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EFFECT OF VIRAL INFECTION ON THE ULTRASTRUCTURAL ORGANIZATION OF BLACKCURRANT LEAF TISSUE CELLS

One of the significant reserves for further increasing the yield of berry crops is to protect them from pests and diseases. Among the latter, viral ones are especially dangerous. Therefore, methods of virus diagnostics and especially electron microscopy are of great importance, which makes it possible to see viral particles, determine their shape, size, localization in tissues, and identify anomalies in affected plant cells. ***Objective.*** *To conduct a comparative study of healthy and diseased blackcurrant leaves in order to determine the degree of influence of the two viruses on the anatomical structure of organelles and inclusions, which can be used in the diagnosis and identification of viruses affecting plants.* ***Methods.*** *The material was blackcurrant plants with symptoms of reversion and green speckles, which are detected visually when examining the plantings of this crop. The morphology of viral particles, the anatomical structure of organelles, and inclusions were studied using the method of electron microscopy of ultrathin sections. Detected ultrastructural changes in cells can be used as diagnostic signs when identifying viruses. Also, viruses were identified by external signs and biological testing.* ***Results.*** *The study of ultrathin sections of leaf tissue and abnormal petals of the blackcurrant flower with symptoms of reversion revealed a bacillus-visible virus (Blackcurrant reversion virus) from the Rabdoviridae family, which is easy to identify due to its large size and appearance, in which it differs from similar features in ordinary cellular components. Typical locations of virus particles are the cytoplasm, nucleus, and perinuclear zone. The size of viral particles on ultrathin sections was 271 ± 7.19 nm long and 78 ± 2.31 nm in diameter. According to electron microscopic methods of studying artificially infected plants of Chenopodium quinoa, an inoculum of affected blackcurrant leaves, virions of Cucumis virus 1 Smith were observed in cells, which were freely located in the cytoplasm of the cell interspersed with ribosomes. Individual areas of the cytoplasm with a high virus concentration were also found in the affected parenchymal cells. Zones surrounded by a double membrane differ in the size and degree of virus saturation. When studying the pathogen morphology in the native preparations, the viral particles had a spherical shape with small, very colored central areas. Measurements of the virus*

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particles showed that they had an average size of 29.6 ± 0.59 nm. During the study of the ultrastructure of blackcurrant plant cells affected by Cucumis virus 1. Smith, myelin-like bodies were found not only in the cytoplasm of affected cells but also in the extra-plasma space. Analysis of the morphology of chloroplasts of blackcurrant plants affected by green speckles and reversion shows that chloroplasts with outgrowths and cup-shaped formations are present in many cells. Under various viral infections, there is a wide variety of mitochondria's shapes: they are elongated, cup- or club-like, etc. At the same time, their internal structure changes as well. We found that at the stage of the neurotization of a Nicotiana tabacum leaf infected with Cucumis virus 1. Smith, the peroxisome matrix is intensively filled with crystalline inclusions that have an electron-dense surface or are a system of rods with different configurations in the form of rectangles and trapezoids. They completely fill the entire matrix. During the development of viral pathology in the cells of diseased plants, destructive processes also cover the nucleus. As a rule, it takes on a lobed or radially elongated shape. Among the viruses we studied, the Blackcurrant reverse virus causes this trait the most. This may be due to the fact that this virus, accumulated in large quantities, exerts mechanical pressure on the nuclei and thereby accelerates the process of their deformation. A peculiar sign of changes in the nucleus ultrastructure is the content of viral particles. Of the viruses we studied, blackcurrant reversal virus particles were the most common in the nucleus nucleoplasm and perinuclear zone. ***Conclusions.*** *The intracellular development of viruses and their use of energy systems and components of plant cells for their reproduction lead to significant morphological and structural changes in the latter. In particular, electron microscopic studies of ultrathin tissue sections of diseased blackcurrant plants in comparison with healthy ones revealed the forms of the nucleus, mitochondria, and plastids modified under the influence of viral infection, namely Blackcurrant reverse and Cucumber mosaic viruses. Chloroplasts noticeably swelled without the existing content of starch grains, and clumping or the absence of gran thylakoids was observed. Plastids with a highly reduced membrane system were found. The results of studies have shown that the species affiliation of the virus does not cause specific changes in the morphology and structure of mitochondria. Their structural transformations under the influence of the viruses under study were the same: changes in shape and swelling, expansion of crists and a decrease in their number, a decrease in the electron density of their matrix, and so on. It was found that in the nuclei of cells infected with the Blackcurrant reverse virus, chromatin forms small, interconnected granular lumps located in different zones of the matrix. When studying ultrathin sections of Nicotiana tabacum leaf infected with Cucumis virus 1. Smith, rather specific crystal inclusions that fill the entire matrix were detected by the peroxisome. If external anomalies are detected in the form of mosaics, spots that can be caused by many pathogens in the absence of mechanical transmission of the pathogen, it is advisable to use the method of electron microscopy of ultrathin sections.*

