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Nematode-transmissible virus disease agents detected in some vegetable crops

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Santrauka

Nematode-transmitted viruses cause economically important diseases in a range of crops worldwide. This study presents the results of detection of nematode-transmitted viruses isolated from some vegetable crops in Lithuania. Samples of cucumber (*Cucumis sativus* L.), zucchini (*Cucurbita pepo* L.) and tomato (*Lycopersicon esculentum* Mill.) crops exhibiting symptoms characteristic of viral diseases were found in various field-grown and greenhouses. Viral diseases were expressed by symptoms of mottling, yellow ringspots, vein necrosis and distortion of leaves. Growth and fruiting of plants were slightly reduced. For identification of virus disease agents traditional (transmission electron microscopy and test-plants) methods and molecular diagnostic (reverse transcription-polymerase chain reaction, RT-PCR) technique were used. Identification of causal agents was based on specific symptoms of infected vegetables, determined experimental host ranges, morphological properties of virions and on cDNA amplified product sizes of nepoviruses in PCRs using virus-specific oligonucleotides. A 499 and 420 bp specific DNA fragments were amplified using isolates from cucumber and tomato samples infected with *Tomato ringspot nepovirus* and *Arabis mosaic nepovirus*, respectively.

Key words: cucumber, identification, nepoviruses, RT-PCR, tomato, zucchini.

INTRODUCTION

Nematode-transmitted viruses were named as nepoviruses. Characteristics of the *Nepovirus* group were described by Harrison et al. [1971]. In 1979, Matthews expanded the group to 18 members and possible members. Properties of nepoviruses have been reviewed by [Murant, 1981]. Nepoviruses do not replicate in the vector, but the nematodes are able to transmit them for weeks or months. Nepoviruses have three types of particles ca 28 nm in diameter. Cytoplasmic vesiculate inclusions and rows of virus particles in tubules occur in many nepovirus infections. The nepoviruses of importance in vegetables are *Arabis mosaic virus*, *Strawberry latent ringspot virus, Tobacco ringspot virus, Tomato black ring virus*, and *Tomato ringspot virus*. The host range of nepoviruses is wide [Murant, 1981; Edwardson and Christie, 1986, 1997; Brunt et al., 1996; sutic et al., 1999; Khan and Dijkstra, 2006].

Tomato ringspot virus (ToRSV), one a member of the *Nepovirus* genus of plant viruses, is found in many perennial crops [Brunt et al., 1996]. It affects 285 plant species in 159 genus of 55 botanical families [Edwardson and Christie, 1997]. ToRSV is readily transmissible by sap inoculation. Seed transmission of ToRSV has been reported in several crops. The virus is also transmitted by vegetative propagation and pollen. Species of both *Xiphinema* and *Longidorus* nematodes are reported to transmit ToRSV. The virions are icosahedral, about 28 nm in diameter, sedimenting as three components. The most characteristic type of foliar symptom induced by ToRSV is ring spotting of leaves. This virus was isolated from many ornamental plants in Lithuania [Navalinskienė and Samuitienė, 2006].

Arabis mosaic nepovirus (ArMV) was described by Murant [1970]. Particle size measurements vary from 21,9 nm to 30–32 nm in diameter. The virus particles are isometric and about 30 nm in diameter. ArMV has a bipartite genome with two RNA-species. The occurence of the spherical inclusions in the phloem should distinguish ArMV infection from those of other nepoviruses. Thermal inactivation points vary from 54–56 °C to 64–66 °C, dilution end points vary from 1/250–1/1000 to 10^{-4}–10^{-5} and longevity *in vitro*

vary from 2–3 days to 62–68 days. Several nematodes of the family *Longidoridae* have been suspected of transmitting ArMV, but only the evidence for *Xiphinema diversicaudatum* is adequate [Trudgill et al., 1983; Kulshrestha et al., 2005]. Dodder transmission has also been reported. Seed transmission of ArMV has been reported in 16 genera and species, with some very high percentages observed in lettuce 60–100 %. ArMV infects a rather wide range of hosts including monocots and dicots in 262 species of 161 genera in 58 families [Edwardson and Christie, 1986]. This virus has also been reported from sugarbeet, celery, cucumber, cucurbita, carrot, horseradish and lettuce plants [Brunt et al., 1996]. A number of other cultivated and wild species has been reported as hosts. This virus was described as agent of virus disease infecting *Crocus* L. plants in Lithuania [Navalinskienė and Samuitienė, 2005].

