Ultrastructure Aspects Of Acquired Resistance In Plants Under Viral Pathogenesis

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Abstract: The reported paper analyzes mechanisms of plant resistance to biotic factors with a focus on virus-induced lesions. Ultrastructure transformations occurring in leaf cells of virus-infected plants are studied. The work provides data on morphological features of cellular organelles in systemic and local viral lesions of virus-hypersensitive plants.

1. Introduction
To date, natural response reactions (immunity) to hazardous biotical and abiogenous environmental factors developing in a plant have become an important issue. However, a systematic understanding of this problem is still lacking. At the same time, a solution to this issue may make a certain contribution to the progress of selection, increase an agricultural yield, further the preservation of biodiversity, as well as facilitate research with a focus on plant protection. The phenomenon of interest has received critical attention because of ever-increasing environmental pollution, global climate changes, and inability of living organisms to adapt to these factors.

To explore the immunity evolvement to biotic factors in plants it is reasonable to consider an ultrastructure aspect of this question.

2. Main part
In co-evolution of plants and pests a variety of natural protective mechanisms have developed to form the adaptive immunity. From our point of view, a plant-virus interaction is a future-oriented research domain for there has been little discussion of virus-induced diseases so far.

The adaptive immunity develops if an organism is in immediate contact with a pathogen and may be either systemic or local.

The systemic acquired resistance of plants shows whether they are re-infected by the same virus (re-infection). It is related to the development of defensive reactions, limiting propagation and accumulation of a virus [1,2,3,4]. However, for the evolution of systemic resistance, developing defensive mechanisms are far to be sufficient to localize an infection in the virus entry area and prevent it from spreading through an entire plant, whereas local acquired resistance may isolate a virus in its entry spot. The adoptive immunity is apparent at different levels of a species: organism, tissue, ultrastructure, and molecule.

At a level of organism an immune response of plants to a virus entry affects their morphological characteristics on the whole, e.g. a variety of diagnostic signs caused by diverse diseases appear on plant leaves. For a variety of plants and pathogens these signs can be in a form of mosaic light yellow or green spots, leaf tissue can tend to curling up or crooking, knobs can develop on leaf ribs, so called yellow sickness – yellowish or light green leaves – chlorosis may be seen.
At a molecular level, the resistance of a plant may show as the synthesis of certain compounds. For instance, PR-proteins are produced in plants to respond to bacteria, fungi, and viruses. These compounds were detected in leaves when systemic or local types of virus-induced resistance developed there. They were discovered for the first time when exploring hypersensitive tobacco plants infected with tobacco mosaic virus [5]. It was established these compounds to connect with processes furthering defensive reactions of plants to a pathogen entry. For now, PR-proteins represent molecular markers used to reveal the systemic induced resistance [6].

There are also other chemical compounds, which influence directly or indirectly the effect of acquired virus-induced resistance. For instance, it is reported on a so-called “anti-virus factor” (AVF) [7] targeting the inhibition of a virus replication process [8]; the research has highlighted activated hydrolases (RNase and protease) in the apoplast for a case of virus-induced resistance [9]. In addition, chemical resistance inducers were detected in plants; e.g. active oxygen forms, salicylates, jasmonates, and ethylene. Jasmonic acid is thought to be a key molecule in the warning system activating defensive mechanisms in plants stressed by damaging factors.

In her research A.M. Polyakova [10] demonstrated that plants may respond to a virus entry in a different way: from morphological to ultrastructure transformations in infected leaves. The researchers reported on a combination of paling ribs and morphological changes in cellular organelles in cut Samsun tobacco leaves totally infected with tobacco mosaic virus. In local and systemic virus infections such responses of plants to pathogen entries were related to the promoting protein-synthetic activity of cells and processes of catabolism (lysosomal activity) [10]. For instance, a high degree protein-synthetic system activity manifested itself in the occurrence of large nuclei with expanded nucleoli in infected cells, as well as numerous ribosomes in cytoplasm, and a well-developed system of mitochondria and chloroplasts. At the same time, a number of researchers [10, 11, 12, 13] pointed at active lytic processes in infected cells (activated Golgi apparatus, a significant number of small vacuoles, residues of lysed cell bulk, etc.).

