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SYMPTOMS OF *PEPINO MOSAIC VIRUS* IN GREENHOUSE TOMATOES AND REACTIONS OF TEST PLANTS ON INFECTION

Abstract. This study was carried out to detect the *Pepino mosaic virus* in various tomato hybrids grown in greenhouses. Total of 194 plant sample were collected from the greenhouse during 2019–2020 years. As a results of DAS-ELISA was found 54 of samples with *Pepino mosaic virus*, which was identified both in monoinfection and in the complex with other viruses: *Cucumber mosaic virus, Tobacco mosaic virus, Tomato mosaic virus* and *Potato virus X*. The possible symptoms of *Pepino mosaic virus* during the growing season of tomatoes include interveinal chlorosis, deformations, mosaic and yellow spots on leaves and also blotchy ripening fruits. The reaction of 10 plant species to the inoculation of *Pepino mosaic virus* was established. The results showed the greatest susceptibility of *Nicotiana rustica* L. and *Datura stramonium* L., where the maximum concentration of viral particles was detected 4 weeks after infection (OD: 0.952–1.013). The results presented in the article can be used to diagnose *Pepino mosaic virus* during the monitoring of greenhouse tomato plantations.

Keywords: *virus*, PepMV, tomato, hybrids, greenhouse, test plants, DAS-ELISA, symptoms

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СИМПТОМЫ ПРОЯВЛЕНИЯ *PEPINO MOSAIC VIRUS* НА ТЕПЛИЧНЫХ ТОМАТАХ И ТЕСТ-РАСТЕНИЯХ

Аннотация. *Pepino mosaic virus* является опасным возбудителем, поражающим растения томата в условиях защищенного грунта. Исследования по выявлению вируса на территории республики, проведенные в период 2019–2020 гг. при маршрутных обследованиях тепличных посадок различных гибридов культуры, показали наличие возбудителя в 54 образцах из 194 проанализированных с применением иммуноферментного анализа. Установлено, что *Pepino mosaic virus* встречается как в моноинфекции, так и в комплексе с такими вирусами, как *Cucumber mosaic virus, Tobacco mosaic virus, Tomato mosaic virus и Potato virus X.* Среди типичных симптомов проявления вируса при инфицировании растений томата отмечали межжилковый хлороз, мозаичность и деформацию листовой пластинки, а также неравномерное созревание плодов. В лабораторных условиях проведена оценка реакции 10 видов тест-растений на инокуляцию *Pepino mosaic virus.* Наибольшую восприимчивость проявили *Nicotiana rustica L. и Datura stramonium L.,* у которых отмечали яркую внешнюю реакцию на заражение патогеном и высокое содержание вирусных частиц спустя 4 недели после заражения: 0,952–1,013 единицы оптической плотности. Представленные результаты могут быть использованы для диагностики *Pepino mosaic virus* при проведении мониторинга посадок томата закрытого грунта.

Ключевые слова: вирус, PepMV, томат, гибриды, защищенный грунт, тест-растения, ИФА, симптомы

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Introduction. In the Republic of Belarus, tomato (*Lycopersicon esculentum* L.) is grown in greenhouses under conditions of long-term crop rotation. The grown assortment of tomato hybrids allows satisfying the demand in the consumer market segment in the country and increasing the volume of exports. Due to the absence of breeding centers in the country, vegetable growers buy seeds from international

vegetable-breeding companies (De Ruiter, Rijk Zwaan, Syngenta, etc.). It is known that many pathogens persist in seeds which contributes to their introduction into new regions [1]. This is the main way for the spread of such dangerous viruses as *Tomato ringspot virus* (ToRSV) or *Tomato brown rugose fruit virus* (ToBRFV) that infects tomato culture [2, 3].