Keywords: *blackcurrant, cell, viral diseases, chloroplasts, mitochondria, nucleus, peroxisomes, ultrastructure, electron microscopy.*

Blackcurrant is exposed to various phytopathogens, among which agents of viral diseases are so dangerous [1, 2]. In the interactions of phytoviruses with nutrient plants, there is a poorly understood mechanism for switching the main physiological processes to the reproduction, spread, or localization of viral particles [3]. Therefore, it is important to study virus-induced ultrastructural changes in the organelles and inclusions in leaf cells, which can be used in the diagnosis and identification of viruses affecting plants.

The most common viral diseases of blackcurrant are blackcurrant reversal virus, and green and yellow mosaic viruses [4, 5]. Their carriers are mites, aphids, nematodes, and cicadas, which infect plants during the most active periods of their ontogenetic development.

Our observations indicate that in some plantings of the studied culture, the infection rate with viruses reaches 60%. This leads to a decrease in the yield by 10—70% along with simultaneously deteriorating the quality of berries.

In order to determine the degree of influence of various viruses on the anatomical structure of affected plants, we conducted a comparative study of healthy and diseased plant leaves.

Materials and methods. The material for investigation was different blackcurrant varieties from industrial and collection plantings of the Institute of Horticulture of the National Academy of Agrarian Sciences of Ukraine, as well as samples with symptoms of reversion and green speckling detected visually when examining the plantings of this crop. The objects of the study were viruses isolated from blackcurrant plants, namely *Blackcurrant reverse virus* and *Cucumber mosaic virus*. To confirm the viral etiology of the detected diseases, identify their pathogens, and detect abnormalities in affected plant cells, diagnostic methods on herbaceous plant indicators and electron microscopy of ultrathin sections were used [6, 7]. As an indicator, there were taken herbaceous plants *Chenopodium quinoa*, *Chenopodium murale*, *Cucumis sativus*, and *Nicotiana tabacum* infected by mechanical inoculation. Buds, young leaves, and petals were used as an inoculum. The inoculum was obtained by rubbing plant tissues with the addition of stabilizing mixtures: 0.03 g phosphate buffer pH 8.0 + 0.2% ascorbic acid + 0.2% sodium sulfate + 0.03 M caffeine; 2% nicotine base solution. The ratio of plant material and stabilizing mixture 1:6. Mechanical infection was carried out in the phase of 4—6 leaves of *Chenopodium quinoa*, *Chenopodium murale*, and *Nicotiana tabacum* (up to 10 plants for each tested sample) and in the cotyledon phase — of *Cucumis sativus* (up to 20 plants). To prepare ultrathin sections, strips of plant tissue were fixed in a 6.5% glutaraldehyde solution, followed by additional fixation with 2% osmium oxide, then dehydrated in alcohols, absolute acetone, and poured into EPON-812. The resulting sections were stained with lead citrate and viewed under an EMV—100A electron microscope [8, 9]. When studying viruses in raw extracts, the methods described by Protsenko, Legunkova, and Larina with co-authors were used [10, 11]. Negative contrast of native viruses was performed using a 2% solution of phosphotungstic acid pH 7.0—7.2 and 3% uranyl acetate [12].