The aim of this study was to present data on occurence and identification of nematode-transmitted viruses isolated from some vegetable crops in Lithuania based on the results of determined biological and morphological properties, and adapt a molecular diagnostic for them in infected plants.

MATERIALS AND METHODS

Samples of vegetable leaves and fruits for investigation were collected in private garden of Vilnius, Kaunas, Kėdainiai districts. Plant samples with expressed visual virus disease symptoms have been investigated for virus presence. Diagnostic study of the pathogen was done at the greenhouse and of Plant Virus Laboratory using standard methods for mechanically transmitted viruses. The viruses were identified by test-plant reaction to inoculation with isolates from infected vegetables under investigation according to [Murant, 1981; Brunt et al., 1996]. In order to differentiate viruses and their isolates from diseased cucumber, tomato and zucchini samples and identify the viruses, test-plants of *Aizoaceae* Rudolphi, *Amaranthaceae* Juss., *Asteraceae* Dumort., *Chenopodiaceae* Vent., *Cucurbitaceae* Juss., *Fabaceae* Lindl and *Solanaceae* Juss. families were used (Table 1).

Table 1. The test-plants reaction to nepoviruses isolated from vegetable crops

1 lentelė. Diagnostinių augalų reakcija į daržovėse aptiktus nepovirusus

Test plant / diagnostiniai augalai	Symptoms induced by nepoviruses / nepovirusų sukelti požymiai	
	ArMV	ToRSV
Amaranthus caudatus L.	0	-
A. paniculatus L.	0	S: TDis, St
A. retroflexus L.	0	-
Atriplex hortensis L.	0	0
Beta vulgaris var. saccharifera Alet.	S: ChlSp	-
Capsicum annuum L.	0	0
Celosia argentea f. cristata (L.) Kuntze	L: NL	L:RL; S: RiSp,Ru
Chenopodium album L.	L: ChlL; S:YSp	-
C. amaranticolor Coste et Reyn.	L:ChlL; S: VC	L:WhL; S: Ru,St
C. ambrosioides L	L: NL; S: LeDis	L:fNL; S: TRu,TDis
C. foetidum Schrad.	0	0
C. murale L.	0	L:ChlL; S: VC,TDis
C. quinoa Willd.	L: ChlL; S: St,TDis	L:ChlL; S: TRu,W
C. urbicum L.	0	-
Cucumis sativus L.	S: VC, ChlSp	L:NL; S: ChlSp
Cucurbita pepo L.	0	S: ChlMo
Datura stramonium L.	L: NL; S: VC, ChlSp	0
Gomphrena globosa L.	L: NL; S: difMo:	L: WhL or NL
Lycopersicon esculentum Mill.	S: VC, Chl, Mo, LeDis	S: VN, ChlMo
Nicandra physalodes (L.) Gaertn.	S: difChlMo, VN	0
Nicotiana debneyi Domin.	S:VB, GrMo,LeDis	S: ChlMo
Nicotiana glutinosa L.	L: NL; S: VC, Mo	0
N. occidentalis L.	L: NL	-
N. rustica L.	L: NL; S: ChlSp	L: RL; S: RiSp,LeDis
N. sylvestris Speg et Comes	L: NL	0
N. tabacum L. 'Samsun'	S: ChlSp	L: RL
'White Burley'	L: NL; S: ChlSp, St	-
Petunia hybrida Vilm.	S: ChlRiSp	-
Phaseolus vulgaris L. 'Bataaf'	L: ChlL; S: difChlSp	L: fNL
Pisum sativum L. 'Žalsviai'	0	L: NL
Tetragonia expansa Murr.	L: ChlL; S: VC, Mo	L:ChlL; S:difYSp,St
Verbesina encelioides Benth et Hook.	0	L: fBlL or BrL
Vigna unquiculata (L.) Walp.	S: VC, ChlMo	-