Previously, *Cucumber mosaic virus* (CMV), *Tobacco mosaic virus* (TMV), *Tomato mosaic virus* (ToMV), *Tomato aspermy virus* (TAV), *Potato virus X* (PVX) and also *Potato virus Y* (PVY) were detected in greenhouse tomato plantings with the development level range of 5.6 to 37.5 % [4, 5]. *Pepino mosaic virus* (PepMV) was noted in tomato plant samples periodically.

PepMV is a *Potexvirus* (family *Alfaflexiviridae*) which infected tomato crops worldwide [6]. For example, the occurrence of PepMV on tomato crops was noted in Germany, Italy, the Netherlands, Poland, Romania and other countries. It is also known that the main host plants of PepMV are pepino (*Solanum muricatum* Aiton), potato (*Solanum tuberosum* L.) and some weed species [7, 8].

The main source of the virus is tomato seeds, where the pathogen remains in the coat [9]. The infection of tomato seeds can vary from 0.005 to 0.057 % [10]. As for most potexviruses PepMV mainly spreads mechanically from plant to plant without the involvement of an obvious vector [11]. There is evidence that bumble bees [12] and the soil-borne fungus *Olpidium virulentus* (A. Br.) Schroet. [13] can function as vectors for PepMV. Also, recent studies suggest that tomatoes pests (e. g. *Trialeurodes vaporariorum* Wetw.), as well as some types of entomophagous (e. g. *Aphidius colemani* Viereck) can act as vectors too [14, 15]. The damage from PepMV is associated with a decrease in the commercial quality of tomato fruits and their quantity, which can vary depending on the hybrid, time, conditions, the way the virus penetrates the plant, as well as its strain composition and the presence of other viral pathogens (mixed infection) [16]. Soler-Aleixandre et al. [17] reported high losses with the collapse of up to 90 % of plants; others describe low yield losses of up to 15 % [18] or no quantitative yield losses, but significant reduction in fruit quality (up to 40 %).

PepMV was first detected in greenhouse tomato plantings in Belarus in 2012, but no further targeted research has been carried out [19]. The objective of this work was to identify the *Pepino mosaic virus* in tomato plants and to study the symptoms of the disease on various test plants.

Materials and method. Phytosanitary monitoring of tomato plantings was performed at 11 greenhouse complexes of the republic during 2019–2020. Inspection and sampling were made according to the recommendations presented in the EPPO diagnostic protocol for PepMV (PM 7/113 (1), 2013) [6]. The samplings were made from tomato plants with a wide range of virus-like symptoms: various types of mosaics on leaves and fruits, lightening of veins, chlorosis, reduction, wrinkling of leaves, etc. The samples were placed inside polyethylene bags and brought to the laboratory.

Identification was performed using the DAS-ELISA method (double antibody enzyme-linked immunosorbent assay) for PepMV (commercial kits BIOREBA AG, Switzerland). Each ELISA test included two positive and two negative controls. Samples were rated positive if the mean optical density at 405 nm (OD) of the sample exceeded three times the mean of two wells containing extract from healthy plants [20]. In the same way, the samples were tested for pathogens such as CMV, TMV, ToMV, PVX, *Tomato spotted wilt virus* (TSWV).

Plants *Nicotiana tabacum* L., *N. glutinosa* L., *N. rustica* L., *Datura stramonium* L., *Capsicum annuum* L., *Lycopersicon esculentum* Mill., *Physalis pruinosa* L., *Phaseolus vulgaris* L., *Cucumis sativus* L. and *Cucurbita pepo* Mill. were tested for their susceptibility to PepMV. The indicator plants were grown under laboratory conditions in pots with a peat substrate. When 5–6 true leaves were formed, the plants were transplanted into 5-liter pots for further keeping in the greenhouse. The distance between the pots did not allow contact between plants. Watering was carried out daily in accordance with the needs of the plants. Individual equipment was used to care for the plants, and the necessary measures were taken to prevent the development of pests.

As inoculants, the juice of the leaves of tomato (*Prunus* F1) infected with PepMV was used. Virus was inoculated locally by standard procedure [21]. Five plants of each cultivar were inoculated with the isolates used and as control 5 plants was inoculated with water.

