

Viral Load Distribution of Cucumber Green Mottle Mosaic Virus in Leaves

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Abstract—Data on the viral load distribution by different leaves parts (upper, middle, lower leaves) in cucumber plants grown in protected ground are presented. Studies were conducted at the time of the visible infection manifestation. It was revealed that accumulation of virions of the cucumber green mottle mosaic virus (CGMMV), with the onset of manifestation, prevailed in the middle and lower leaves, which is probably due to the “slow” redistribution of the virus along the external phloem. This pattern is confirmed by a high correlation coefficient ($r = 0,99$). It was also found that the viral load indicator has high variability ($C_v = 63,77\%$). A similar coefficient of variation may indicate the fact that under constant environmental and nutritional conditions, which are provided under the conditions of protected ground, the individual susceptibility of cucumber plants to the phytopathogen affects the number of viral particles in the plant.

Keywords—viral load, cucumber green mottle mosaic virus (CGMMV), phytopathogens, cucumbers, polymerase chain reaction (PCR), protected ground.

I. INTRODUCTION

In the conditions of modern agricultural production, the issue of protecting plants from viruses, which are poorly studied and difficultly identified in phytopathogens, is an acute issue. Due to the lack of applied work on the development of viral infection in plants, it is impossible to give unambiguous recommendations for the disease prevention in a protected ground. A small amount of information about the localization of various viruses in the plant organism leads to the fact that antiviral measures do not take into account the specifics of a particular infection, and technological methods of cultivation exacerbate the spread of the virus [1].

Viral infections cause various lesions of the leaf surface (mosaic, chlorosis, necrotic spots), corolla falls and fruit deformation, which is accompanied by a yield decrease, which can reach up to 48-50%, and in some cases up to 70%. Due to the deterioration in the commercial quality of fruits, such products are sold at low cost, which has an economic significance [2]. An increase in the number of plants per unit area accelerates the formation of generative organs, and at the same time contributes to an increase in the number of mosaic plants. In some cases, there is a latent infection, when under certain conditions the symptoms of the disease do not appear [3]. Infection of single plants under overcrowded conditions, insufficient disinfection of equipment and lack of timely

diagnosis can take on the nature of epiphytotics, which is why viral infections are widespread in greenhouse facilities [4].

Infection of plants with the virus usually leads to a systemic infection in which the host plant manifests symptoms of the disease. If timely laboratory diagnostics are unavailable, the main way to determine the presence of a viral infection in greenhouse facilities is to visually evaluate the disease signs. Visual evaluation allows you to register a viral infection only with the manifestation of characteristic symptoms, when measures of plant treating are already ineffective [5].

The main indicator of the viral infection development is the viral load, which is characterized by the number of viral particles per unit volume of the sample material. One of the methods for its determination is the polymerase chain reaction (PCR) [6]. Information on the accumulation and distribution of virions in various plant organs obtained during quantitative PCR can help to correct the methods of cultivation of protected soil cultures, and also help to create a set of measures aimed at limiting epiphytotics even before the appearance of pronounced infection signs, and thereby maintain a high quality of farmed products. Thus, the widespread use of PCR in the practice of early diagnosis of viral infections in greenhouse complexes will help reduce crop losses due to the spread of viral diseases.

In a number of articles devoted to the study of viral load distribution in various plant organs, the authors note that the massive accumulation of viral particles occurs in the upper leaves and nearby organs [7]. According to the results of a study conducted on tobacco plants infected with cucumber mosaic virus, it was found that a large number of CMV virus particles were found in the cells of young developing leaves in the early periods of infection, which later led to the development of mosaic and spotted leaves [8]. This phenomenon is also confirmed by studies on the distribution of viral particles in cucumber plants infected with the cucumber green mottle mosaic virus (CGMMV), when the first organs that undergo a systemic infection are young upper leaves, and a large number of viral particles are found in them [9]. In systemic infections, the virus is transported through the vascular system from the initially infected leaf to the roots, and then to the area close to the apex. The virus does not penetrate the apical meristem, but remains localized in the area immediately below it, from where it penetrates the developing rudiments of leaves, which may explain the high concentration of viral particles in the upper leaves [10].

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The cucumber green mottle mosaic virus is one of the most common viruses that infect cucumber plants in greenhouse facilities. CGMMV causes losses up to 50% of the crop, reducing the intensity of photosynthesis and quantity of fruits, which are deformed and acquire a bitter taste [11]. The spread of the virus in a plant begins with inoculation of leaf mesophyll cells, then it goes from mesophyll cells to the phloem, then it is transported to the satellite cells and sieve elements. Then the virus enters the external and internal phloem. The internal phloem mediates a more rapid movement of the virus into the upper organs of the plant, while the external phloem transports to the lower organs at a lower speed [12]. This explains the fact that CGMMV, which is transported by the phloem, is redistributed over time from the upper leaves organs to the lower leaves [13]; thus, predominance of the viral particles quantity should be noted in the middle and lower leaves at the disease manifestation stage.

The objective was to determine the viral load in the upper, middle and lower leaves in a cucumber plant at the time of visible manifestations of CGMMV.

II. METHODS

The studies were carried out in the Laboratory of Enzyme Analysis and DNA Technologies of the Novosibirsk State Agrarian University. The material for the study was cucumber plants with viral infection manifestation, which were selected in the current greenhouse facility of the Novosibirsk region. A total of 160 plants were studied. A visual condition evaluation was carried out for each plant. Symptoms were evaluated in the lower, middle and upper leaves according to a three-point system (0 – no signs of infection; 1 – slight manifestation; 2 – high manifestation). Viral load was determined by PCR. Upper, middle and upper leaves for the study were selected from each plant. Virus genomic RNA was isolated from leaf tissues with the subsequent step of reverse transcription and polymerase chain reaction using the authors’ point system. When setting up the reaction, a quantitative method using standards to determine virus copies in 100 µl of samples.

