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THE HOST RANGE FOR UKRAINIAN ISOLATE FOR HOSTA VIRUS X (HVX)

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Abstract. *The goal of this project was to identify the host range of HVX. The Tobacco plants Nicotiana bentamiana were estimated to be the most susceptible to the pathogen using ELISA and experimental inoculation.*

Key words: *Hosta Virus X, susceptible host plants, Nicotiana bentamiana*

Hosta virus X (HVX) is rapidly becoming a serious pathogen of commercially important hosta plants worldwide. HVX is transmitted by contaminated cutting tools and vegetative propagation and is also could be transmitted by the seed. HVX symptoms in hostas including: leaf mosaic, leaf twisting, plant stunting and death.

Mass viral infection of plants through the mechanical inoculation and long period of asymptomatic persistence of the virus in plants (from several weeks to several years), causes the complexity in early HVX diagnostics. Thus, the probability of infection of healthy *hosta* plants and other plants increases, resulting into the rise of genetic variability of HVX due to viral genome mutations. HVX is transmitted with the planting material. Nowadays, globalization and growth of international trade of the ornamental plants increase the risks, associated with the viral widespread worldwide, consequently causing economic loss in different states [1].

Initially, HVX was detected on the territory of Ukraine in 2012 [2]. Virus caused manifestation of various typical symptoms in *hosta* plants, associated with the virus. Depending on the class, the symptoms included: leaf deformation of the infected plants, discoloration and necrosis. The researches, which were carried out,

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showed, that Ukrainian isolate of the virus is genetically close to the isolates, [illegible] in the USA and Poland [3].

The virus is easily transferable from one plant to another by mechanical means and as a result of vegetative propagation. This is confirmed by the presence of viral symptoms as well as positive serological and molecular tests results.

Thereby, in order to avoid contamination of healthy plants and for detection of already infected ones, the virological control of planting material is needed for successful diagnostics of the viral infection. Consequently, the aim of our research was to find out and describe a number of hosts of Ukrainian HVX isolate. Experimental infection of several indicator-plants was conducted using Ukrainian HVX isolate, derived from *hosta sum and substance genus and taken from Grishko National Botanical Garden in Kiev.*

Materials: Ukrainian HVX isolate, derived from *hosta sum and substance genus and taken from Grishko National Botanical Garden in Kiev.* Following list of indicator-plans was used/verified for the research:

1. Zuccini, “Tsukisha” genus (*Cucurbita pepo ssp. pepo*)
2. Momordica genus (*Momordica chrantia*)
3. Tabacco, shag (*Nicotiana rustica*)
4. Tomato (*Lycopersicon esculentum*)
5. Pepper (*Capsicum annuum ssp. grossum FiL.*)
6. Melon (*Melo sativus*)
7. Indian Datura (*Datura metel L.*)
8. Tobacco *(Nicotiana bentamiana)*

Inoculation of the plans was done by mechanical means using standard methodology [4].

Methods: biological testing methodology was used as well as ELISA with specific polyclonal antibodies to HVX.

Results and discussion. Results of experimental infection showed the presence of the symptoms only in tomato (*Lycopersicon esculentum*), pepper (*Capsicum annuum ssp. grossum FiL.*) and tobacco *(Nicotiana bentamiana)***.**

Leaves of tomato (*Lycopersicon esculentum*) appeared to have relatively big area of necrosis (1-1,5 мм) with light-brown color; pepper (*Capsicum annuum ssp. grossum FiL.*) had the same symptoms: light-brown leaves necrosis (0,5-1,5 мм); tobacco *(Nicotiana bentamiana)* showed symptoms, associated with leaves mosaic.

Other plants, used for the tests, didn't manifest any visible symptoms (Table 1).