Results. Using an electron microscopic method of analysis, a bacillus-like virus *Blackcurrant reversal virus* localized in the nucleus, perinuclear zone, and cytoplasm was detected in the tissues of blackcurrant leaves with double-flowering symptoms. The particle was 271 ± 7.19 nm long and 78 ± 2.31 nm in diameter (Fig. 1). Symptoms of mottled and pale green mosaic were found on blackcurrant bushes during leafing, and pale green «watermark» patterns were seen on aging leaves, which are clearly visible in transmitted light. On herbaceous indicator plants, the following symptoms were noted: ochre necrosis with a diameter of 2—3 mM on inoculated leaves of *Chenopodium quinoa*, light brown necrosis in the form of concentric rings on *Chenopodium murale*, yellow spotting with weak deformation of the leaves of *Cucumis sativus*, and mosaic on the leaves of *Nicotiana tabacum*. The signs on naturally infected blackcurrant bushes and on test plants with artificial infection made it possible to identify the pathogen as virions of *Cucumis virus 1***.** Smith. Virions of *Cucumis virus 1.* Smith were observed using electron microscopic methods to study artificially infected *Chenopodium quinoa* plants. They were freely located in the cytoplasm of the cell interspersed with ribosomes. Individual areas of the cytoplasm with a high virus concentration were also found in the affected parenchymal cells. The zones surrounded by the double membrane differ in size and degree of virus saturation (Fig. 2, a). When studying the pathogen morphology in the native preparations, the viral particles had a spherical shape with small, very colored central areas. Measurements of the virus particles showed that they were 29.6 ± 0.59 nm in size (Fig. 2, b). They were well preserved in the preparations stained with a 3% uranyl acetate solution (pH 5) for 2—3 sec, after which the remaining contrast agent was carefully removed with filter paper. Virions can occur in cells even before the appearance of external signs of the disease. With the development of the infectious process in diseased cells, the concentration and size of viral aggregates increased.

A comparative study of the ultrastructure of cells in normal and infected plants showed that the affected cells contain significantly

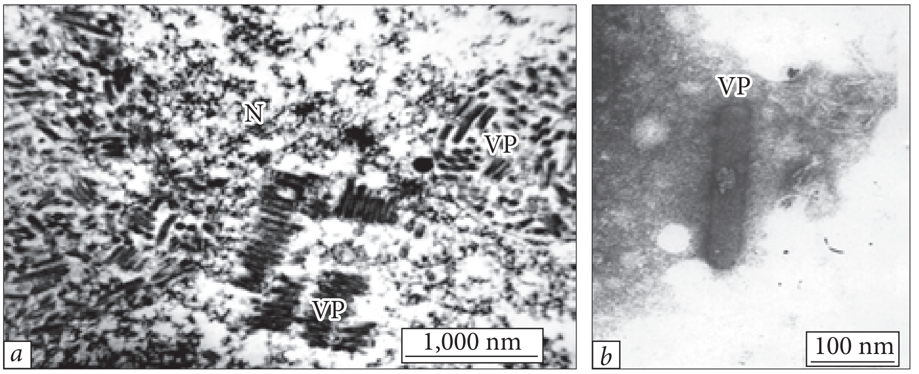

Fig. 1. *a* — Bacillus-like particles of the *blackcurrant reverse virus* in the nucleus of blackcurrant leaf cells (VP — viral particles, N — nucleus). Marker size — 1000 nm; *b* — Bacillus-like viral particle with rounded ends in a suspension of abnormal blackcurrant flowers. Marker size — 100 nm

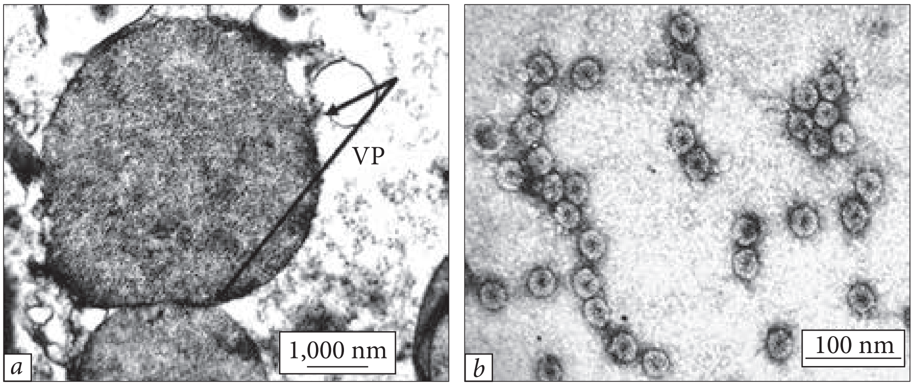

Fig. 2. Viroplasmas of *Cucumber mosaic virus 1.* Smith: *a* — in the cytoplasm of *Chenopodium quinoa* cells (VP — viral particle). Marker size — 1000 nm; *b* — isometric particles of *Cucumber mosaic virus* 1. Smith, from *Chenopodium quinoa* leaves. Marker size — 100 nm

