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ВЫЯВЛЕНИЕ ВИРУСОВ МОЗАИКИ АРАБИСА И АСПЕРМИИ ТОМАТА СРЕДИ ОРХИДНЫХ ПРИРОДНОЙ ФЛОРЫ УКРАИНЫ

Проведено обследование террестриальных орхидных природных биоценозов Украины и коллекции орхидных Национального ботанического сада имени Н.Н. Гришка. Идентифицированы два вирусных патогена среди исследованных растений: вирус мозаики арабиса и вирус аспермии томата.

Ключевые слова: вирусы орхидных, вирус мозаики арабиса, вирус аспермии томата

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THE INFLUENCE OF ANTROPOGENIC LOAD AND OF THE PHYTOVIRAL INFECTION ON THE SYNTHESIS OF PHENOLIC COMPOUNDS

Have been shown a direct relationship between the degree of anthropogenic load and the degree of infestation by viruses, and the content in these plants phenolic compounds. Also, shown that the reproduction of TMV in sugar beet plants inducses the synthesis of phenolic substances that accumulating, probably, start to inhibit virus accumulation.

Key words: anthropogenic load, infestation by viruses, phenolic substances.

Introduction. One of the ecological and biochemical mechanisms of plant adaptation in the complex influence of anthropogenic factors and viral infections. are the changes in the composition and in the quantitative ratio connections of the compounds in the antioxidantal group, in particular-phenolic nature [1].

Phenolic compounds – one of the most important classes of secondary metabolites, widely represented in plants. Various functions of phenolic compounds in the plant cell, and at the same time a wide range of biological effects on humans and animals, justify study of its participation in the development of protective reactions of organisms in adverse conditions of existence [2]. The literature on the effect of stress factors on the content of phenolic compounds in plants is fragmentary and contradictory. However, it clearly indicate the variability of these parameters [3].

This fact confirms the assumption of importante role of phenolic compounds in the development of resistance of plants to changes in the intensity of natural and anthropogenic stress factors [4].

According to the all written above, the goal of the research was:

– to analyze the influence of the location of the place of grown about the factors of anthropogenic load on the fitovirusological state of plants of sugar beet (*Beta vulgaris* L) and content of phenolic compounds in them;

– to confirm the in vitro correlation between virus reproduction and synthesis of phenolic compounds.

Materials and methods. Method of random samples from agrocenosis with varying degrees of anthropogenic load on visual symptoms were selected sugar beet plants, which further were investigated by undirected enzyme-linked immunosorbent assay for the presence of viral antigens and achohol extraction method for determining the concentration of phenolic compounds [5, 6]. Leaf blades, which were selected for the ELISA, were dried and measured their content of phenolic compounds.

Analysis of results. ELISA was demonstrated the dependence of the distribution and antigen detection percent in plants from the place of sampling. The highest content of phenolic compounds in plants we recorded in the areas with a high degree of anthropogenic load and, accordingly, the greatest defeat by viral pathogens.

This interconnection has been saved in relation to other locations.

To confirm the interconnection between the accumulation of viral particles and the change in the level of concentration of phenolic compounds, we made a laboratory experiment on the model of "sugar beet plant – TMV."

The content of phenolic compounds in plants infected by a virus increased irregularly, compared with the control.

When we compared the dynamics of accumulation of viral antigens and phenolic compounds in sugar beet plants infected with TMV, this dependence can be traced as: in the period between 3 and 6 day after an infection we observed a slight increase of the concentration of viral antigens against the background of the concentration of phenolic compounds. From 6 to 10 day a number of fenolic compounds increases even faster than in the previous period. The concentration of the TMV antigens in this time wasn't changing.

Findings. This way, we have shown a direct relationship between the degree of anthropogenic load and the degree of infestation by viruses, and the content in these plants phenolic compounds.

It is shown that the reproduction of TMV in sugar beet plants inducses the synthesis of phenolic substances that accumulating, probably, start to inhibit virus accumulation.

Sugar beet – biennial plant and can't be used for laboratory model "virus – a plant" and is not suitable for laboratory researches we are doing the selection of the optimal model system for the study of interference of viruses and synthesis of phenolic compounds in the plant and the use of phenolic compounds by the plants when there are some stress factors, like abiotic and biotic nature.

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ВПЛИВ АНТРОПОГЕНИХ ФАКТОРІВ НА СИНТЕЗ ФЕНОЛЬНИХ СПОЛУК ПРИ ФІТОВІРУСНІЙ ІНФЕКЦІЇ

Було показано прямий взаємозв'язок між рівнем антропогенного навантаження та рівнем інфікованості вірусом, а також вмістом фенольних сполук в цих рослинах. Також, показано, що відтворення ВТМ в рослинах цукрового буряку індукують синтез фенольних сполук які акумулюючись, можливо, починають пригнічувати накопичення вірусу.

Ключові слова: антрпогене навантаження, рівень інфікованості вірусом, фенольні сполуки.

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ВЛИЯНИЕ АНТРОПОГЕНЫХ ФАКТОРОВ НА СИНТЕЗ ФЕНОЛЬНЫХ СОЕДИНЕНИЙ ПРИ ФИТОВИРУСНОЙ ИНФЕКЦИИ

Была показана прямая взаимосвязь между уровнем антропогенной нагрузки и уровнем инфицированности вирусом, а также содержанием фенольных соединений в этих растениях. Также, показано, что воспроизведение ВТМ в растениях сахарной свеклы индуцируют синтез фенольных соединений которые аккумулируясь, возможно, начинают подавлять накопление вируса.