1. Indicator-plants reaction on the HVX infection

№	Indicator-plants	Type of reaction on the HVX infection
1.	(*Cucurbita pepo ssp. pepo*)	Inoculated leaves didn't show any observable symptoms; next generation of leaves didn't show any signs of infection symptoms either. Immunity.
2.	(*Momordica chrantia*)	Inoculated leaves didn't show any observable symptoms; next generation of leaves didn't show any signs of infection symptoms either. Immunity.
3.	(*Nicotiana rustica*)	Inoculated leaves didn't show any observable symptoms; next generation of leaves didn't show any signs of infection symptoms either. Immunity.
4.	(*Lycopersicon esculentum*)	A relatively big area of necrosis (1-1,5 мм) with light-brown color was observed on the leaves after 2-nd day of the infection. Next generation of leaves showed the same mosaic/necrosis (выбери правильное) symptoms.
5.	(*Capsicum annuum ssp.grossum FiL.*)	Light-brown leaves necrosis (0,5-1,5 мм) was observed on a 2-nd day of inoculation; next generation of leaves didn't show any visible symptoms.
6.	(*Melo sativus*)	Inoculated leaves showed no visible symptoms; next generation of leaves didn't show any visible symptoms. Immunity.
7.	(*Datura metel L.*)	Inoculated leaves showed no visible symptoms; next generation of leaves didn't show any visible symptoms. Immunity.
8.	*(Nicotiana bentamiana)*	Inoculated leaves showed mosaic symptoms.

The samples with productive infection were identified using ELISA.

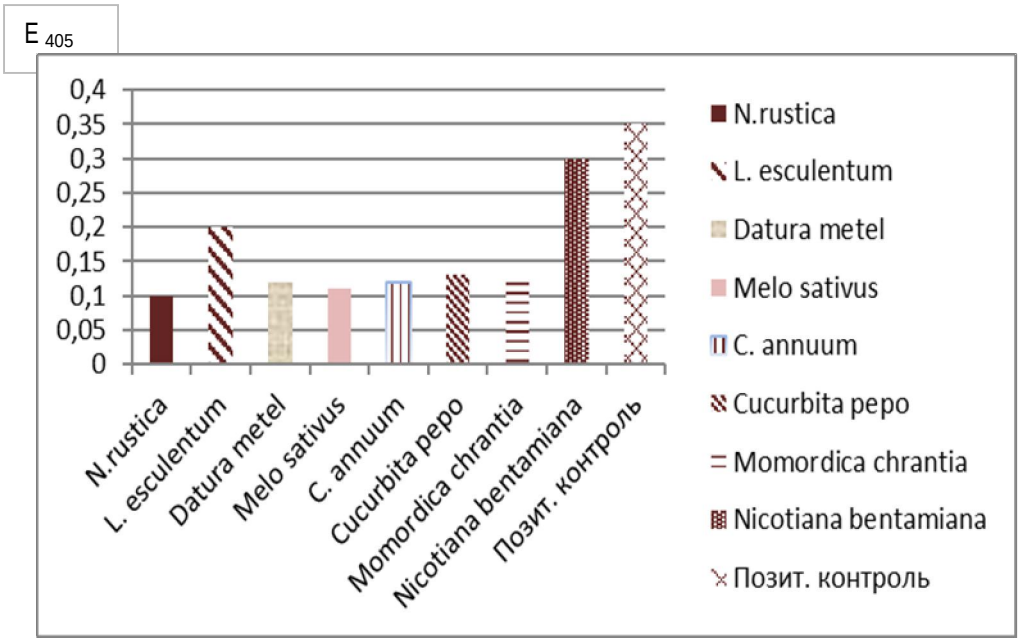

Fig. 2. ELISA results for HVX diagnostics in indicator-plants

Only tobacco plants (Nicotiana bentamiana) showed productive HVX infection, according to the test (Fig.2).

During the experiment, multiple light-brown necrosis were diagnosed on tomato (*Lycopersicon esculentum*) leaves (1-1,5 mm) and pepper *Capsicum annuum ssp. grossum FiL.*) leaves (0,5-1,5 mm).

It is known that local necroses appear as a result of death of infected cells, where pathogen penetrated into susceptible plants. During the necrosis process, the signal substances are made, which activate the defensive mechanisms in neighbor and distant cells. Thus, death of the cell initiates the production of anti-pathogenic ferments and metabolites into intercellular space, which block the infectious spread in the plant, resulting into elimination of the virus. Consequently, above-mentioned processes lead to the plant's recovery [5].

