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Culture des plantes

MOVEMENT OF CUCUMBER MOSAIC VIRUS IN RESISTANT PEPPER LINES

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Abstract

Cucumber mosaic virus (CMV) movement in L113 and L57 pepper lines resistant to this virus was studied. The virus presence was confirmed only in the blades and petioles epidermis of CMV inoculated leaves while the rest parts of the plants were virus free. On these leaves of L113, necrotic local lesions (typical hypersensitive response – HR) were formed whereas in L57 chlorotic spots appeared and a few days later the leaves fell. Despite the fact of only L113 possesses HR-mediated resistance to CMV, virus long-distance movement was similarly restricted in both pepper lines. El-microscopic observations have found regions consisting of optically almost empty dead cells in symptomless areas near the necrotic (L113) and chlorotic (L57) spots. The walls of these cells are incidentally crossed by dark-coloured plasmodesmata. The structural changes in plasmodesmata in still alive leaf cells also testify for their active participation in the virus movement through the leaf cells of CMV inoculated leaves. These cell changes were observed only in CMV inoculated leaves of both resistant pepper lines.

Key words: cucumber mosaic virus, disease resistant, cell-to-cell and long-distance virus movement cell senescence, *Capsicum annuum*

Introduction. Host (cultivar) resistance occurs when genetic polymorphism for susceptibility exists in the plant taxon, i.e. some genotypes show heritable resistance to a particular virus, whereas other genotypes in the same gene pool are susceptible. Resistance to plant viruses is often associated with a hypersensitive response (HR). In host resistances, the virus may not multiply or may multiply to some extent, but spread of the pathogen through the plant is restricted, and disease symptoms are generally highly localized or are not apparent [1].

In some accessions of the genus *Capsicum* and related wild species, different forms of CMV resistance: tolerance [2] and partial resistance [3], have been established. The tolerance is known based on reduced virus multiplication and/or restriction of CMV installation in host cells. Partial resistance to CMV is usually associated with some mechanism of reduction of the CMV long-distance movement [3, 4].

Two Bulgarian pepper lines resistant to CMV were developed through continuous selection – L57 and L113 [5]. Both lines differ in their response after being inoculated by CMV. The chlorotic (L57) or necrotic (L113) spots were developed on CMV inoculated leaves. In both cases, the inoculated leaves fall down and the other parts of the plant develop normally [5].

As stress effectors, viruses are known to provoke premature host senescence and more rarely death of some cells. The induced host cell death has usually been regarded as a mechanism of the infection localization. That is why the provoked collapse of tissue (HR) is usually regarded as an important antiviral defence strategy of the plant. However, the roles of HR (as a form of programmed cell death) in the resistance are under discussion [6]. To determine the role of HR in the virus spread, it is of interest to follow the structure of the cells and their PD nearby to HR-expressing tissues.

In the study, we looked for provoked changes in the general leaf morphology, plasmodesmata development and the link between changes in the leaf tissues after CMV infection in susceptible and resistance pepper plants expressing or not HR. The presence of virus particles was checked in the inoculated and systemic leaves, petioles and stems.

Material and methods. *Plant material and virus inoculation.* Two pepper lines – L57 and L113, resistant to CMV and the susceptible cv. Albena were used. The inoculations were realized with CMV strain CMV-PB at concentrations of 50 (L113) and 25 (L57) µg/ml virus in 0.01 M phosphate buffer, pH 7 [5]. The plants at the stage of 3rd or 4th leaves were mechanically inoculated by rubbing (two leaves) with 20 µl inoculum per leaf. The readings of symptoms and a presence of virus were followed on the 3rd, 7th, 14th and 21st days post inoculation (dpi).

Virus location in the plant. The DAS-ELISA [7] with CMV-specific IgG and conjugate with alkaline phosphates was performed. The tissue print analysis using a DIG-labeled RNA probe complementary to CMV RNA (19 dpi)

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was made. RNA probes for detecting CMV RNA were prepared by T7 RNA polymerase $Hind$III-linearized plasmid p40, which contains the 270 nt of the 3′-end of CMV RNA4. Nylon membranes were hybridized with positive strand-specific digoxigenin (DIG)-labelled CMV RNA4 probes. Blots were washed, incubated with antidigoxigenin antibody, and the DIG-bound RNA was visualized with CDP-star, according to the manufacturer's instructions (Roche Diagnostics).

