
Viruses of cucumber plants and identification of their agents

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Viral diseases of cucumber plants are characterized by various symptoms: vein clearing, mosaic, mottling and malformation of leaves, deformation and stunting of plants. The present paper reports the results of identification of viruses from cucumber plants. Identification of viruses was based on host range, symptoms on naturally infected cucumber plants and susceptible test plants and morphological properties of virus particles. Three viruses: *Cucumber mosaic cucumovirus* (CMV), *Cucumber green mottle mosaic tobamovirus* (CGMMV) and *Tomato ringspot nepovirus* (ToRSV) were isolated and identified from cucumber plants in Lithuania. Isometric particles of CMV and ToRSV and rod-shaped of CGMMV were found in the samples of preparations from naturally infected cucumber as well as from test plants. The purified preparations of CMV had A_{\max} at 260 nm and A_{\min} at 240 nm, A_{260}/A_{280} ratio was 1.05–1.12. The yield of CMV was 58.5–61.4 mg/1 kg infected plant tissue. The purification of ToRSV and preparation of antiserum were presented in [1].

Key words: cucumber, viral diseases, viruses, identification, purification

INTRODUCTION

The yield of the crop of cucumber plants (*Cucumis sativus* L.) depends not only on agricultural and meteorological conditions, but on the diseases including those caused by plant viruses. Cucumber seeds of cultivars of various origin have been brought to the country in the latter decade, therefore viruses affecting cucumbers might inevitably be spread. Timely detected viral diseases of cucumber plants and their agents as well as ascertained biological properties would enable to use corresponding measures restricting their spread. Common and green mosaics of cucumber plants and their agents have been described [2]. In the lists of plant viruses 8 economically important viruses belonging to different taxonomic groups, which were isolated from cucumber plants, have been specified [3, 4]. *Cucumber necrosis tobusvirus*, CMV, *Cucumber leaf spot carmovirus*, CGMMV are considered to have a world-wide distribution. CMV is reported to infect 1287 plant species in 518 genera in 100 families [5] and induces a very diverse spectrum of symptoms on them [6]. The virus has some reservoirs in wild perennial flora. The infection is transmitted by over 60 aphid species, it spreads through the sap of infected plants during leaf contact, through seeds of 19 plant species and *Cuscuta sp.* [3, 7]. ToRSV affects 518 plant

species in 236 genera of 65 families [5]. In neighboring Latvia indoor cucumber plants were damaged mostly by CGMMV. Sometimes CMV, *Tobacco necrosis necrovirus* and *Arabis mosaic nepovirus* were identified. CMV was most dangerous to outdoor cucumber plants. Other nepoviruses have also been found [8]. The first investigations on viral diseases of cucumber plants in Lithuania were related to the influence of the mentioned diseases on tomato plants cultivated in indoor soil next to cucumber plants. Investigations on virus diseases of cucumber plants and their agents have been continued in Lithuania since 1993 and supplementary data on viruses and their biological properties have been obtained.

The aim of the preset work was to isolate viruses from different cultivars of cucumber plants with virus-like symptoms, to identify them, define experimental host range, symptoms, shape of virions and for CMV to prepare the antigen suitable for obtaining the diagnostic antiserum.

MATERIALS AND METHODS

The object of the investigation was cucumber plant samples collected in private gardens, experimental fields of the Kaunas Research Station of plant crop species (Mūniškės, Kaunas r.) and Lithuanian Institute of Horticulture (Babtai, Kaunas r.) with dis-

tinct viral symptoms: mosaics, spotting, deformation, growth disorders. Viruses were isolated from leaf extracts and identified following the methods of transmission electron microscopy (EM) and test plants according to the presented scheme [9]. In order to differentiate virus isolates and identify viruses, test plants of *Aizoaceae* Rudolphi, *Amaranthaceae* Juss., *Asteraceae* Dum., *Chenopodiaceae* Vent., *Cucurbitaceae* Juss., *Fabaceae* Lindl and *Solanaceae* Juss. families (Table 2) were used based on the data of corresponding plant viruses. To stabilize virions in the infected plant sap, 0.1 M phosphate buffer pH 7.0 was used. Antigens of CMV were prepared from fresh or frozen at -20°C plant material (*Nicotiana glutinosa* L. and *Tetragonia expansa* Murr.) of a corresponding isolate infected by viruses. CMV