The evolving local acquired resistance is proposed to be one of the most important criteria indicating the developing acquired virus resistance.

The local acquired resistance results in the necrosis on infected leaves and a hypersensitivity reaction, which localizes a pathogen in its entry area, blocking its propagation from a necrosis through the plant [14]. Therefore, no particles of virus are detected in tissues between necroses on infected leaves [15]. However, a great number of virus particles were found in a local necrosis, they are in a form of small or large blocks with a varying number of intact virions [13].

The re-infection (re-inoculation) of leaves with hypersensitivity to a pathogen caused by a homologous virus initiated less extended secondary necroses between primary necrosis zones with a space above 1-2 mm from their edges. On the contrary, neither secondary necroses nor virus particles are detected 1-2 mm from primary local necroses. Investigating local necrosis areas infected with tobacco mosaic virus in 1961, A. Ross suggested the resistance to a virus infection to develop in these tissues. He offered to refer to these zones as ad-necrosis ones. K. Yarwood [16] and Ross [17] demonstrated the so-called acquired resistance to evolve in ad-necrosis areas infected with tobacco mosaic virus.

At the same time, the resistance is induced between ad-necrosis zones in tobacco leaves infected with tobacco mosaic virus; a number and diameter of secondary necroses decrease in re-inoculation. This type of resistance was offered to refer as a systematic one.

So far, there has been no detailed investigation on the realization of systemic and local virus-induced resistance. Recent studies have established that under the local acquired resistance ultrastructure transformations of cells in and out of local necroses and ad-necrosis areas are quite different, indicating a degree and target of their metabolic activity. The important finding to emerge from the study is that these transformations depend upon the position of cells relative to a local necrosis edge [11]. Thus, the most significant destructive changes in the ultrastructure of cell organelles were detected in the necrosis center, where most of cells were subject to necrotization. In a varying degree necrotic cells were found...
close to a necrosis edge, although ultimately intact cells with considerable destructive changes of organelles formed there.

At a distance from a necrosis edge (in a space between necroses) the morphology of cell organelles was quite normal, demonstrating no signs of necrotization [11]. For instance, nucleoli in some cells close to the necrosis edge suffered from necrotization, but in a varying degree. At the same time, necrotization of nuclei was not registered in more distant cells in an ad-necrosis zone (1-2 mm), although they displayed considerable morphological deviations. These transformations represented characteristics of organelles typical for higher metabolic activity of a cell, i.d. a twisted nucleolemma, coarse nucleoli, and heterochromatin.

Endoplasmic reticulum in Datura Stramonium L. leaf tissue infected with tobacco mosaic virus had a variety of destructive changes. For instance, cisternas of agranular endoplasmic reticulum in a local necrosis and close to it were swollen. Here, swollen cisternas in the middle of a necrosis were somehow brazed-in into the dark collapsed cytoplasm. Swollen cisternas of endoplasmic reticulum may evidence deteriorating defensive properties in their membranes and suggest entering of hydrolases into cytoplasm [18]. It is a most probable reason for a cytoplasm collapse.

Furthermore, researches pointed at destructive transformations of vacuoles close to a necrosis edge for their tonoplast was unusually electron-dense. Destructive changes were also detected in chloroplasts. Their edges looked amorphous. Mitochondria also deviated from the norm since their crista was expanded or narrowed, other changes were found as well.

No necrotization signs were reported at a further distance from the necrosis edge (1-2 mm). The crista in mitochondria was well-developed and embedded in a relatively dense matrix, that fact may be considered as an evidence of its activity. There were more membranes of rough endoplasmic reticulum detected in palisade cells there than in non-infected cells. Moreover, the well-developed granular endoplasmic reticulum was found in cells of ad-necrosis areas. It was reported on an increased amount of Golgi bodies and activation of its apparatus; hence, a lot of vesicles were formed. The study highlighted a heightened number of peroxisomes, multivesicular bodies, and coated vesicles. In addition, a morphometric analysis disclosed a high volume of cytoplasm and volume density of mitochondria, micro-bodies, membranes of granular endoplasmic reticulum in cells of ad-necrosis areas, except for chloroplasts.