Tissue processing for electron microscopy. The samples for microscopy observations were collected from several leaf areas of 2nd rubbed (with necrotic or chlorotic spots) and 4th systemic leaves of CMV inoculated plants and the same leaves of control plants at 19th dpi. The leaf pieces (1–2 mm^2) were fixed in 3% (v/v) glutaraldehyde, postfixed in 1% (w/v) OsO_4 and embedded in Spurr's medium (Spurr, 1996). The ultra-thin sections were stained with uranyl acetate and lead citrate for transmission electron microscopicexamination (el-microscope Zeiss EM-109 (Zeiss, Jena, Germany)

Results. The main differences between the two pepper lines were relevant to the phenotype expression of CMV resistance. On the CMV inoculated leaves of L113, necrotic local lesions (HR) were formed, whereas in L57 chlorotic spots appeared, i.e. HR was missing. The first symptoms on CMV inoculated leaves of the resistant pepper lines developed on the 12–16 dpi and fell down 3 to 6 days later. The development of CMV inoculated plants of L113 and L57 was the same as that of non-infected control plants. By contrast, inoculated leaves of susceptible cv. Albena were symptomless and had a limited fall-down as an exception to the rule, but typical CMV symptoms developed on systemic (young) leaves on the 12–14 dpi and the whole plant development was retarded.

Both methods of virus detection – DAS-ELISA and tissue print test (Figs 1 and 2) gave similar pattern of the virus spread through the plants. The virus accumulation in the CMV inoculated leaf blades of cv. Albena is slower than that in the L57 and L113 (Fig. 1). In contrast with the susceptible control cv. Albena, in the petioles of CMV infected leaves of both resistant lines, a later and smaller virus quantity was found. This presence of CMV was obviously possible via cell-to-cell viral movement but not via the petiole phloem flow. The stem, including the nodes of inoculated leaf attachment and systemic leaves of both resistant lines, did not contain the virus. The CMV distribution was detected in all parts of inoculated plants of the susceptible cv. Albena (Figs 1 and 2).

The branched PD situated in groups are typical (over 90% of all) of palisade cells in visibly non-damaged blade regions around the just formed necrotic (L113) and chlorotic (L57) spots on CMV inoculated leaves. By contrast, the leaf cells of regions situated away from visibly damaged regions in the inoculated leaf of the CMV resistant lines, and those of inoculated leaves of cv. Albena contain no more than 45% branched PD. PD with dark-coloured (to one or another extent)

aperture are another type seen in both L113 and L57 (Fig. 3D). One could suppose that they are filled up with osmiophilic substances, which resulted in full or partial lack of PD functioning; a view supported by the fact that often the adjacent cells seem more or less damaged. Despite the lack of HR in L57 leaf with apparently dead cells, single dead cells or (rare) small in area regions consisting of 2–3 optically empty dead cells with PD filled with dark-coloured material were found in and around the chlorotic spots, like in cells of visibly no-damaged areas of L113.

The PD of spongy mesophyll cells are in smaller number. Sometimes dead cells with damaged PD are seen in the regions near the leaf vein.