Statistical data processing was carried out by generally accepted methods [14] using the Excel program.

III. RESEARCH RESULTS AND DISCUSSION

When we visually assessed plant damage, the following signs of the CGMMV development were most often observed: focal necrosis, wrinkled leaf, elongated leaf edges, reduced leaves and plant growth retardation. The degree of manifestation was estimated mainly at 1 point (Fig. 1).

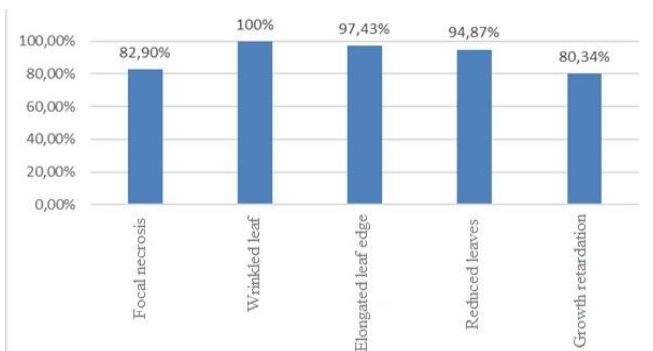


Fig. 1. Viral manifestation prevalence in plants

According to the results of molecular-genetic studies, it was found that during visual manifestation, the CGMMV particles accumulate mainly in the middle and lower leaves. Three types of curves can describe the distribution of viral particles in the studied plants (Fig. 2).

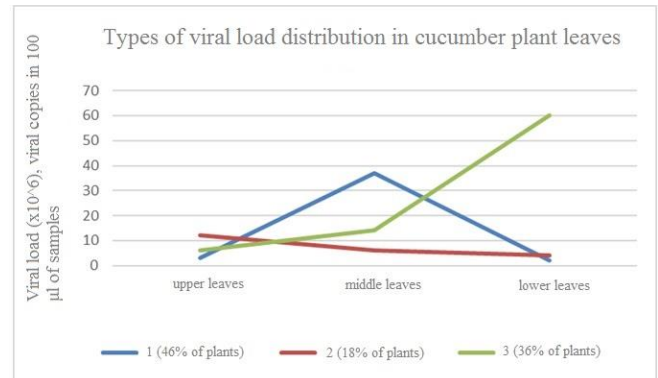


Fig. 2. Types of viral load distribution in leaves

Table I presents the averaged data on the degree of manifestation of viral infection and viral load by different leaves parts, which indicate the presence of a connection between the viral particles quantity and the leaves part of the plant; this dependence is confirmed by a correlation coefficient of 0,99. The coefficient of variation, calculated for leaves parts, shows a significant variability of this trait (Cv = 22,09%) in different parts.

TABLE I. THE AVERAGE VALUES OF MANIFESTATION DEGREE AND VIRAL LOAD IN DIFFERENT LEAVES PARTS

Leaves part	Manifestation degree, averaged point	Mean value of viral load (x10 ⁸), particle quantity in 100 µl of samples
Upper leaves	0	3.03
Middle leaves	1	4.67
Lower leaves	1	4.47

The results are consistent with patterns established by other researchers that relate to the transport of Tobamovirus viruses in plants. The early detection of a large number of virions in the upper leaves is associated with a more “fast” transport of viral particles through the internal phloem, and the further accumulation of viral particles in the middle and lower leaves and registration of a large number of them are associated with “slow” transport through the external phloem [15]. This explains the fact of the accumulation of a greater number of viral particles in the middle leaves at the time of the CGMMV manifestation.

The “Viral load” indicator in the studied plants ranged from $2,8 \times 10^4$ to $78,7 \times 10^9$; these limits may indicate that the viral load has high variability from plant to plant (Cv = 63,77%). A high coefficient of variation indicates a high variability of the viral load, which depends on the individual susceptibility of the plant, provided that environmental factors such as temperature, humidity and light are constant. However, in the absence of data on the effects of these factors, their influence on the accumulation of virions in plant organs cannot be completely ruled out.

Statistical processing of the total data array showed mixed results. The values of the correlation coefficient (for the upper leaves $r = 0,210$; middle leaves $r = -0,036$; lower leaves $r =$

0,194) indicate an insignificant relationship between the viral load and the score for the appearance of plants.

However, the values of the Fisher's test (for the upper leaves Fact. = 34,66; middle – 48,308; lower – 45,35; Fcrit. = 4), which estimates the degree of influence of a particular factor, indicate a high degree of significance of the viral load on the infection manifestation.

IV. CONCLUSION

According to the results of the study, it was found that high values of viral load in cucumber plants in the vast majority of cases are observed on the middle (46%) and lower (36%) leaves. A correlation was established between the viral load and the part of the plant ($r = 0,99$). It was also shown that the viral load values are highly variable ($Cv = 63,77\%$). The high variability of viral load, subject to the constancy of external factors, is presumably associated with the strength of protective reactions of each single plant. The obtained results may indicate the presence of a connection between the viral load and the degree of infection manifestation, however, the influence of temperature, humidity and illumination is not excluded, which is confirmed by correlation indicators. Aside from that, additional studies on influence of environmental factors on the viral load expression will give an understanding of the virus-organism-environment interaction.

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