more myelin-like formations than healthy ones. Their number, size, shape, and location are not the same for different viral diseases. They were found in the cytoplasm, chloroplasts, and vacuoles. In blackcurrant plants infected with the cucumber mosaic virus, myelin-like bodies are found not only in the cytoplasm of the affected cells but also in the extra plastic space and are characterized by unstable packaging of swirls placed parallel to the membranes (Fig. 3).

In blackcurrant plants infected with the *Blackcurrant reversion virus*, amorphous and crystalline inclusions are often found. The presence of electron-dense material in the cytoplasm of cells, externally granular or finely fibrillary, was observed in studying the strain of *Tomato mild mottle virus* (ToMMmoV) using an electron microscope [13].

A characteristic structural component of mesophyll cells is peroxisomes. They are found in all eukaryotic cells and are highly metabolic organelles surrounded by a single membrane [14, 15]. The involvement of peroxisomes in maintaining oxidized-reduced homeostasis has been

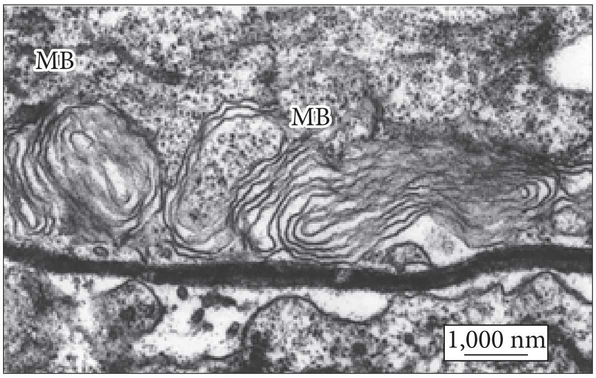

Fig. 3. Myelin-like bodies for damage of blackcurrant plants infected with *Cucumber mosaic virus* 1. Smith. The marker size is 1000 nm

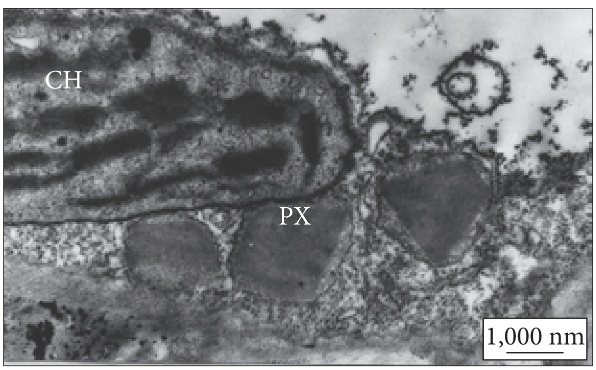

Fig. 4. Crystalline inclusions in the peroxisomes of tobacco leaf cells (PX — peroxisomes, CH — chloroplast) infected with *Cucumber mosaic virus 1.* Smith. Marker size — 1000 nm

noted in [16]. We found that at the stage of necrotization of a *Nicotiana tabacum* leaf infected with the *Cucumis virus 1. Smith*, the peroxisome matrix is intensively filled with crystalline inclusions, which have an electron-dense surface or are a system of rods with different configurations in the form of rectangles and trapezoids. They completely fill the matrix (Fig. 4).

In studying the ultrastructure of plant cells affected by various viruses, characteristic signs of morphological changes in plastids were established. Analysis of their morphology in plants affected by cucumber mosaic and currant double viruses indicates that the largest amount of outgrowths and cup-shaped formations is contained in the plant cells of *Nicotiana tabacum*. At the same time, their internal structure is being rebuilt. In one of the affected cells, you can see chloroplasts with signs of degeneration and a well-developed lamellar system. In destructive chloroplasts, it is difficult to detect a membrane system, and gran thylakoids are poorly distinguished or completely absent. Instead, «blocks» can be formatted as modified structures that arise as a result of the destruction of grains (Fig. 5). This leads to a sharp decrease in the volume of photosynthetic membranes, that is, there is a typical pattern of chloroplast aging, which is usually accompanied by a decrease in the leaf photosynthesis intensity.