Discussion. According to CARANTA et al. [3], restriction to the long-distance movement of CMV is a common resistance mechanism in *Solanaceous* crops, but hypersensitive or extreme resistance against this virus is never observed. However, our results strongly showed that L113 possessed HR-mediated resistance to CMV which was inherited as a dominant trait. CMV resistance was closely linked to the tobacco mosaic virus resistant gene L (L1) and both resistances were transmitted together (our unpublished data). CMV concentration was almost the same in inoculated leaves of L57 and susceptible cultivar but the virus quantity in L113 inoculated leaves reached higher value. Therefore, both
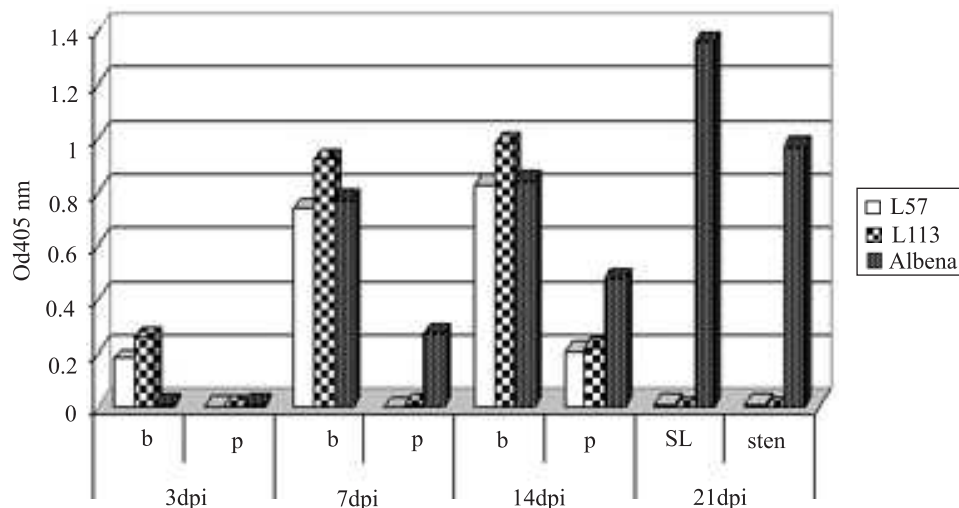

Fig. 1. Dynamics of CMV distribution in inoculated and systemic (non-inoculated) leaves of L57 and L113 resistant pepper lines and susceptible control cv. Albena. The quantity of CMV (OD_{405nm}) estimated by DAS-ELISA: The blades (b) and petioles (p) of CMV inoculated leaves were tested at 3rd, 7th and 14th dpi. The youngest leaves (SL) and stem below them were analyzed at 21th dpi

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investigated pepper lines did not restrict CMV installation in the host cells (described by Caranta et al. [8] in pepper cv. Perennial), or virus accumulation in the initially infected cell is not rapidly arrested [9]. These experiments confirmed that CMV long-distance movement was restricted in plants of resistant pepper lines. It is also known that the production of reactive oxygen species, particularly hydrogen peroxide, in response to virus attack is a key event in HR [10]. The development of CMV-induced HR of L113 was found supported from changes in the hydrogen peroxide quantity in inoculated and systemic (non-inoculated) leaves. The hydrogen peroxide in CMV inoculated leaves of L113 reached two peaks in progress of virus infection. The first peak preceded the formation of necrotic local lesions and the second one coincided with the time of fall of the leaves [11]. In L57 inoculated leaves, hydrogen peroxide quantity was higher (up to 118%) only during the time of leaf fall and at the rest of the time it was lower than that in mock-inoculated leaves [12]. These data are in correspondence with the observed type of resistance – CMV-induced HR in L113 and HR lack in L57.

A common feature of viral movement are modifications of the PD facilitating its spread. The main CMV-provoked change found in both resistant lines is the enhanced branching of PD of the leaf palisade cells. The processes of lateral fusing of both neighbouring primary PD or by the de novo addition of new cytoplasm strands to such already present in the cell walls are known
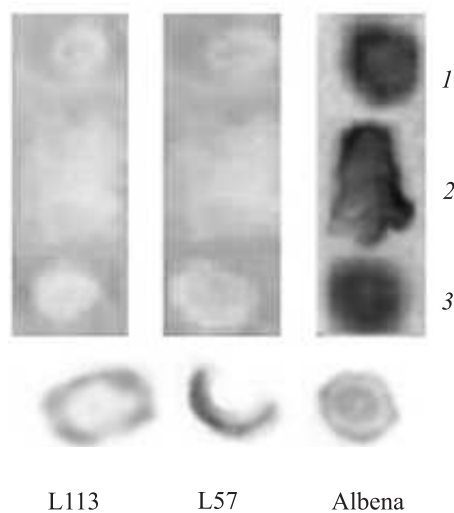