Abbreviations / santrumpos: L – loca reaction / vietinė reakcija, LL – local lesions / vietinės žaizdos, NL – necrotic lesions / nekrotinės žaizdos, M – mosaic / mozaika, Mo – mottling / margumas, Chl – chlorosis / chlorozė, S – systemic reaction / sisteminė reakcija, RL – red lesions / raudonos žaizdos, WhL – white lesions / baltos žaizdos, f – fine / smulkios, TN – top necrosis / viršūnės nekrozė, TRu – top rugosity / viršūnės raukšlėtumas, TCr – top curling / viršūnės lapų garbanė, Dis – deformation/deformacija, St – stunting / žemaūgė, VC – vein clearing / gyslų išryškėjimas, VB – vein banding / gyslų apvadai, VN – vein necrosis / gyslų nekrozė, dif – diffuse / difuzinis, BlL – black lesions / juodos žaizdos, BrL – brown lesions / rudos žaizdos, Sp – spots / dėmės, Gr – green / žalias, RiSp – ring spotting / žiediškasis dėmėtumas, YSp – yellow spotting / geltonas dėmėtumas, Le – leaf / lapas, W – wilting / vytimas, 0 – no symptoms / be simptomų, – not tested / neinokuliuota.

The specimens for mechanical inoculation were prepared by homogenizing tissues of affected vegetable and test-plant samples in 0,1 M sodium phosphate buffer, pH 7,0–7,2 (1:3 wt/vol), containing a stabilising agents 0,02 % 2-mercapthoethanol or 0,01 M sodium diethyldithiocarbamate (Na DIECA). The inoculations were performed with an aid of carborundum powder as an abrasive [Dijkstra and de Jager, 1998]. Symptom development on indicator hosts was recorded every 2–3 days during the 12 days following inoculation and twice a week in the subsequent 30 days. The possibility of symptomless infection was checked by electron microscopically (EM) or back-inoculation to healthy test-plants.

Presence of virus particles and their morphology was determined by investigation of negatively stained with 3 % uranyl acetate dip preparations using transmission electron microscope JEOL JEM–100S at instrumental magnification of 25 000^{x} [Hayat, 1970; Harris and Horne, 1994; Dijkstra and de Jager, 1998].

For confirmation of ArMV and ToRSV detection in experimentally infected test-plants the reverse transcription-polymerase chain reaction (RT-PCR) was used [Saiki et al., 1988; Henson and French, 1993].

ArMV-RNA extraction was carried out according to the instruction of "Quick Prep total RNA extraction kit for the direct isolation

of total RNA from most eukaryotic tissues or cells" (produced by Amersham Pharmacia Biotech.). Frozen tissue samples (25–50 mg) of infected plant tissues were grounded in liquid nitrogen and transferred to 1,5 ml microfuge tubes. 150 μl volume of the extraction buffer was poured in the tube and 3 μl of 14,3 M β-mercaptoethanol was added. The solution was mixed to obtain a homogeneous suspension. 350 μl of lithium chloride (LiCl) solution was added to the homogenized samples. 500 μl of caesium trifluoracetate (CsTFA) dispensed into the homogenized samples. The tubes were placed on ice for 10 min and later spined for 15 min at 14 000 g. The RNA formed a pellet at the bottom of the microtubes. The proteins form a coat at the top of the tubes and DNA remains in the liquid phase. The protein coat and the liquid phase were removed and proceeded to wash the total RNA pellets. They were washed with three "kit" components: 75 μl extraction buffer, 175 μl LiCl solution and 250 μl CsTFA solution added to the tubes containing the RNA pellet. The samples were spined in a microcentrifuge at full speed for 5 min. The supernatants were discard without disturbing the pellets. 1 ml of 70 % ethanol was added to the samples. The samples were spined in a microcentrifuge at 14 000 g for 5 min. The pellet was air dried for 10–15 min. DEPC-treated (Diethyl Pyrocarbonate) water containing 1 % of RNAse inhibitor was added to the RNA pellets. The pellet was broken by pulse vortexing. For disvolving the pellets the samples were placed on ice for 15–30 min. Then the samples were heated at 65 °C for 10 min and stored at –20 °C.

