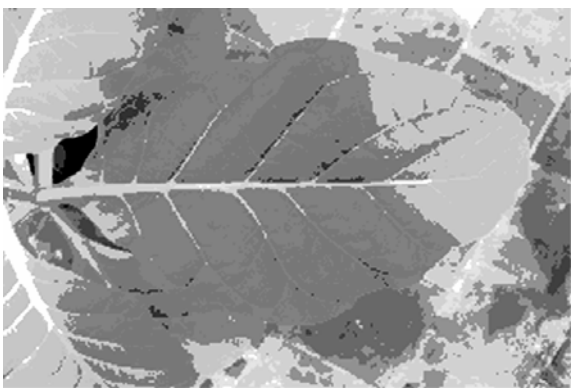


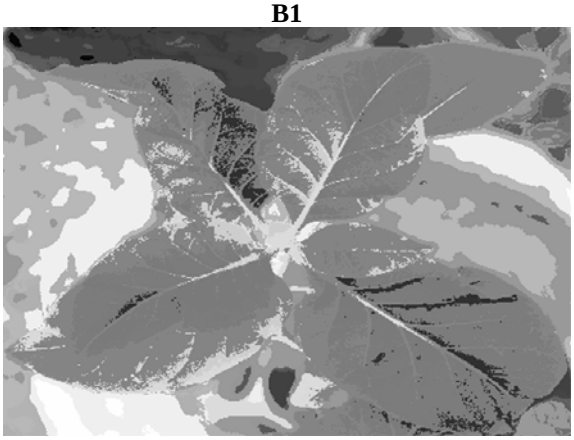
Fig. 1. Concentration of a virus in transgenic tobacco plants, that expressed the genes of heterological secretory nucleases:
1 – bovine pancreatic ribonuclease, 2 – ribonuclease of *Zinnia elegans,* 3 – nontransgenic tobacco

A1

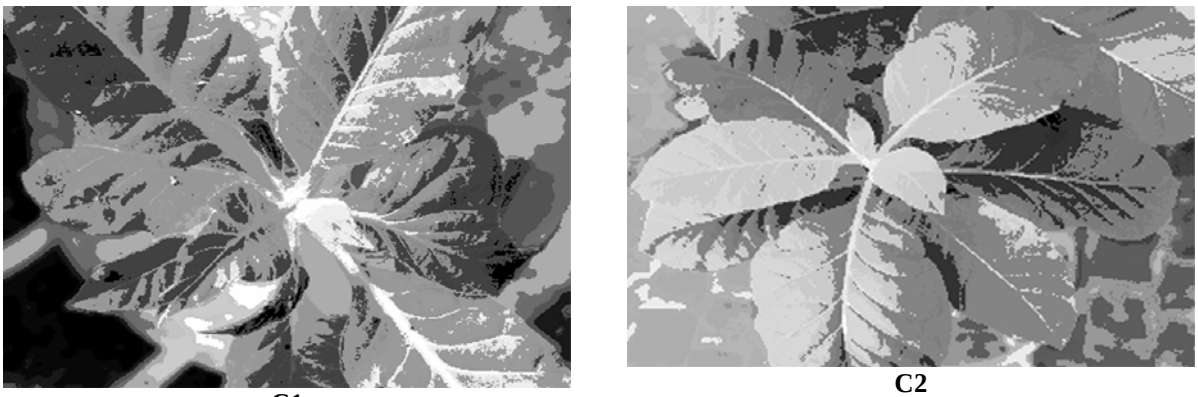


A2

B1



B2



C1 **C2**

Fig. 2. Symptoms of disease induced by VOG on transgenic and nontransgenic tobacco plants (1 – infected by VOG; 2 – control, mok-inoculated): A. – transgenic tobacco, that expressed a gene of bovine pancreatic RNA-ase; B. – transgenic tobacco plants, that expressed a gene of extracellular nuclease of *Zinnia*; C. – nontransgenic tobacco plants

In control nontransgenic tobacco plants at 14-th day after infection more than triple accumulation of a virus, in comparison with transgenic tobacco plants was fined. As a whole the level of virus concentrations in control plants was higher in comparison with its contents in transgenic tobacco plants. At the same time it is necessary to note, that transgenic plants of tobacco, that expressed a gene of bovine pancreatic ribonuclease were more tolerant to VOG in comparison with transgenic tobacco plants, that expressed a gene of extracellular RNA-ase of *Zinnia* (Fig. 1).

Transgenic tobacco plants, that expressed a gene of bovine pancreatic ribonuclease were characterized not only an essential delay of virus accumulation, but also by exhibiting symptoms of an infection in comparison to tobacco plants, that expressed a gene of extracellular ribonuclease of *Zinnia* and those of the control (Fig. 2, A, B, C).

Analysis of nuclease activity of transgenic and control tobacco plants in SDS-polyacrylamide gel with addition of RNA in matrix of dividing {sharing} gel has been done. As it is seen from fig. 3, in transgenic plants of tobacco, that expressed genes of bovine ribonuclease bands are present, that correspond to the position of RNA-ase, degrading RNA in gel matrix, which correspond with a line designating degradation of bovine commercial pancreatic RNA (Fig. 3).

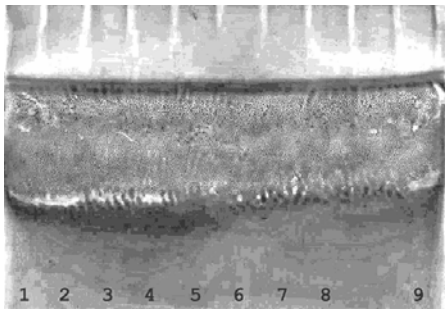


Fig. 3. Analysis of nuclease activity of transgenic and control tobacco plants in SDS-polyacrylamide gel with addition of RNA in matrix of dividing gel. Drawings on lines: 1, 2, 3 – extracts of total proteins of transgenic plants of tobacco at 14, 21, 28 days after infection by VOG; 4 – extract of proteins from a transgenic tobacco which has been not infected by VOG; 5, 6, 7 – protein extract from nontransgenic tobacco, isolated at 14, 21, 28 days after infection by VOG; 8 – protein extract of nontransgenic, noninfected tobacco plant; 9– commercial preparation of bovine pancreatic ribonuclease

Thus it is possible to make the assumption, that heterological nuclease – bovine pancreatic ribonuclease participates in antiviral protection from VOG of transgenic tobacco plants, without dependence from nonspecific character of a mediated nuclease stability.

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