and ToRSV were purified according to [6, 10] and [11] methods, respectively. The purification of CMV isolates N 9009 and N 9109 was applied. Systemically affected plants of *N. glutinosa* were homogenized in 0.2 M phosphate buffer pH 7.2 with 0.1% 2-mercaptoethanol. The homogenized material was cleared up by adding 10% of chloroform and emulsified by 1% of Triton X-100. Virions were precipitated by 10% polyethylenglycol M.w. 6000 and 0.2 M sodium chloride. Further on, CMV was purified by two cycles of differential centrifugation through a 20% sucrose cushion. The amount of the viruses in the purified preparations was estimated by electron microscopy and spectrophotometrically. The methods of ToRSV purification and preparation of diagnostic antiserum have been described earlier [1].

Table 1. A comparison of host range and symptoms caused by virus isolates from cucumber plants

| Nr | Test plants | CMV | CGMMV | ToRSV |
|----|-------------------------------------------------|-----------------------------------------------------|----------------------------------------------------|------------------------------------------------------------------------------------------------------|
| 1 | <i>Amaranthus caudatus</i> L. | 0 | 0 | 0 |
| 2 | <i>Atriplex hortensis</i> L. | L: ChlLL | 0 | – |
| 3 | <i>Capsicum annuum</i> L. | S: M, Dis | 0 | 0 |
| 4 | <i>Celosia argentea f. cristata</i> (L.) Kuntze | – | 0 | L: RLL; S: RiSp, Ru |
| 5 | <i>Chenopodium amaranticolor</i> Coste et Reyn. | L: ChlLL | 0 | L: WhLL; S: TDis, St |
| 6 | <i>Chenopodium ambrosioides</i> L. | L: NLL | 0 | L: SmNLL; S: TDis |
| 7 | <i>Chenopodium foetidum</i> Schrad. | 0 | 0 | L: ChlLL |
| 8 | <i>Chenopodium murale</i> L. | (L: ChlLL) | 0 | L: ChlLL; S: VC, TDis |
| 9 | <i>Chenopodium quinoa</i> Willd. | L: ChlLL | (L: ChlLL) | L: ChlLL; S: TDis, W, D |
| 10 | <i>Cucumis sativus</i> L. | ‘Alfa’ ‘Movir’ ‘Libelle’ ‘Trakų pagerinti’ | S: M, Dis S: VC, YM S: Dif ChlSp S: M, Mo | S: M, Mo (L:NL); S: GrMo, Dis L: NLL; S: DifMo L: LaNLL; S: ChlSp S: RiSp S: Mo, RiSp |
| 11 | <i>Cucurbita pepo</i> L. ‘Black Beauty’ | S: YM, Dis | S: VC, MiMo | S: ChlMo |
| 12 | <i>Datura stramonium</i> L. | S: DifMo, Dis | (L: LL) | 0 |
| 13 | <i>Gomphrena globosa</i> L. | L: NLL | 0 | L: Wh,N,RiLL |
| 14 | <i>Lycopersicon esculentum</i> Mill. | ‘Nevskij’ ‘Ryčiai’ | S: M, Dis S: Mo | 0 0 S: VN, ChlMo S: VN, ChlMo |
| 15 | <i>Lupinus albus</i> L. | S: Mo, Dis, TW | 0 | – |
| 16 | <i>Nicandra physalodes</i> (L.) Gaertn. | L: NLL | 0 | 0 |
| 17 | <i>Nicotiana debneyi</i> Domin. | S: YMo | 0 | S: ChlMo |
| 18 | <i>Nicotiana glutinosa</i> L. | S: VC, Mo, TDis | 0 | 0 |
| 19 | <i>Nicotiana rustica</i> L. | S: DifMo | (S: ChlMo) | L: RiNLL; S: RiSp |
| 20 | <i>Nicotiana tabacum</i> L. ‘Samsun’ | S: Mo, Dis, St | 0 | L: LaRiLL |
| 21 | <i>Phaseolus vulgaris</i> L. | ‘Bataaf’ ‘Red Kidney’ | S: ChlSp, Mo, Dis S: NSp, M, St | 0 0 L: SmNLL (L: YLL) |
| 22 | <i>Pisum sativum</i> L. ‘Žalsviai’ | S: DifMo, M | 0 | 0 |
| 23 | <i>Tetragonia expansa</i> Murr. | L: ChlLL | 0 | L: LaChlLL; S: DifChlSp, St |
| 24 | <i>Vicia faba</i> L. ‘Aušra’ | S: M, Mo | 0 | 0 |
| 25 | <i>Vigna sinensis</i> Endl. | S: ChlMo | 0 | – |
| 26 | <i>Zinnia elegans</i> Jacq. | L: NSp | 0 | 0 |