Nucleic acid from plants infected by ToRSV was extracted using the small-scale procedure as proposed for extraction of nucleic acids from woody plants [Zhang et al., 1998] with slight modifications. Tissue samples of infected plants were ground in liquid nitrogen and transferred to microtubes. 600 μl 1xSTE buffer (0,1 M NaCl, 0,001 M Tris, 0,001 M NaEDTA, pH 6,9), 80 μl of 10 % SDS and and 800 μl of 2xSTE-saturated phenol was added to the tissues. The mixture was centrifuged 5 min at 16 000 g. Aqueous phase was removed and transferred to a clean microfuge tube. Ethanol to a final concentration of 30 % was added, then ~10 mg cellulose (whatman CF-11). Cellulose was washed by vortexing 3 times with 1 ml of 1xSTE/30 %

ethanol, collecting cellulose by centrifugation between washes and discarding supernatants. RNA from cellulose was eluted by adding 200 μl of 1xSTE buffer, and centrifugation for 5 min. Supernatant was transferred to a clean tube. For precipitation of the RNA 40 μl of 3 M sodium acetate and 1 ml of ethanol was added. The tube was incubated at –20 °C for 2 h, centrifuged for 10 min at 16 000 g, and the pellet was incubated with 80 % of ethanol at –20 °C.

Two specific oligonucleotide primers of the coat protein gene of ArMV were designed using published sequences for ArMV [Pantaleo et al., 2001] from GenBank Accession №. # 55460, X81814, X81815. These primers were called AP1[2] (5' – AAT ACC CCG GGT GTT ACA TCG – 3') and AP2[2] (5' – CAT TAA CTT AAG ATC AAG GAT TC – 3').

Primers used in RT-PCR were designed from ToRSV viral sequence information [Griesbach, 1995]. These primers included U1, (5' to 3') GAC GAA GTT ATC AAT GGC AGC nt 1,078 to 1,098) and D1, TCC GTC CAA TCA CGC GAA T (nt 1,506 to 1,527) of the putative viral polymerase gene.

The dissolved RNA was used in experiments for detection of ArMV and ToRSV by RT-PCR. Pellets of RNA were resuspended in the solution containing 0,4 μM of primers AP2[2] (for ArMV) or D1 (for ToRSV) and PCR water. The samples were denaturared at 70 °C for 5 min and called at 4 °C for 5 min.

For the first strand cDNA synthesis the RNA pellet solution to the mixture containing 5x reaction buffer, 1 % of RNAse inhibitor, 10 mM deoxynucleoside triphosphates (dNTP) mixture and 10 Units of RevertAid™ M-MuLV reverse transcriptase (MBI *Fermentas*, Vilnius, Lithuania) were added. The first strand cDNA synthesis was carried out at 37 °C for 60 min and 70 °C for 10 min. DNA amplification was performed in 55 ml reaction mixtures containing each of the four dNTP at a concentration of 200 μM, each primer at a concentration of 0,4 μM, 10xPCR buffer, 25 mM $MgCl_2$ and 0,25 U of recombinant *Taq* polymerase (MBI *Fermentas*) using Eppendorf Mastercycler Personal. PCRs were carried out for 40 cycles using the following parameters: 1 min at 94 °C (4 min for the first cycle), 2 min at 52 °C and primers extension for 2 min (10 min in

the final cycle) at 72 °C.

Resulting PCRs products were analysed by electrophoresis in 5 % polyacrylamide gel, stained with ethidium bromide, and DNA bands were visualized using a UV transilluminator. DNA fragment size standard (DNA Ladder) was Phix174 RFI DNA Hae III digest (MBI *Fermentas*). Fragment sizes (base pair, bp) from top to bottom: 1353, 1078, 872, 603, 310, 281, 271, 234, 194, 118, 72.

RESULTS AND DISCUSSION

In the course of survey of virus diseases of vegetable crops, viruses with isometric particles were detected by EM investigation and mechanical transmission from infected plants or fruits. Preliminary observations indicated that these viruses had properties resembling those of nepoviruses [Murant, 1981]. In the tissue extracts of naturally affected tomato, cucumber and zucchini plants isometric virions predominated.

Arabis mosaic virus (ArMV). Naturally infected some tomato plants exhibiting of general interveinal-yellowing of leaves, bright mottling, reduced leaf size (Fig. 1) and stunted plant growth were observed in Vilnius district.