The plants were inoculated by PepMV at the stage of 3–4 full-grown leaves. The inoculated plants were observed regularly in a long period post inoculation. DAS-ELISA testing was performed 4 and 20 weeks

after inoculation to confirm viral infection in the test plants and to determine the accumulation of viral particles. Diagnosis of the species of viruses by ELISA according to the protocol of research presented by the manufacturer BIOREBA (Switzerland) was carried. The analysis results were evaluated by photometer Multiskan MS (Labsystem) at a spectrum of 405 nm.

Results and discussions. Plants with virus-like symptoms on different tomato hybrids were noted in greenhouses. Also the symptoms that charactered by PepMV on tomato plants were noted. Symptoms such as interveinal chlorosis, leaf deformation, mosaic and yellow spot on the leaves, shoots and even pedicels of tomato have been observed. In addition, yellowish stripes covering the entire stem, up to the point of growth and inflorescences tomato were noted. Various types of mosaic, cracking or deformation were observed in fruits, in particular on cherry tomato hybrids (Figure 1).

DAS-ELISA tests were carried out on the leaf samples collected from 194 plants with virus infections symptoms in order to determine the existence of PepMV. The results showed that 54 samples of 6 tomato hybrids grown in different greenhouses were infected with PepMV. Thus, the incidence of PepMV infection for 194 samples was 27.84 % of which 11.34 % of the samples contained monoinfection. Complex PepMV infection with other viruses was found in 16.5 % of the samples studied. At the same time, the species composition of viruses involved in pathogenesis and the level of their accumulation in tomato plants varied in the same hybrids.

The possibility of PepMV development in tomato plants together with other viral pathogens is noted in the works of many authors. Thus, PepMV was detected with CMV, *Tomato chlorosis virus* (TCV),

a *b*

c *d*

Figure 1. Symptoms *Pepino mosaic virus* on tomato plants: *a* – yellow mosaic on the leaves; *b* – yellow leaf spot and streakiness on the shoots; *c, d* – spotting and deformation of fruits

Species composition of viruses co-occurring with *Pepino mosaic virus* in tomato plants (determined by DAS-ELISA method, 2019–2020)

Complex infections		
2-component	3-component	4-component
PepMV + TMV	PepMV + TMV + CMV	PepMV + ToMV + TMV + CMV
PepMV + CMV	PepMV + TMV + PVX	–
PepMV + ToMV	PepMV + ToMV + CMV	–

N o t e. PepMV – *Pepino mosaic virus*, CMV – *Cucumber mosaic virus*, TMV – *Tobacco mosaic virus*, ToMV – *Tomato mosaic virus,* PVX – *Potato virus X.*

Tomato torrado virus (ToTV), etc. [22]. In our studies PepMV was detected together with CMV, TMV, ToMV and PVX in different combinations (Table).

In most cases, a complex infection in a plant leads to a change in the nature of the phenotypic manifestation of the disease: an increase in symptoms or a weak development of external signs. Co-infection of tomato with PepMV and TMV showed symptoms of venous chlorosis (Figure 2, *a*) and reduction of leaf blades.

On susceptible tomato, plants were infected with PepMV together with CMV manifested interveinal chlorosis and mosaic (Figure 2, *b*), and on the fruit – deformation and blackening. PepMV in combination with ToMV was manifested in the form of a pale green leaf spot of the upper layer of tomato or on young shoots.

This wide variation in symptoms observed with viral infections in tomato suggests that both positive and negative interference can occur between species. It is known that under conditions of mixed infections, the pathological effect of viruses is due to the nature of the interaction of pathogens with the host plant and the relationship with each other. In this regard, the fact of establishing a high frequency of occurrence of PepMV in combination with other viruses requires a more detailed study of the specificity of accumulation and translocation of the pathogen, depending on the composition of the infection.