The study results have shown that the species affiliation of the virus does not cause any specific changes in the morphology and structure of mitochondria. Their structural transformations under the influence of the viruses studied were the same: changes in shape and swelling, expansion of crysts and a decrease in their number, a decrease in the electron density of their matrix, etc. (Fig. 6).

Clusters of mitochondria are observed on ultra-thin sections of individual cells. In the formed aggregates, there are 4—6 organelles. It is possible that in infected plants, the activity of mitochondria during respiration increases not due to the complication of the internal structure, but due to the fact that the number of organelles contained in the cell has increased.

During the development of viral pathology in the cells of diseased plants, destructive processes cover the nucleus as well. As a rule, it takes on a lobed or radially elongated shape. Among the viruses studied, the blackcurrant double virus most induces this trait, which may be due to the fact that, accumulateed in large quantities, it exerts mechanical pressure on the nuclei and thereby accelerates the process of their deformation. In this case, the electron density of the nu-

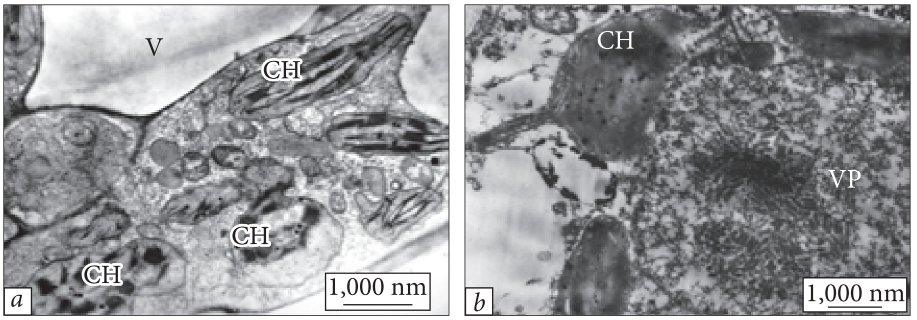

Fig. 5. Modified forms and structures of plastids in the cells of blackcurrant leave affected by *cucumber mosaic virus 1.* Smith — *a*; Blackcurrant plant affected by *Blackcurrant reversion virus* — *b*; V — vacuole, CH — chloroplast, VP — viral particles

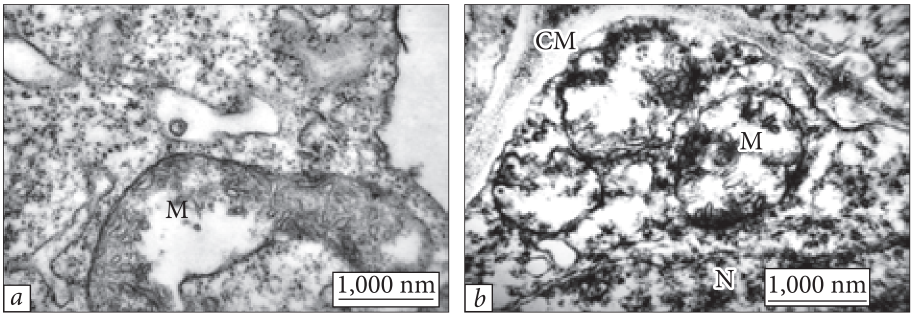

Fig. 6. Morphological changes of mitochondria in the cells of blackcurrant leave affected by the viruses: *a — Cucumber mosaic virus 1.* Smith; *b* — double *Blackcurrant virus* (CM — cell membrane, M — mitochondria, N — nucleus)

cleoplasm decreases. Small cores are very rare. As a result of the violation of the placement of chromatin matter in the matrix, electronic light zones are formed.

A specific sign of changes in the nucleus ultrastructure is the content of viral particles. Of the viruses studied, blackcurrant double particles were most often found in the nucleoplasm of the nucleus and perinuclear zone.

Thus, under the viral infection, a number of morphological and structural changes occur in the nuclei, which may be common to all viral pathogens or only to some of them. The fact that certain signs of pathology are more pronounced in some viral diseases and weaker in others can be explained by the degree of virulence of this pathogen and plant resistance to it.