Ключевые слова: антрпогенная нагрузка, уровень инфицированности вирусом, фенольные соединения.

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ISOLATION OF *INFECTIOUS PANCREATIC NECROSIS VIRUS* FROM WILD-LIFE RAINBOW TROUT *ONCORHYNCHUS MYKISS* IN WESTERN UKRAINE

During routine sampling and testing of catched wild-life fish species from Siret river of Chernivtsi region, an aquatic birnavirus was isolated from diseased rainbow trout Onhorhynchus mykiss. Preliminary examination of diseased fish revealed a range of lesions particularly in pancreatic tissue. Virus isolate grew in fish cell line RTG-2, FHM and EPC. IPNV caused morphological changes, such as vacuole enlargements and cells rounding. Subsequently cells scaled from a surface and characteristic cytopathic effect (CPE) of virus on cells was visible. Examination by electron microscopy demonstrated that the isolated virus was ultrastructurally similar to IPNV. In addition after amplification of viral RNA incorporating three different primer pairs the IPNV specified PCR products were visible on agarose gel stained with ethidium bromide. The nucleotide sequences of amplified fragments were analysed and the prevalance of Ukrainian isolate, which was named "Karpaty", to Sp strain was revealed. The comparison of sequences of IPNVs VP2 and NS genes from NCBI and amplified fragments of IPNV strain "Karpaty" confirmed the high identity of 95-99% with Sp strain, firstly isolated in Denmark. Among the isolates of Sp strain the most related to IPNV "Karpaty" were viruses found in Great Britain, Norway, France, Turkey and Iran.

Key words: Infectious pancreatic necrosis virus, cell culture, RT-PCR, sequence analysis.

Introduction. Infectious pancreatic necrosis virus (IPNV) belongs to the family *Birnaviridae* and is an agent of an acute, contagious fish disease causing high mortality not only in juvenile salmonids but also in non-salmonid fishes. Members of the family *Birnaviridae* are icosahedral viruses of approximately 65 nm in diameter composed of five polypeptides and two strands of double-stranded RNA [1].

In young salmonid fish, IPNV can cause high mortalities followed by a life-long, chronic infection in the survivors. Persistently infected fish are asymptomatic that have virus in many visceral organs and can shed live virions [2]. The IPNV is widely distributed in Europe and there are severel new reports about virus isolation in neighboring countries of Ukraine such as Poland, Czech Republic, Slovakia and Russia [3,4]. Since salmonids breeding are mainly located in the west region of Ukraine the IPNV is an economically important fish pathogen for all Ukrainian trout farms.

The **aim** of present study was to isolate IPNV from salmonids in Ukraine. Therefore the goals of the present study were to provide preliminary characterization of isolated strain in cell culture and electron-microscopy, to select valid oligonucleotide primers and test it in PCR assay; to provide sequencing of amplified products in way of verification of target amplification; and to accomplish the phylogenetic analysis of Ukrainian IPNV strain.

Materials and methods. During June 2011, a total of 14 fish samples (body weight 0.4-0.7 kg) were continuously collected from a diseased wild-life rainbow trout catched in Siret river, west region of Ukraine. Each fish was dissected, and the samples of pancreas, kidney and spleen were removed from individual fish and placed into a 1.5 ml microcentrifuge tube. Samples were transported on ice to the laboratory and processed immediately.

RTG-2, FHM and EPC cell lines were maintained in DMEM medium (SIGMA) supplemented with 100 U ml^{-1} penicillin, 100 μg ml^{-1} streptomycin and 10% fetal bovine serum (FBS). The samples of kidney and spleen of tested fish were homogenized with DMEM and filtred through the 0,45 μm membrane (Millipore). Then the virus suspension was inoculated onto 24-hours cell monolayers growing in 25 cm^2 flasks. After adsorption for 60 min at 20°C, DMEM medium supplemented with 2% FBS was added to the monolayers. When a complete viral cytopathic effect (CPE) was evident, the tissue culture supernatant was harvested and centrifuged at 2500 × *g* for 10 min at 4°C to remove cell debris. The 50% tissue culture infective dose ($TCID_{50}$ ml^{-1}) of the resulting supernatant was determined [5].

The IPNV was purified both from collected organs and the tissue culture supernatant by the method of ultracentrifugation. Briefly, after cell debris was separated by centrifugation at 2500 × *g* for 10 min at 4°C the pellet was discarded and the supernatant was centrifuged in ultracentrifuge Beckman L5-50B in a rotor SW-40 for 60 min at 70500 × *g* at 4°C. The virus pellet was suspended in TNE (50 mM Tris-HCl, 150 mM NaCl, 1 mM disodium ethylene diaminetetracetic acid [EDTA], pH 7.5) and centrifuged at 2500 × *g* for 5 min at 4°C. Then the virus suspension was used for electron-microscopy investigation and viral RNA extraction [6].

For electron-microscopy investigation the viral suspension was stained with 2% uranyl acetate and studied in electron microscopy EM-125.

Genomic viral RNA was extracted from collected fish organs, viruses-infected cells culture supernatant and purified virus suspension using GeneJET™ RNA Purification Kit (Fermentas) as described in manufacturer's protocol. After elution of the RNA in DEPC-treated water, it was kept at -20°C until required. The cDNA synthesis was conducted using RevertAid™ Premium First Strand cDNA Synthesis Kit (Fermentas) following the manufacturer's instructions and subjected to PCR amplification.