Abbreviations: L – local reaction, S – systemic reaction, LL – local lesions, R – red, Wh – white, Gr – green, N – necrotic, Chl – chlorosis, Y – yellow, Ri – ring, Sm – small, La – large, VC – vein clearing, VN – vein necrosis, TW – top wilting, Dis – deformation, TDis – top deformation, Dif – diffuse, D – dying, St – stunt, Sp – spot, Ru – rugosity, M – mosaic, Mo – mottling, O – no symptoms, ‘–’ plant is not inoculated, () – symptoms were questionable

Table 2. Virus isolates and viruses identified from cucumber plants

| Cultivar or select. number | Symptoms | Isolate number | Location | Morphology of virions | Virus identified |
|---------------------------------|----------------|----------------|----------------|--------------------------|------------------|
| 'Alfa' | M, YMo | 9009 | Varėna r. | Isometric | CMV |
| 'Movir' | M, Sp | 9109 | Kėdainiai r. | Isometric | CMV |
| 'Rodničok' | GM, Ru | 9111 | Kaunas r. | Rod-shaped | CGMMV |
| 'Estafeta' | TDis, Mo | 9302 | Kaišiadorys r. | Isometric | ? |
| 'Libelle' | YSp | 9404 | Kėdainiai r. | Isometric | ? |
| 'Trakų pagerinti' | M, Mo, St, Dis | 9405 | Kėdainiai r. | Isometric and rod-shaped | CMV ? |
| 'Rodničok' | M, difMo | 9602 | Širvintos r. | Isometric | CMV |
| 'Niežinskij miestnyj' | VN, Mo | 9706 | Vilnius r. | Isometric | CMV |
| 'Kauniai-4' | Chl Sp | 9709 | Kaunas r. | Isometric | ? |
| RS93246F ₁ (Holland) | G difMo | 9717 | Kaunas r. | Rod-shaped and isometric | CGMMV ? |
| 'Ventura' | YRi, St | 9802 | Kaunas r. | Isometric | ToRSV |
| 'Ventura' | YMo, Dis | 9804 | Kaišiadorys r. | Isometric | ToRSV |
| 'Polan' | St, YSp | 9805 | Vilnius r. | Isometric | ToRSV |
| 'Restima' | Dif chl Sp | 9807 | Vilnius r. | Isometric | ToRSV |
| 'Ventura' | Dif GMo | 9812 | Kaišiadorys r. | Rod-shaped | CGMMV |
| 'Krukiai' | Dif YSp | 9903 | Kaunas r. | Isometric | ? |

“?” – virus is not identified.