Discussion. By using electron microscopy of ultrathin sections of blackcurrant tissues with typical double symptoms, we found bacillus-like particles with a size of $271 \pm 7.19 \times 78 \pm 2.31$ nm, which differed in the size and structure from ordinary cellular components. Virions are generally localized in the phloem tissues. The most typical site of localization of the virus is the nucleus and perinuclear space, rarely - the cytoplasm. Tubular structures were found in the nucleoplasm, the size of which corresponds to the inner rod of the particles are the nucleocapsid of mature virions.

Our electron microscopic studies of the affected tissues of the leaves and abnormal petals of blackcurrant flowers confirmed the data of other scientists on the detection of rhabdovirus particles in the tissues of this culture with characteristic symptoms of double-flowering [17—19]. From the literature, it is known that the diagnosis of cucumber mosaic virus (*Cucumis virus 1.* Smith) is complicated by the fact that its particles are difficult to differentiate from cytoplasmic ribosomes, the size of which is close to that of vibrios [20]. Using the electron microscopy for the tissue cells of artificially infected plants of *Chenopodium quinoa*, separate areas of the cytoplasm surrounded by a double membrane with different degrees of saturation with viral particles were detected. During the further study of the pathogen morphology in native preparations, isometric particles with small strongly colored central regions, the size of which averaged 29.6 ± 0.59 nm, were observed.

The intracellular development of viruses and their use for the reproduction of energy systems and components of the cell lead to significant morphological and structural changes in the latter. However, intracellular reproduction of the virus in subliminal and latent infections does not cause significant changes in host cells but can cause a very wide range of structural and functional changes in other types of infections: local, systemic, acute, chronic, or persistent.

Such myelin-like formations were established when raspberry plants were infected [21]. Some researchers have found similar myelin-like bodies when infecting plants with the soybean mosaic virus [22]. There is a small amount of information in the literature about the causes and function of myelin-like formations in the pathological state of cells caused by the aging of plant organs [23].

Similar changes in the structure of peroxisomes were observed in other viral infections in the plant cells [24—26]. Almost all the studied phytoviruses violate the ultrastructure of plastids in some way. One of the characteristic features of morphological changes in these organelles caused by viral infection is their shape. On ultra-thin sections of infected tissues, they are most often characterized by spherical, amoeboid, cup-shaped, and other configurations [20]. We observed such forms of plastids in cells of blackcurrant infected with the *Blackcurrant reversal virus* and *Cucumber mosaic virus*. Various outgrowths were formed on them (Fig. 5).

Analogous phenomena are described by a number of other scientists for other viral diseases [27—30]. Such anomalies can also occur in other plant lesions, as evidenced by literature data [31, 32].

Almost all studied phytoviruses in some way violate the ultrastructure of plastids. One of the characteristic features of the morphological changes in these organelles caused by a viral infection is their shape. On ultrathin sections of infected tissues, they are most often characterized by spherical, amoeboid, cup-shaped, and other configurations [17]. We observed such forms of plastids in blackcurrant cells infected with doubled-flowering and cucumber mosaic viruses. On the various plastids, outgrowths were formed (Fig. 5).

We have also revealed the effect of the viruses studied on the ultrastructure of mitochondria. Their action, first of all, was manifested in the form of these organelles. In infected cells, in contrast to healthy ones, we observed elongated, mace — and horseshoe-shaped mitochondria (Fig. 6).

At the same time, their internal structure changes. Extensive electron-light zones are found in the matrix of many mitochondria and the cristae are short and greatly expanded. Large mitochondria, characterized by a small number of cristae, are quite common.

Similar degenerative changes in the structure of mitochondria were observed under the influence of other viral diseases in host plant cells [33—36]. Other researchers have observed the involvement of the plant nucleus in viral infections [37—39]. Such changes in the nucleus occur in other plant lesions [40].

Conclusions. If external anomalies are detected in the form of mosaics and spots, which can be caused by many pathogens, and mechanical inoculation of the pathogen with juice is absent, it is advisable to use electron microscopy, which allows one to identify structures in the affected cell that are absent in a healthy one. This method has made it possible to reveal a bacillus-like virus on blackcurrant bushes and to investigate its structure and nature of localization.

A number of morphological and structural changes in cellular organelles have been identified. Such changes indicate an inhibitory effect of the virus on all physiological processes in plants, which dramatically reduces their productivity.

