

Dynamics of Cucumber Green Mottle Mosaic Virus Accumulation and its Association to the Disease Manifestation

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Abstract—The article presents data from laboratory experiment on the artificial infection of cucumber plants with the green mottle mosaic virus in different concentrations. The dynamics of virus accumulation in each of the experimental groups is described. There is an association for which stage of viral load that correlates with the onset of visible signs of infection and a further increase of the disease symptoms. The obtained data showed that the virus accumulation in the plant obeys the exponential equation. When constructing a graph of the accumulation of viral load, it was noted that the onset of visible manifestations of the disease falls on the linear segment of the curve, and the mosaic manifestation on the exponential segment. The dependence of daily average growth on the virus concentration has not been established. Based on the results of the experiment, we can give reasonable recommendations on the use of quantitative PCR analysis in predicting outbreaks of viral infections in greenhouse complexes based on the viral load in the plant during disease symptoms absence.

Keywords—CGMMV, cucumber diseases, cucumber viruses, protected ground, phytopathogens, viral load, epiphytotics.

I. INTRODUCTION

In recent years viruses infecting vegetable crops have been intensively studied in Siberia [1]. This is especially true for vegetable growing using protected ground. Challenging-regulated environmental factors, technological methods used in greenhouses, lead to outbreaks of viral diseases. One of these techniques is interplanting – an event when you plant young plants to the aging ones. This technique is often used with greenhouse plants and contributes to the spread of infections. The lack of technological gaps between rotations, which does not allow a complete disinfection of locations and equipment of greenhouses, or poor-quality disinfection, also contributes to the preservation of pathogens and their circulation among plants [2-4].

According to the Ministry of Agriculture of the Russian Federation, 70.0% of all greenhouse vegetable harvests in 2018 were the cucumbers. Viral diseases of which, despite the large number of resistant hybrids, are recorded everywhere. The pathogen characteristic for the protected ground is the cucumber green mottle mosaic virus (CGMMV) [5-6]. It is an RNA-containing, highly specialized virus of plants of the gourd family. Signs of the disease are: spotting, bloating and deformation of the leaves, growth retardation. Fruits are usually not changed, however,

a change in color, shape, taste can be observed. CGMMV infection can cause crop losses up to 40%. The causative agent is extremely resistant to adverse environmental factors: virions remain viable even when heated up to 90 °C, when dried and frozen. In dry leaves, this phytopathogen can persist for up to a year. However, even in the absence of a large amount of plant residues, as in the case of large greenhouse plants, the virus can be stored on inventory, on the surface of cultivation facilities and on the staff clothes [7-8]. Vaccination could be an effective method for controlling CGMMV in a protected ground, however, the high mutational variability of viruses significantly hinders the development of effective vaccines that work stably for a long time [9]. Taking into account all the above mentioned, the most optimal way to prevent the development of epiphytotics in a closed micro-agrobiocenosis of greenhouses is the timely detection of plants, which carry viral infections.

The complex of diagnostic measures used in greenhouse facilities includes a visual inspection of plants for the detection of signs of the disease and laboratory tests. The use of modern diagnostic methods, for example, PCR analysis, makes it possible to detect the presence of viruses in a plant even during the latent course of infection (latent infection) or in the early stages when signs of infection are poorly expressed [10-11]. Since the ability to carry out the necessary diagnostic laboratory tests does not exist in all complexes, they begin to talk about the presence of viral diseases only when their symptoms become visible to the bare eye. This approach allows you to take measures only restraining the further spread of the virus among plants, which are also not always successful due to the high concentration of the circulating pathogen.

The purpose of this study was to identify the dynamics of virus accumulation in plant cells and correlate it with the time when signs of the disease become visible. When observing the viral load development from the very beginning of the infection, it is possible to identify up to what point the latent virus accumulation occurs, and how many virions make the infection signs noticeable. The obtained data are of practical importance, since this will make it possible to predict outbreaks of infection in greenhouse facilities and will make it possible to take adequate measures in time for the prevention or relief of the infection process.

II. METHODS

The studies were carried out on the basis of the Laboratory of Enzyme Analysis and DNA Technologies of the Novosibirsk State Agrarian University. The experiment was conducted on cucumbers of the Mewa F1 variety (Rijk Zwaan). We used plants in phase 4 of these leaves for the experiment. The subject of the study was the cucumber green mottle mosaic virus, as the most common virus in Siberia.

The essence of the experiment was the artificial infection of 5 groups of cucumber plants with CGMMV in different concentrations. The infection solution was prepared by serial dilutions starting from 10^6 to 10^2 viral copies in 100 μ l. Plants were infected by spraying with 100% covering of leaf surface. Plants of the control group were not infected. To prevent the transmission of infection between groups, they were placed in isolated locations.

To assess the degree of mosaic damage, a scale with the following five-point system was used: 0 or 1 – plants are apparently healthy; 2 – slight mosaic of apical leaves; 3 – distinct mosaic of apical leaves, signs on the leaves of the middle and lower levels are absent, slight growth retardation; 4 – vivid signs of mosaic on the leaves and corolla, general yellowing of the plant, growth retardation, dipping ovaries [12].

In order to confirm infection and further determine the viral load (viral copies/100 μ l), the method of quantitative PCR was used. Leaves were taken from the top with an interval of a week. Virus genomic RNA was isolated from leaf tissues with the subsequent step of reverse transcription and polymerase chain reaction using the authors' test system.

Statistical processing of data was carried out by generally accepted methods [13-15] using the programs Excel and Statistica 10. We used data on the entire experimental population of plants for analysis.