RESULTS AND DISCUSSION

Sixteen isolates have been chosen out of various cultivars of cucumber plants infected by viruses (Table 2). In the tissue extracts of affected cucumber plants isometric virions predominated, however, rod-shaped virus particles were also present. Observations and investigations proved that CMV infected mostly outdoor cucumber plants. The symptoms caused by this virus in cucumbers varied (typical leaf mosaic and decreased leaves, shortened stem internodes, brittle stems and slow growth). Fruits were smaller, often crooked with protuberant dark green areas. CMV distinguishes itself from other known viruses by the widest spectrum of affected plants. Besides cucumber plants, CMV naturally affects tomato (*Lycopersicon esculentum* Mill.), pepper (*Capsicum annuum* L.), parsley (*Petroselinum crispum* Num.), celery (*Apium graveolens* L.), potato (*Solanum tuberosum* L.), fruit and ornamental plants, as well as some plants from the family *Fabaceae* (peas (*Pisum* L.), beans (*Phaseolus* L.), lupine (*Lupinus* L.), clover (*Trifolium* L.) and lots of weed plants. CMV penetrates into a seed germ and remains infectious for some years. A few infected plants can cause a mass distribution of CMV infection. Several isolates were investigated, but CMV was identified in isolates N 9009, 9109, 9405, 9602 and 9706. The most distinct symptoms were noted

in isolates N 9009 from cucumber cv. Alfa and N 9109 from cucumber cv. Movir. CMV infected practically the whole set of mechanically inoculated plants, with the exception of *Amaranthus caudatus* L. The pathogen caused vein brightening, mottling and various deformations (filaments, blistering, development of only one leaf side) of growing leaves of *N. glutinosa*. On infecting *N. tabacum* L. 'Samsun', a systemic reaction (growth disorder, mosaic or mottling and deformation of young leaves) was also noticed. The reaction of *Lycopersicon esculentum* Mill. to our isolate of CMV infection was small and narrow leaves and their mosaicism. In the leaves of *Chenopodium amaranticolor* Coste et Reyn., *C. ambrosioides* L., *C. foetidum* Schrad., *C. quinoa* Willd., *C. murale* L. a local reaction in the form of tiny chlorotic or necrotic spots was revealed. Under the influence of CMV only local necrotic lesions formed on the leaves of *Nicandra physalodes* (L.) Gaertn. In the upper leaves of *C. sativus* diffusive chlorotic spots developed, which later on widened and formed up a clear mosaic picture. The upper leaves of infected *Capsicum annuum* L. became mosaic, and their blades got deformed. The most distinct symptoms of infection (diffusive change or light and dark green areas and crooked blades) developed in the leaves of *Datura stramonium* L. A rather specific reaction could be observed in the infected plants of *Lupinus albus*, *L. N. physalodes*, *N. glutinosa*, *D.*

stramonium, *L. albus* and *C. sativus* are the plants suitable for differentiation of CMV infection. Isometric virus particles (~ 30 nm in diameter) were detected in plants infected by investigated isolates of CMV. The ratio of optical density A_{260}/A_{280} of the purified viral solution was 1.05–1.12. The yield of CMV from 1 kg of *N. glutinosa* tissue was 58.5–61.4 mg. In the negatively stained preparations polyhedral virus particles with more densely stained centers were revealed (Fig. 1). This morphological property is characteristic of the representatives of *Cucumovirus* group [12]. The identity of CMV was confirmed by the molecular method of reverse transcription – polymerase chain DNA amplification reaction [13].

It has been confirmed that CGMMV is more harmful to cucumber plants grown in greenhouses. The virus particles are stable, they remain infectious for a long time on implements, greenhouse constructions or remnants of cucumber plants. It localizes in the integument of seeds and the membrane covering the seed-lobe. CGMMV distinguishes itself by a narrow specialization, in nature it damages exceptionally plants the family of *Cucurbitaceae*: cucumber (*C. sativus* L.), muskmelon (*Cucurbita melo* L.), watermelon (*Citrullus vulgaris* L.), zucchini squash (*C. pepo* L.), custard marrow (*C. pepo* L. var. *patissonina* Greb.). The upper leaves of infected cucumber plants are wrinkled, mottle greenish and small. Sometimes necrotic edgings around the veins appear. Fruits tend to be deformed, covered with white or yellowish star-like spots. Fruit embryos most often die. Cucumber plants of cvs. Ventura, Rodničok, RS93246 F₁ (Holland) infected by green mottle mosaic were detected in the greenhouse of Babtai and Neveronys (Kaunas r.), Kietaviškės (Kaišiadorys r.) and others (Table 2). Symptoms characteristic of CGMMV developed in mechanically inoculated