To determine the response to infection and assess the level of its accumulation, we inoculated a number of test plants with PepMV isolate under laboratory conditions. The test results showed that 8 out of 10 species tested were susceptible to the virus.

It should be noted that *D. stramonium* L. plants showed the fastest and brightest response to inoculation with PepMV isolate. On the 7th day after infection, a yellow mosaic was observed on the inoculated leaves plant. Local chlorotic lesions, leaf deformities, or systemic yellow vein streak were then noted (Figure 3).

a *b*

Figure 2. Symptoms on tomato leaves at complex infection *Pepino mosaic virus* with *Tobacco mosaic virus* (*a*) and *Cucumber mosaic virus* (*b*)

Figure 3. Reaction of *Datura stramonium* L. to infection with *Pepino mosaic virus* (laboratory experiment, 2019)

It is known that the reaction of plants of the genus *Nicotiana* to infection with PepMV is variable and strongly depends on the strain composition of the pathogen, the type and even the variety of tobacco. For example, the reaction to mechanical inoculation with the polish isolate of *N. tabacum* cv White Burley plants manifested itself as vein chlorosis and mosaic. *N. tabacum* cv Xanthi reacted in the same way [23].

In other studies, *N. tabacum* cv Xanthi plants did not respond to inoculation by such strains PepMV as EU-tom, Ch2 or US1 [24, 18]. Fakhro et al. (2011) unrecorded any symptoms on *N. tabacum* L. cv Samsun after mechanical inoculation by European isolate of PepMV [25]. However, in our experiments, a positive reaction of *N. tabacum* cv Samsun to the virus was noted already on the 7th day after inoculation in the form of a chlorotic mosaic. Among other *Nicotiana* species, *N. rustica* was also susceptible to the PepMV isolate, where a systemic mosaic was observed. The reaction of *N. glutinosa* plants to PepMV inoculation was asymptomatic.

The results of laboratory experiments by some researchers showed that pepper plants of various varieties were not infected with PepMV or the manifestation of symptoms was local [7, 26]. Overall, the scientists concluded that pepper is not a systemic host for the three viral strains (EU-tom 1066, Ch2 PCH06/104, US1-PRI) used in the study, and it is likely that *Capsicum annuum* L. is not an important host in the epidemiology of PepMV.

In our studies, to assess response to infection of PepMV, *C. annuum* cv Alesya (Belarusian selection) was used. Despite the same conditions of infection and maintenance of pepper plants, mixed results were obtained. So, 10 days after inoculation of the plants, local symptoms in the form of a light-yellow mosaic 2 out of 5 test pepper plants appeared. Other plants were asymptomatic even 4 weeks after inoculation with PepMV. After 4 months, the response of susceptible pepper plants was divided into soft mosaic and marginal chlorosis.

It should be noted that tomato plants are highly susceptible to virus infection. One week after inoculation and throughout the entire study period on *L. esculentum* cv Lyana systemic symptoms of lesion were noted: yellow or light green spotting, chlorotic lesion and leaf deformation (Figure 4, *a*). Plants of *Physalis* genus normally are not infected by PepMV. Cases of local and systemic reactions of *P. floridana* L. to the Polish isolate of the PepMV-SW virus are known [27]. In our studies we used *P. pruinosa* cv Yantar'. As a result, the reaction in the form of deformation and swelling of the leaf blade manifested itself only in 2 plants on the 30th day after inoculation (Figure 4, *b*).

When infected with different PepMV isolates, symptoms on *Phaseolus vulgaris* L. plants may be absent or appear as small-spotted spots [23, 28]. The same spotting was observed in *P. vulgaris* cv Motolskaya White in our experiments.

