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SUMMARY OF LAB DIAGNOSTICS FOR VIRUS PESTS ENDANGERING GLASSHOUSE FARMING OF VEGETABLES IN UKRAINE: THE OUTCOMES, SOURCES OF INFECTION, AND CONCLUSIONS

Данная работа обобщает результаты проведенных лабораторией научных экспертиз растительного, семенного и посадочного материалов овощных культур, которые выращиваются в тепличных условиях. Приведены данные относительно инокулюма и рекомендации для предотвращения развития вирусных инфекций.

This work summarizes the outcomes of conducted laboratory testings of plant, seed and seedling material for vegetable cultures grown in glasshouse conditions. Here we provide data of the source(s) of infection and recommendations regarding preventive measures against virus diseases.

Introduction. The Department of Virology at Taras Shevchenko' Kyiv National University is a known expert in monitoring and diagnostics of virus diseases of agricultures and in plant virus epidemiology. During the last decade we have been extensively involved in studying virus diversity and spread for both natural and manmade ecosystems for fundamental issues (virus ecology, virus epidemiology and evolution). This is reflected in vast number of publications [1-4] and related joint international projects with leading institutions from Czech Republic, Germany, Bulgaria, Hungary, Turkey, Belarus and the USA.

Glasshouse farms represent so called 'closed ground' systems and having stable temperature, light and humidity conditions are posed to significantly higher risk of development of bacterial, virus and fungi diseases. Artificial conditions of glasshouses not only favor propagation of plant virus vectors – insects and nematodes – but also seriously intensify mechanical transmission of the pathogens due to the elevated density of plants and fungi growing there. Because of indicated reasons, mixed infections of plants or fungi with viruses and other pathogens are rather common for glasshouses and endanger the commercial outcomes of farming. Analysis of plant/seed material and soil, fungi and substrate for viruses will allow reacting adequately, introduce prophylactic measures and minimize losses of commercially valuable production.

During the last 5 years we have faced a growing demand from conscious farmers looking for possible means of improvement of their farming business. Glasshouse farming has become an important branch of agriculture in Ukraine and is growing with a very fast pace. Among the major plant cultures grown in glasshouse conditions are typical vegetables: tomato, cucumber, sweet pepper, eggplant, cabbage and zucchini. It is worth saying that new glasshouses are mostly state-of-the-art both in terms of the structure itself and in terms of technology, and virtually every possible measure for plant cultivation (the light, watering regime and technologies, use of special substrates, nutritional norms, etc.) and defense (use of certified seed material, use of sterile substrate, vector control, etc.) has been put into place.

In spite of such measures, we are regularly contacted by farmers asking to check their plant/seed/substrate material for viruses infecting different cultures. Here we represent major outcomes of such experiments and provide our conclusions.

Materials and methods.

Sample preparation

Samples of vegetative tissues were homogenized in sterile mortars with autoclaved 0.1M PBS buffer, pH 7.4 (1:2-1:4, w/v), at 4˚C. Then the extracts were subjected to low-speed centrifugation at 5000 rpm for 20 min (4˚C) on RS-6 centrifuge ('TNC DASTAN', USSR) to remove the debris [5]. Obtained clarified extracts were further used for virus detection via ELISA.

In case of seeds, these were soaked in sterile water at room temperature for 24-72 hours and then treated as described above. Soil and substrate samples have been homogenized the same way.

ELISA

We have used DAS-ELISA as recommended by EPPO [6]. In this work we have employed commercial antisera for different viruses of Loewe and DSMZ (Germany), Prime Diagnostics (The Netherlands), and INRA (France) following the manufacturers' instructions.

Briefly, 96-well sterile plates ('Labsystems', Finland) were coated with unconjugated virus-specific antibodies and incubated for 4 h at 36˚C to allow to adhere to the wells. Then the samples (clarified sap) were deposited and incubated overnight at 4˚C. Then the conjugated virus-specific antibodies were applied and incubated for 4 h at 36˚C. Each step was followed by triple washing of the plates with 0.2% Tween in 0.1M PBS, pH7.4. The last step was the application of substrate solution (n-nitrophenyl phosphate) for alkaline phosphatase ('Sigma', USA) to visualize positive reaction, and incubation for 60 min at room temperature in the dark. The reaction was stopped with 3M NaOH. Finally, the results were counted at the wavelength of 405 nm (OD405) on ELISA reader ('Dynatech', Germany).