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ВПЛИВ ВІРУСНОЇ ІНФЕКЦІЇ НА УЛЬТРАСТРУКТУРНУ ОРГАНІЗАЦІЮ КЛІТИН ТКАНИН ЛИСТКА СМОРОДИНИ ЧОРНОЇ

Вірусні захворювання є дуже небезпечними для ягідних культур, тому точна і вчасна діагностика їх має велике значення для боротьби з ними. Використання електронної мікроскопії для діагностики дає змогу побачити вірусні частки, встановити їхню форму, розмір, локалізацію в тканинах і виявити аномалії в уражених клітинах рослин, а тому є ефективним методом розпізнавання збудників таких захворювань. **Мета.** Провести порівняльне дослідження здорових і хворих листків смородини чорної з метою визначення ступеня впливу двох вірусів на анатомічну структуру органел і включень, яке можна буде використати в діагностиці та ідентифікації вірусів, котрі уражують рослини. **Методи.** Матеріалом слугували рослини чорної смородини з симптомами реверсії та зеленої крапчастості, які виявляють візуально при обстеженні насаджень цієї культури. Морфологію вірусних часток, анатомічну структуру органел і включень вивчали із застосуванням методу електронної мікроскопії ультратонких зрізів. Виявлені ультраструктурні зміни в клітинах доцільно використовувати як діагностичні ознаки при ідентифікації вірусів. **Результати.** При дослідженні ультратонких зрізів тканин листка та аномальних пелюсток квітки смородини чорної з симптомами реверсії виявлено бациловидний вірус *Blackcurrant reversion virus* із родини *Rabdoviridae,* який легко ідентифікувати завдяки зовнішньому вигляду і великим розмірам, за якими вони відрізняються від подібних ознак у звичайних клітинних компонентах. За результатами електронно-мікроскопічних методів дослідження штучно заражених рослин *Chenopodium quinoa* інокулюмом уражених листків чорної смородини, у клітинах знаходяться віріони *Cucumis virus 1.* Smith, що вільно розміщуються в цитоплазмі клітини вперемішку з рибосомами. В уражених паренхімних клітинах виявлено також окремі зони цитоплазми з високою концентрацією вірусу. Під час дослідження ультраструктури клітин рослин смородини чорної, уражених *Cucumis virus 1.* Smith, мієліноподібні тіла зустрічалися не лише в цитоплазмі вражених клітин, але і в екстра плазматичному просторі. Аналіз морфології хлоропластів смородини чорної, уражених зеленою крапчастістю та реверсією, свідчить про те, що у багатьох клітинах є хлоропласти з виростами та чашоподібними утвореннями. За різних вірусних інфекцій спостерігається велика різноманітність мітохондрій за формою і внутрішньою структурою. Встановлено, що на стадії невротизації листка *Nicotiana tabacum*, інфікованого *Cucumis virus 1.* Smith, матрикс пероксисом інтенсивно і повністю заповнюється кристалічними включеннями, які мають електронно-щільну поверхню або є системою паличок з різною конфігурацією у вигляді прямокутників і трапецій. З розвитком вірусної патології у клітинах хворих рослин, деструктивні процеси охоплюють і ядро. Виявлено характерні ознаки ураження ядра збудником вірусу реверсії чорної смородини, що, можливо, пов'язано з тим, що цей вірус, накопичуючись у великій кількості, здійснює механічний тиск на ядро і тим самим прискорює процес деформації аж до радикальної зміни ультраструктури ядра через появу високого вмісту вірусних часток у нуклеоплазмі ядра та перинуклеарній зоні. **Висновки.** При виявленні зовнішніх аномалій у вигляді мозаїчностей, плямистостей, викликаних багатьма патогенами, та за відсутності механічної інокуляції збудника соком доцільно використати метод електронної мікроскопії, який дає змогу виявити в ураженій клітині структури, відсутні у здоровій. Цей метод дав нам можливість знайти бациловидний вірус на кущах смородини чорної, дослідити його структуру та характер локалізації. У процесі досліджень виявлено низку морфологічних і структурних змін клітинних органел, які свідчать про інгібуючий вплив вірусу на фізіологічні процеси у рослинах, що різко знижує їхню продуктивність.

Ключові слова: *смородина чорна, клітина, вірусні хвороби, хлоропласти, мітохондрії, ядро, пероксисоми, ультраструктура, електронна мікроскопія.*