L113   L57   Albena

Fig. 2. CMV presence analyzed by tissue print test. Longitudinal sections of the nodes of leaf attachment (2); cross sections of stems under (1) or above (3) the nodes, and of petiole (p)
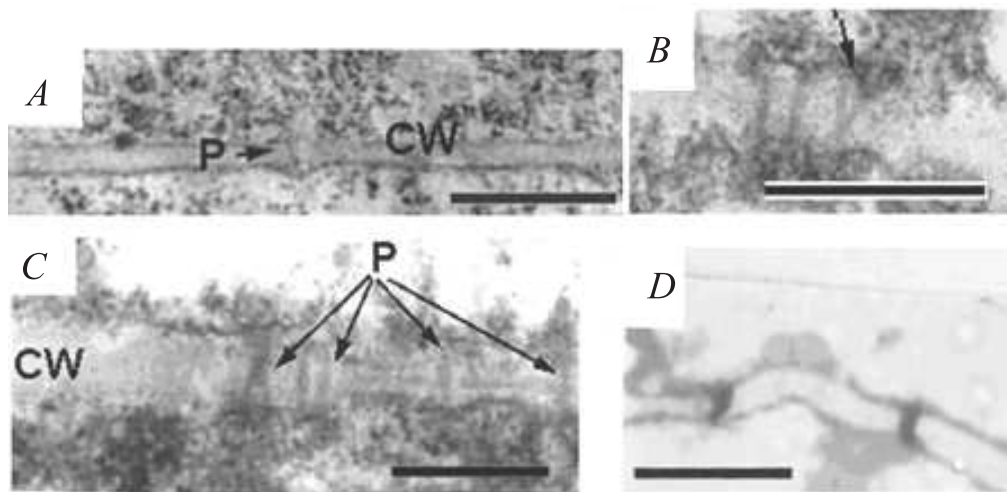
Fig. 3. Sections of palisade cell walls bearing typical plasmodesmata. Mock-inoculated (control) leaf (*A*); CMV inoculated leaf; L57 pepper plant (*B*); CMV inoculated leaves; L113 plant (*C, D*). Bars = 1 μm

as responsive for PD branching. The involvement of PD in viral systemic infection has long been accepted. Such structural changes of PD in susceptible plants are regarded as viruses-provoked facilitating cell-to-cell virus transport (reviewed by WAIGMANN et al. [13]). For viruses, specific movement proteins (MPs) are thought to play a role in the modification of the PD facilitating the virus spread. The more branched PDs were now found located in regions close to virus-provoked necrotic spots characterized by the presence of dead cells. The cell death is usually supposed to prevent further spread of the virus, thus restricting infection to the initially infected cells. On the other hand, whereas (complex) branched secondary structures of PD are characteristic of old leaves [13], the highly damaged cells of the spots could be supposed to enhance senescence of the neighbourly cells. However, the fast reaching of high virus concentrations in the infected blades of both lines (L113 and L57) points to lack of early effective restriction of cell-to-cell movement. The cell death during pathogen attack is a useful tool in understanding disease resistance [13]. In the light of our results, the death of cells not overcoming a pathogen pressure could not significantly prevent the cell-to-cell spread in the leaf blades of the resistant lines.

As it was shown, the tissue print of petioles of CMV inoculated leaves of resistant plants testify for some CMV available in the epidermal layer of cells only, while all petiole tissues of susceptible cv. Albena contain virus (Fig. 2).

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The limited CMV presence in petiole epidermis of resistant lines could be a result of the low speed cell-to-cell virus transport. The events of cell death and the appearance of non-functioning plasmodesmata could be one of the mechanisms responsible for the prevention of the virus penetration into the leaf-petiole phloem flow. All data proved that CMV long-distance movement was strongly restricted in L57 and L113 pepper lines. The faster fall of CMV inoculated leaves in resistant line obviously eliminates the infection pressure for the rest plant parts being an important part of this resistance.

It is most likely that a plant factor(s) responsible for CMV transport through different cell types of the vascular system in the resistant pepper lines is(are) mutated. These changed factors prevent CMV passing through the respective barriers during its long distance movement. HR, the accelerated aging and the fall of inoculated leaves, decreasing the virus infection pressure, favour the CMV resistance in these pepper lines.

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