During the experiment, there were no visual signs of infection PepMV in plant cucumber (*Cucumis sativus* cv Verasen') and pumpkin (*Cucurbita pepo* var *clypeata* cv Malyshka). The results of enzyme-l inked immunosorbent assay of test-plant samples also confirmed the absence of PepMV virus particles.

a *b*

Figure 4. Symptom development of *Pepino mosaic virus* in *Lycopersicon esculentum* cv Lyana (*a*) and *Physalis pruinosa* cv Yantar' (*b*)

PepMV is mainly accumulated in *D. stramonium* L. and *N. rustica* L. plants, where the content of viral particles 4 weeks after infection reached 1.013 and 0.952 units OD (optical density), after 20 weeks – over 2.400 OD. In plants *L. esculentum* Mill. and *C. annuum* L. high virus concentration only 20 weeks after inoculation was observed (Figure 5).

The research results allow to recommend *D. stramonium* L., *N. rustica* L. as accumulators of PepMV for subsequent isolation and use in the development of immunochromatographic tests or molecular diagnostics.

Conclusions. As a result of the ELISA-test of 194 tomato plant samples, PepMV was detected in 54 samples. The virus was identified as both mono-infection and in combination with other viruses from *Bromoviridae, Virgaviridae* and *Alphaflexiviridae* families. The most specific symptoms of PepMV on tomato plants are yellow spot on the leaves, shoots and pedicels; spots on fruits and deformation. In conditions of complex damage to tomato plants, chlorosis, reduction of leaf blades and mosaic were noted, in addition, an asymptomatic course of the disease is also possible.

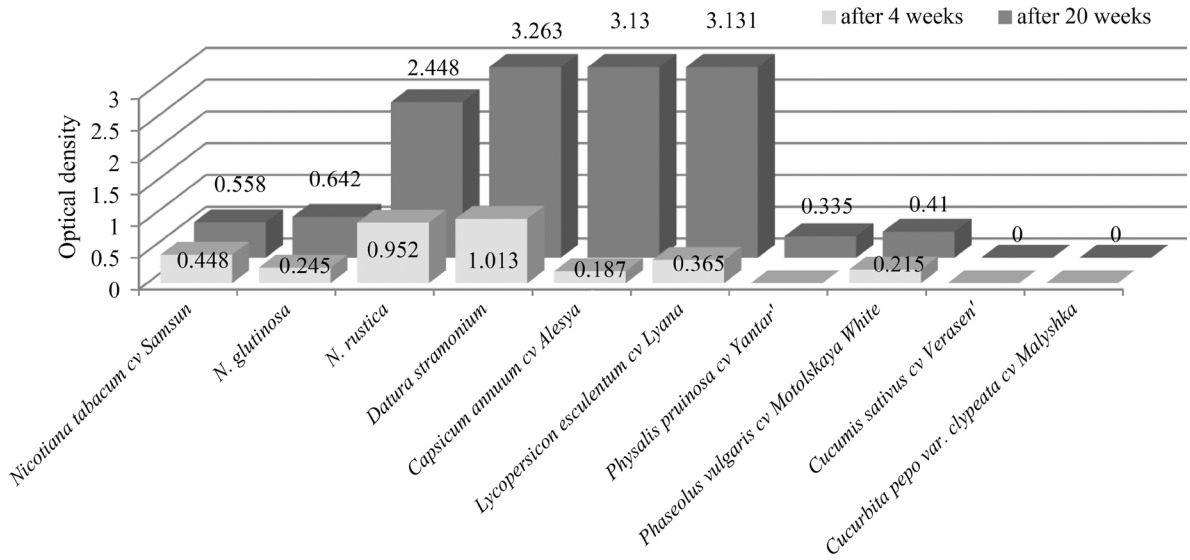

Figure 5. The content of viral practices *Pepino mosaic virus* in the test-plants ("0" – results OD ≤ negative control)

During artificial infection of 10 species of indicator plants, 8 showed various kinds of mosaic lesions. The highest susceptibility to PepMV of plants by *D. stramonium* L. and *N. rustica* L. was established. Also, after a long time of cultivation the maximum concentration of viral particles were detected on these plants.

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