Results and discussion. Among the major cultures grown in glasshouses are: *Lycopersicon esculentum*, *Capsicum annuum*, *Cucumis sativus*, *Solanum melongena*, *Brassica oleracea* and *Cucurbita pepo* (zucchini). These can be infected by a vast list of viruses. We have carried out the testing for major and most dangerous viruses only, which are most typical for these plants and found regularly in Europe (and Ukraine) both in glasshouses and open fields. The results are given in Table 1.

***Table* 1. Summarized outcomes of testing of major vegetable cultures grown in glasshouse conditions for virus diseases (2008-2012 yy)**

Culture	Viruses found	Overall percentage of infected plants	Tentative source of infection
Lycopersiconesculentum	CMV, PMMV, TRV, TMV, TRSV, AMV	5-50%	Mechanical transmission or vector
Capsicumannuum	CMV, AMV, PMMV, ToMV, TMV, TRSV, TSWV	5-60%	All possible means of infection
Cucumissativus	CGMMV, CMV	10%-100%	Mechanical transmission, seed material (CMV)
Solanum melongena	CMV, TRV, TSWV, TRSV, AMV, ToMV	10-90%	All possible means of infection
Brassicaoleracea	AMV	5-50%	Mechanical transmission or vector
Cucurbita pepo (zucchini)	ZYMV, CMV, WMV-2	10-100%	Vector or mechanical transmission, seed material (CMV)

As can be seen from this table, many viruses are found to invade these cultures in glasshouses. As normally only diseased (symptomatic) plants are brought into the lab for subsequent analysis, the infection rate varies from moderate (5-10%) to high or extreme (50-100%).

We should also say that relatively many plant samples (up to 10%) were infected with 2 viruses or more (mixed infection).

Analyzing the sources of infection(s) we came to surprising conclusion. In most cases the seeds of respective cultures were virus-free, which is rather logical as the seeds are mostly imported being certified as virus-free. There are several exceptions and *Cucumissativus* seeds are the most prominent example here. Several batches of seeds of this plant obtained from different sources (i.e., farmers) contained CMV, and they were all imported from Russia.

The most common source of infection, again surprisingly, was mechanical transmission of the virus in the glasshouse. In such a way, something (tools? equipment?) or somebody (workers) brought the virus in the glasshouse where the initial infection then happened. Further on, initially infected plant has become a source of virus for its neighbours.

Vectors (flying insects, predominantly aphids) are a rare case as can be relatively easily monitored and controlled.

Analysis showed that soil and substrate are uncommon sources of infection, as they are regularly changed (substrate) or sterilized (soil).

Insofar we may conclude that major problems with viruses we encounter in glasshouse farming are the non-compliance with hygienic and sanitary norms.

Conclusions. In this work we have demonstrated that plants cultivated in 'controlled' glasshouse conditions are often infected by different 'uncontrolled' viruses. Surprisingly, even when using certified virus-free seed material and controlling vector populations in the glasshouse (virtually every farmer has confirmed that they have no vectors at all!) we still can see rather high level of virus infections in major vegetable cultures. This is reflected in millions of dollars lost annually in total.

Therefore, the neglect of proven hygienic and sanitary norms is the major cause of such losses. There two points every farmer can do in order to improve this situation, ensuring:

1) proper training of its personnel;

2) regular random-based diagnostics of plant material for most common viruses.

Sporadic testing (that's what our lab does, when we've sent already diseased plants for testing) can only witness moderate of high level of infection. Regular analysis will help eradicate diseased plants when they are not a source of infection yet (i.e., when there are no symptoms yet, and hence there's no reason to eradicate such plant).

Proper knowledge and conducting diagnostics in advance will help avoiding crop losses in future.

In addition, we may add that the cultivation of plants in the open field faces similar problems. However, the typical rate of infection for such plants is much lower. Same recommendations apply here as well – be prepared and act in advance.