III. RESULTS

At the beginning of the study, all plant groups were tested for the presence of CGMMV by the method of quantitative PCR. Single virions were found on plants of the experimental and control groups. An interesting fact is that regardless of the virus concentration with which the plants were infected, the final quantity of virus particles was approximately the same and amounted up to 10^{15} - 10^{16} (Fig. 1).

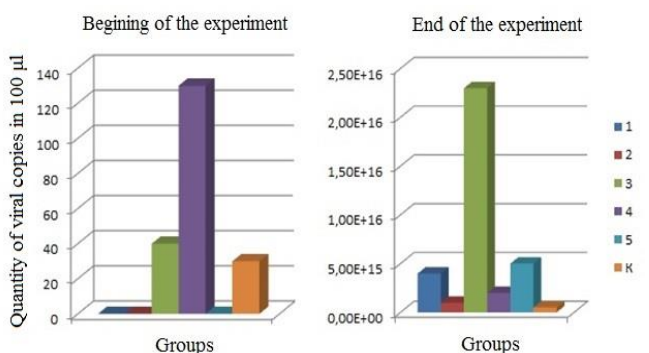


Fig. 1. Quantity of viral particles at the beginning and end of the experiment in different groups of infected plants

Nevertheless, a uniform trend in the development of viral infection in each group was observed: within 5 weeks, it passed to the exponential stage (Fig. 2).

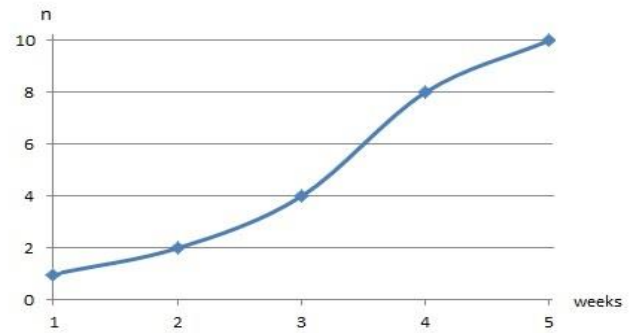


Fig. 2. Trend of viral load accumulation within 5 weeks after infection (10^n copies/100 μ l)

The accumulation of viral load in plants does not depend on the initial number of viral particles with which the plant was infected, which proves a low correlation coefficient ($r = 0.289$), and depends on the time period elapsed since infection. Factors such as growing conditions and variety can affect the manifestation of viral disease symptoms, but the exponential tendency to accumulate viral load will continue.

Before the artificial infection, the plants were well developed; the root system was healthy, with no signs of disturbance.

At the second week of the experiment, the first symptoms of the disease began to appear. They were characterized by pointed edges at the leaves apex; the leaf surface became wrinkled during growth, the edges bent inward. At the fourth week, weakly expressed, disorganized light green spots on the leaf surface were added to the symptoms listed above, which at the end of the experiment formed a clear mosaic (Fig. 3).

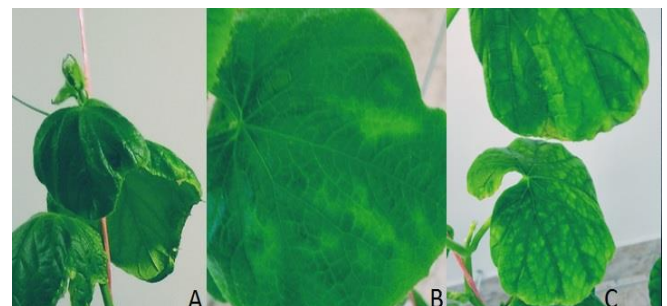


Fig. 3. Signs of CGMMV during infection: A – 2 weeks of the experiment; B – 3 weeks; C – 4 weeks

In the course of the experiment, it was found that various symptoms appear on different segments of the curve of viral load accumulation. Mosaic is more tied to the exponential segment of the curve and the deformation of the leaf surface to the initial.

Some plants showed significant growth retardation. Nevertheless, regularities of the average daily increase in the virus concentration in the plant and the amount of virus during infection were not observed. This is confirmed by the uneven growth graphs for different groups (Fig. 4), and the fact that all samples belong to a single population ($\chi^2 = 6,807$; $df = 28$).

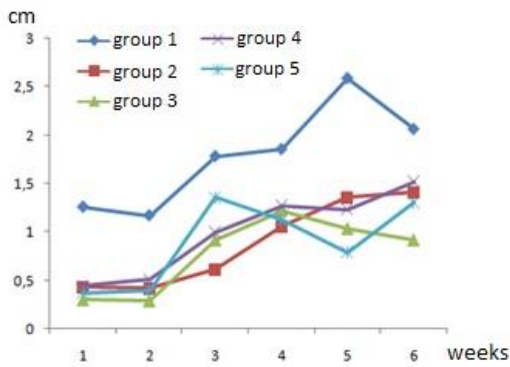


Fig. 4. Dynamics of average daily growth of plants in different groups

IV. CONCLUSION

In the course of laboratory studies, it was found that the initial virus concentration practically does not correlate ($r = 0.289$) with the dynamics of the viral load at the time of plant infection. The accumulation of viral load obeys the exponential equation and depends on the length of time period. Under laboratory conditions, it was found that noticeable mosaic formation is shaped on the exponential segment of the viral load accumulation, and curved edges and wrinkling of leaves – at the initial stages of infection. In this case, the dependence of plant growth rate on the magnitude of the viral load was not detected.

Based on the abovementioned, in the absence of preventive measures in the production cycle, the viral load in plants will develop in a straight line with a sharp transition to the exponential stage. The use of a combined system for the diagnosis of viral infection, based on visual assessment and control of viral load in plants, will optimize the method of antiviral treatments.

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