cucumber cvs. Alfa, Movir, Trakų pagerinti, Libelle and zucchini squash cv. Black Beauty (Table 1). In a week's time after mechanical inoculation a systemic reaction appeared on leaves of the mentioned plants: dark green mottling and deformation of upper leaves. Along with systemic symptoms, local lesions (round necrotic spots) were also observed in the leaves of seed-lobes of cucumber cv. Movir. In some cases in the leaves of *C. quinoa*, *D. stramonium*, *N. rustica* primary local chlorotic spots resembling viral infection developed after mechanical inoculation. Transmission of the supposed viral infection from the mentioned plants onto the healthy ones of the same species gave no results. The CGMMV isolates studied (N 9111, 9717 and 9812) by their biological properties practically did not differ from the typical strain of this virus [3, 14, 15]. Rod-shaped particles 300 nm long were detected in EM preparations from leaves of the mentioned cultivars of cucumbers and test plants inoculated with their infectious sap (Fig. 2). This virus possibly ori-

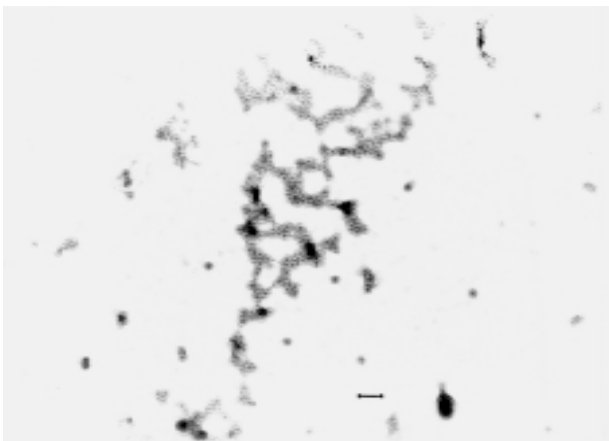


Fig. 1. Particles of *Cucumber mosaic cucumovirus* in *Datura stramonium* plant extract. Bar represents 100 nm

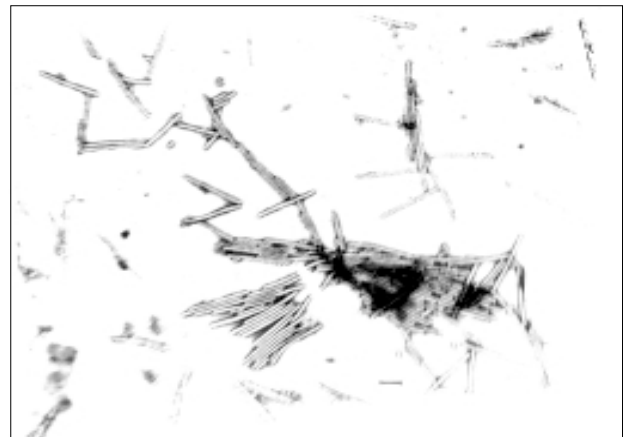


Fig. 2. Rod-shaped virions of cucumber green mottle mosaic tobamovirus from cucumber plants. Bar represents 100 nm

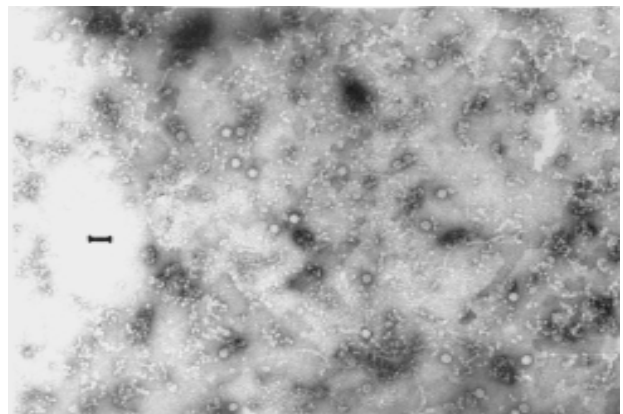


Fig. 3. Particles of tomato ringspot nepovirus in the sap of cucumber plants cv. Restima. Bar represents 100 nm

ginales from seeds gathered from affected cucumber plants and the substrate in which cucumber plants affected by green mottle mosaic were cultivated.