Additionally, the possibility of limited viral accumulation in susceptible plants is present, which manifests into formation of local necroses: (tobacco, shag (*Nicotiana rustica*); pepper (*Capsicum annuum ssp. grossum FiL.*). As a result of defensive mechanisms of the plants, this led to the death of infected cells, which in turn, oppressed viral transport in the infected plant and helped to form specific antiviral defense. The main role of defense in resistant plants was played by substances with anti-viral activity, which may be present before or/and after the

infection took place. Principles of inhibitors action differ – they may affect viral particles directly or they may affect metabolites of the host plants. It is believed, that the viral spread in inoculated leaves of hyper-sensitive plants is blocked because of the local necroses [6, 7].

The localization of the viruses is not necessary related to the plant's necroses, which are being infected. It is believed, that "neighbor viral transport" happens only in the period of local necroses growth in infected hyper-sensitive plants and virus doesn't proliferate further out of formed necroses [8].

N.I. Vavilov called it specific immunity when plant isn't a natural pathogen host, which later has been re-named to non-specific plant resistance to the pathogen. If we consider viral infection, above-mentioned type of immunity is typical for the plants, where viral particles aren't able to reproduce themselves. Conclusively, immunity could be seen as absolute plant non-compliance to the viral infection.

Plants, which have immunity to the infection, the time of viral reproduction is heavily inhibited. Later, this is followed by almost full absence of the visible symptoms of the disease. In our case, these were such asymptomatic infected plants as: zucchini (*Cucurbita pepo ssp. pepo*); momordica (*Momordica chrantia*); melon (*Melo sativus*); Indian datura (*Datura metel L.*) (Table 1)

During the visual inspection of experimental plants, it was noted that a group of plants, which was virally infected, differs from the control group, particularly a following leaves generation in tobacco (*Nicotiana rustica*) and pepper (*Capsicum annuum ssp. grossum FiL.*) - their size significantly exceeded the size in the asymptomatic control group. The immunity of infected plants activated specific defensive mechanisms and ferments, which gave the plants opportunity to develop more actively than in a control group.

In conclusion, from 8 of the studied indicator-plants, HVX was only visually observed on tomato (Lycopersicon esculentum) and pepper (Capsicum annuum) in the form of necroses. At the same time, tobacco (Nicotiana bentamiana) showed mosaic-like symptoms. Using ELISA, it was showed the presence of HVX antigens only in tobacco (Nicotiana bentamiana).

Limited spread of HVX in tomato (Lycopersicon esculentum) and pepper (Capsicum annuum) with local necroses only, appears as a result of defensive plant reaction, followed by the death of infected cells and eventually restricts viral accumulation. Ukrainian HVX isolate, which was studied on the range of plant-indicator hosts, showed minor symptoms on several plants; however, ELISA showed that antigens were present only in tobacco (Nicotiana bentamiana). Derived data match to the data from scientific literature [9].

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СПЕКТР РОСЛИН ІНДИКАТОРІВ ДЛЯ УКРАЇНСЬКОГО ІЗОЛЯТУ Х ВІРУСУ ХОСТИ (ХВХ)

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Анотація. *Метою даного дослідження було встановлення спектру рослин індикаторів для ХВХ. За результатами експериментального інокулювання та імуноферментного аналізу було встановлено, що рослини тютюну Nicotiana bentamiana виявилися найбільш чутливими та сприйнятливими до даного збудника.*

Ключові слова: *Х вірусу хости, рослини індикатори, Nicotiana bentamiana*

СПЕКТР РАСТЕНИЙ ИНДИКАТОРОВ ДЛЯ УКРАИНСКОГО ИЗОЛЯТА Х ВИРУСА ХОСТЫ (ХВХ)

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Аннотация. *Целью данного исследования было определение спектра растений индикаторов для ХВХ. По результатам экспериментального инокулирования и иммуноферментного анализа было установлено, что растения табака Nicotiana bentamiana оказались наиболее чувствительными и восприимчивыми к данному возбудителю.*

Ключевые слова: *Х вируса хосты, растения индикаторы, Nicotiana bentamiana*