To sum up, despite the fact cell organelles remained morphologically intact in ad-necrosis areas, where the local acquired resistance developed, their ultrastructure differed somehow from not-infected tissues. These changes, as apparent from morphological parameters, are typical for catabolism processes, evolving immediately at a necrosis edge, and anabolism processes occurring at a distance from a local necrosis area.

Such ultrastructure transformations in the morphology of cell structures revealed in various parts of Datura Stramonium L. leaves infected with tobacco mosaic virus might indicate the realization of acquired resistance mechanism with a focus on virus localization and damaging.

Hence, the research into mechanisms of responses evolving in cells of virus-infected plant leaves at the ultrastructure level may provide data to determine a degree of plant resistance to pathogen due to a direct correlation between plant lesions and developing lytic processes [11].

To date, there are published data suggesting that under the local acquired resistance a virus is localized thanks to necrotization of tissues, as a consequence, a local necrosis forms, and other processes taking place in tissues around it. This assumption was confirmed as virus particles were detected beyond a local necrosis, close to its edge, in cells remaining morphologically intact [18]. Therefore, mechanisms furthering tissue necrotization restrict a propagating virus but fail to localize it totally [19]. There is still no consensus concerning mechanisms, which initiate the development of a local necrosis area. Some data [20,21] suggest necrotization is caused by products of phenols oxidation – quinones accumulating in tissues. However, the further research pointed out this is not a main reason for the development of necroses for they originate before the activation of polyphenol oxidase [22] and peroxidase [23] ferments is detected. H.W.J. Ragetti’s experiments [24] showed hydrolytic ferments, etc. contribute to the development of hypersensitivity reaction. It was suggested local necroses may be caused by mechanical
factors, such as blocked plasmodesm with callose [25, 26] However, this phenomenon is also observed if a systemic infection develops [26] In some works it was reported on the formation of lignin under evolving necrosis [27].

To conclude, there has been no comprehensive investigation of mechanisms initiating the development of necroses, biological importance of this process, as well as mechanism isolating viruses so far; therefore, they should be closely examined. Nevertheless, there are published data suggesting the potential biological importance of necrotization is not limited by virus isolation in its entry zone but it promotes the synthesis of some signal molecules under cell death. They might initiate reactions which totally localize infections. This assumption is indirectly proved by findings of ultrastructure studies on local necroses developing on Datura Stramonium L. leaves infected with tobacco mosaic virus; they demonstrated both collapsed cells and intact cells, which were not subjected to the hypersensitivity reaction [11, 13]. The ultrastructure remained in these cells in spite of being in the center of a local necrosis; in addition, there were virus particles in them.

Several researchers suggested that completely collapsed and intact cells may co-exist in a local necrosis as fully necrotized cells produce enough signal molecules, which initiate reactions of total pathogen isolation [11, 13], so destruction of other cells is unnecessary. In some studies oligosaccharines are suggested to be such signal molecules [28, 29].

When isolating a virus, so-called antivirus proteins may be very important [14, 29, 8]. In their work Moser et al. [31] assumed that the isolation of a virus may be associated with the destruction of a transport protein. A search of literature revealed findings of other researchers making their contribution to the understanding of this process. However, a systematic understanding of the virus-induced local resistance is still lacking. Therefore, an ultrastructure aspect of this phenomenon may provide new insights into mechanisms of acquired systemic and local resistance development.

3. Conclusions

To sum up, a study with a focus on mechanisms of acquired resistance in plants to harmful biotical environmental factors has revealed ultrastructure transformations in cells of plants are in line with principles and orientation of metabolic processes. Furthermore, morphological characteristics of transformations attributed to the development of local acquired resistance in Datura Stramonium L. leaves infected with tobacco mosaic virus are different due to prevailing anabolism (ad-necrosis cells) or catabolism (necrosis cells) processes. This fact might be used for assessment of plant resistance to viruses.

The target of both systemic and local acquired resistance is to isolate a virus in a plant and damage it.

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