From leaves of cucumber cvs. Restima, Ventura, Polan with yellow ringspot (isolates N 9802, 9804, 9805, 9807), ToRSV, one of the most harmful virus in *Nepovirus* group, was isolated. It is easily transmitted through the diseased plant sap and seeds. In the soil it is spread by nematodes of the genus *Xiphinema*. ToRSV experimentally affects plants of 35 botanical families [11]. ToRSV is rather harmful – if the virus gets into the plant the latter may even die. It is especially dangerous if valuable cucumber cultivars or their selective numbers are to be maintained. The data on plants infected by ToRSV and character of symptoms (Table 1) indicate the harmfulness of this virus. In EM preparations of the virus-affected cucumber leaves and inoculated test plants, virions 28 nm in diameter, morphologically corresponding to ToRSV, were discovered (Fig. 3). Having compared the main plant species indicated in the literature as susceptible to ToRSV and the character of symptoms in them as well as the host range and symptoms of the affected plants, our virus isolates from cucumber plants in which ringspot symptoms were prevailing, the isolates were identified as typical for ToRSV [3, 11, 16]. Data on identification of this virus, conditions of the purification of antigens and the properties of the obtained diagnostic antiserum have been described earlier [1].

The obtained results concerning the biological properties of the agents of cucumber viral diseases (specific symptoms of naturally affected cucumber plants, wide or very limited host range of infected plants and symptoms in them) and the morphology of virus particles (isometric or rod-shaped virions) have proved that CMV, CGMMV and ToRSV are the viruses that affect cucumbers in Lithuania. The agents of some isolates from cucumbers have not been identified; preliminary investigations indicated that they differ in their biological properties from the viruses identified.

Received 18 March 2002

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AGURKŲ VIRUSINĖS LIGOS IR JŲ SUKĖLĖJŲ IDENTIFIKAVIMAS

S a n t r a u k a

Agurkų virusinės ligos pasireiškia įvairiais simptomais: išryškėja lapų gyslos, mozaika ar margligė, susmulkėja lapai, deformuojasi augalai. Straipsnyje pateikiami agurkų virusų identifikavimo augalų-indikatorių ir peršviečiamosios elektroninės mikroskopijos (EM) metodais rezultatai. Pagal natūraliai pažeistų agurkų ir eksperimentinių augalų požymius, viruso eksperimentiškai pažeidžiamų augalų spektrą ir virusinių dalelių morfologines savybes Lietuvoje iš agurkų buvo išskirti ir identifikuoti trys virusai: agurkų mozaikos (*Cucumber mosaic cucumovirus*, CMV), agurkų žaliosios margligės-mozaikos (*Cucumber green mottle mosaic tobamovirus*, CGMMV) ir pomidorų žiediškosios dėmėtligės (*Tomato ringspot nepovirus*, ToRSV). Natūraliai virusais pažeistų agurkų ir augalų-indikatorių EM preparatuose nustatytos CMV ir ToRSV izometrinės, o CGMMV – lazdelinės virusinės dalelės. Išgryninto CMV preparato absorbcijos A_{\max} buvo esant 260 nm bangos ilgiui, o A_{\min} – 240 nm. A_{260}/A_{280} santykis lygus 1,05–1,12. CMV išeiga vienam kg augalinės žaliavos buvo 58,5–61,4 mg. Apie ToRSV gryninimą ir specifinio diagnostinio antiserumo paruošimą paskelbta anksčiau [1].