Our rich experience, trained scientific personnel and state-of-the-art equipment for plant virus diagnostics allow to propose:

Diagnostics of virus diseases:

- Cereals (rice, wheat, barley, rye, oat, maize)
- Oil and industrial cultures (sunflower, cotton, sugar beet, sugar cane, rape, tobacco)
- Vegetableand cucurbitaceous cultures (potato, tomato, cucumber, pepper, eggplant, zucchini, marrow, melon, cucurbit)
- Legumes (soybean, bean, pea)
- Fruitcultures (apple, prune, cherry, sweet-cherry, реасняблуня, слива, вишня, черешня, персик)
- Decorative and aromatic cultures (orchid, rose, carnation, lavender).

Virus diagnostics may be carried out in plant material, soil, etc. Knowledge about which virus infections prevail in Your crops will allow You conducting: i) better and more reliable planning of land use and crop rotation, ii) optimal selection of virus-resistant or tolerant plant variety, iii) define most proper time for culture planting and harvesting. Moreover, virus diseases are prerequisites for further developments of fungal and bacterial pathologies, and hence their timely diagnostics would enable You to utilized plant protection means more efficiently.

Testing seed material on virus infections

Many virus diseases are efficiently transmitted by plant seeds. This is of special importance for wheat, rice, rye, barley, sugar beet, sunflower and legumes. Presence of virus in the seed pose a serious problem as every plant grown from this seed stock will be infected. Furthermore, seed control for the absence of virus pathogens is an acting international standard of sanitary epidemiological control of plants. Diagnostics of seed material in our lab will save You time and valuable relationships with Your partners.

Obtaining of virus-free planting material

In our lab, we adopted techniques for microclonal propagation of plants. The technology allows obtaining virus-free plant material even from virus-infected plants. Such approach is the only reliable mean to preserve unique decorative plants including roses, carnations, etc. The technique enables commercial growing rare or disappearing plant species independently from the season of the year. Microclonal plant propagation also allows to re-establish collections of planting material for genetic selection of agricultures.

Training and professional development in:

- Visual monitoring of crops of agrarian and decorative cultures for virus diseases
- Evaluation of pathogen spread on a given territory

- Methods for complex diagnostics of plant virus infections (theoretical and practical training)
- Analysis of plant material for presence of virus pathogens utilizing serological techniques (enzyme-linked immunosorbent assay, its modifications)
- Analysis of plant material for presence of virus pathogens utilizing molecular techniques (polymerase chain reaction, its modifications)

- Consulting servicesin diagnostics and monitoring of plant virus diseases
- Raising of specific antiserums to common plant viruses (for Your use inself-dependent serological diagnostics of virus disease via enzyme-linked immunosorbent assay)
- Design of molecular probes to any plant virus (for Your use inself-dependent serological diagnostics of virus disease via polymerase chain reaction).

Working with us, You may rely on our individual approach to Your needs, timely and accurate accomplishment of the work, and mutual benefit as reflected by our friendly and long-lasting relations with major plant growers in Ukraine.

1. Dmitriev A., Shevchenko O., Polischuk V., and Guscha N. 2009.Effects of low dose chronic radiation and heavy metals on plants and their fungal and virus infection. Data Science Journal, 8(24): 49–57. 2. Polischuk V.P. 2005. Effects of radioactive and chemical pollution on plant virus frequency / V.P. Polischuk, T.P. Shevchenko, I.G.Budzanivska [et al]. NATO Security through Science Series, Environmental Security: Vol.2., Equidosimetry (F. Brechignac and G. Desmet, eds.). P.87-92. 3. Polischuk V.P., Budzanivska I.G., Shevchenko T.P. Abiotic environmental factors: effects on epidemiology of plant virus infections Cooper J.I. et al. (eds.).2006. Virus Diseases and Crop Biosecurity. NATO Security through Science Series, Springer. P.121-132. 4. Dmitriev A.P., Shevchenko O.V., Polischuk V.P., Guscha N.I. Chapter XVII: Effects of low dose chronic radiation and heavy metals on plant-pathogen interactions, pp.229-243. In: Burlakova E.B., Naydich V.I. (eds.) The lessons of Chernobyl: 25 years later. – Nova Science Publishers, 2012. 5. Hill S.A. Methods in plant virology. Oxford: Alden Press, 1984. 167 p. 6. http://www.eppo.int/.

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ASSESSMENT OF THE REACTION OF WHEAT TO THE STREAK MOZAIK VIRUSES INOCULATION ACCORDING TO MALONDIALDEHYDE CONTENT

Изменения перекисного окисления липидов в растениях озимой пшеницы сорта "Память" оценивали на содержание малонового диальдегида в 1 и 14 день после инфицирования вирусом полосатой мозаики пшеницы. Были обнаружены специфические черты реакции в корнях и листьях при инокулировании растений вирусом. Соотношение между содержанием малонового диальдегида в листьях и корнях могут быть использованы для ранней непрямой диагностики инфекции.

Changes in lipid peroxidation in plants of winter wheat variety "Pamyat'" were evaluated for content of malon dialdehyde in 1 and 14 days after infection with the streak mosaic virus. The special features of the reaction in the roots and leaves to the inoculation with the virus were discovered. The relationship between malon dialdehyde content in leaves and roots can be used for early indirect diagnosis of the infection.

Patosystems with wheat streak mosaic virus (WSMV) became often the object of successful research involving both fundamental and applied areas of virology. The application of methods assessing the physiological state of plants is important in a comprehensive approach for determining sensitivity of host plants, the indirect, rapid diagnosis of viral infection [5]. Malondialdehyde (MDA) that is one of the end products of lipid peroxidation (LPO) is also one of the universal criteria for early diagnosis of stress [2–4]. Therefore, the MDA content is a convenient measure of plant response to the initial action as abiotic and biotic factors.

The aim of our work is evaluating the physiological state of plants of wheat according to the contents of MDA under the conditions of artificial inoculation WSMV.

Materials and methods. The seeds of winter wheat variety "Pamyat'" were treated potassium permanganate solution. The plants were grown on moistened paper in Petri dish, covered glass microbiological caps with Knopp solution added. Two-weeks plants were infected WSMV. The MDA content was determined based on the reaction of 2-tiobarbiturova acid [1, 6]separately in the leaves and roots after 1 day and 14 days after inoculation. The difference between variants of the experiment is accurate at $p<0,05$.

Results and discussion. The nature of infection and its symptoms in model systems is somewhat different from those observed in agrocenoses under the conditions of natural infection. This is largely due to the influence of buffer solution and carbide which are used for artificial inoculation of plants. In our experiment plants of winter wheat variety "Memory" even on the 14th day after the infection did not show the external signs of disease, although after artificial inoculation characteristic symptoms are usually recorded earlier than in the field. However, by changing the intensity of LPO the signs of stress caused by WSMV could be found within 18 hours after inoculation (fig. 1).

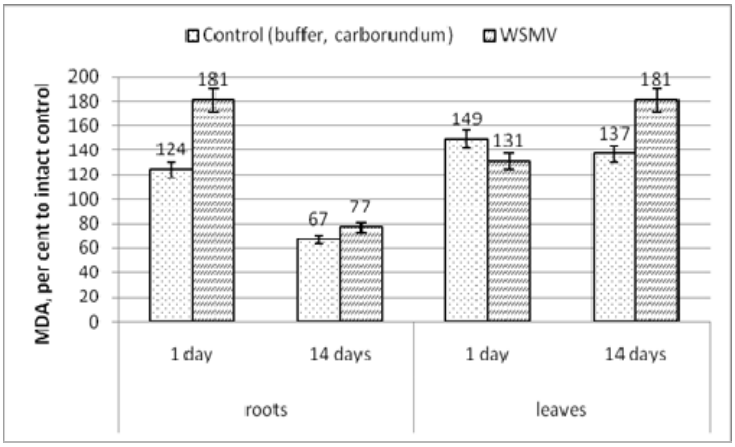

Fig.1. Lipid peroxidation (MDA content) in winter wheat plants variety "Pamyat'" as correlation (% to intact control) between MDA contents on 1 day and 14 days after inoculation by WSMV ($p<0,05$)