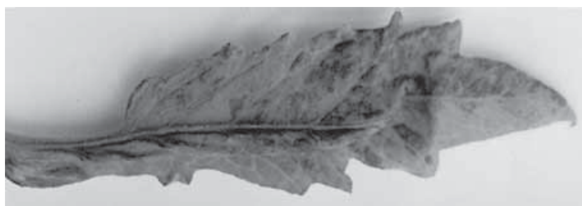

Fig 1. Tomato leaf affected by ArMV

1 pav. ArMV pažeistas pomidoro lapas

Symptoms associated with ArMV were described as reduced growth, shoots with short internodes, and leaf chlorosis and distortion [Murant, 1970]. Samples originating from these tomato plants were inoculated onto the test-plants. The symptomatology of test-plants is shown in Table 1. This virus infected 22 out of 32

mechanically inoculated test-plants. Several herbaceous indicator plants reacted with typical local and systemic symptoms that are reported for ArMV. Inoculated plants of *Chenopodium* L. genus were developed chlorotic local lesions on leaves. Systemic reaction was expressed by stunting and top distortion of these plants. Symptoms characteristic for this virus were found on some infected plants: *Datura stramonium* L., *Phaseolus vulgaris* L. Similar results were obtained with test-plants from *Nicotiana* L. genus. Virus isolates induced different systemic symptoms on leaves of *N. debneyi* Domin. plants: light and dark green mottle followed by vein-necrotic banding of older plant leaves. As the affected leaves turned brown and shrivelled, growth declined and the plant usually reduced in size until only a small central cluster of malformed leaves remained. Middle-aged inoculated leaves of tomato showed interveinal chlorosis, while more mature leaves showed more intense interveinal chlorosis and leaf size distortion in some cases. All tomato cultivars which were inoculated by virus were susceptible to this virus. The symptoms induced by virus on various test-plant species were typical of ArMV. Basing on the test-plant reaction results we can conclude that investigated isolates belong to typical strain of ArMV, because their biological properties correspond to literary data [Murant, 1970]. High concentration of isometric virus particles approximately 28 nm in diameter were found in the preparations made from inoculated and infected test plants (Fig. 2). Morphology of such particles is characteristic for ArMV from genus of *Nepovirus.*

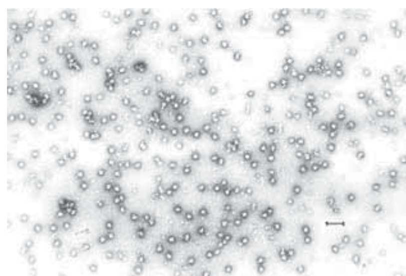

Fig. 2. Electronomicrograph of ArMV particles. Bar represents 100 nm

2 pav. ArMV dalelių elektronomikrografija. Brūkšnelis – 100 nm

Tomato ringspot virus (ToRSV) was isolated from naturally infected cucumber, tomato and zucchini plants expressing specific for this virus symptoms. Naturally infected *Cucumis* cv. 'Ventura', 'Polan' and 'Restima' plants showed chlorotic ringspots on leaves (Fig. 3).

Fig. 3. Yellow ringspotting of cucumber leaf

3 pav. Agurkų lapo žiediškasis geltonas dėmėtumės

ToRSV was detected in the cultivars of tomato 'Olan', 'Dombello' and 'Sonata' which showed specific ringspotting symptoms on fruits (Fig. 4).

Fig. 4. Ringspots on tomato fruit

4 pav. Žiediškosios dėmės pomidorų vaisiuje

Four samples of zucchini plants expressing virus disease symptoms were observed in field-grown in Vilnius district. Diseased zucchini plants showed yellow ring spots on young leaves and chlorotic mottle on older leaves (Fig. 5).

Fig. 5. Zucchini leaf affected by ToRSV
5 pav. ToRSV pažeistas cukinijų lapas

Growth of plant and leaves was slightly reduced, but fruits showed no symptoms. The investigated virus disease agent induced typical severe symptoms (chlorotic or necrotic local lesions and systemic apical deformation) on the main diagnostic plant species (Table 1). Our investigation revealed that ToRSV as the ArMV infected a wide experimental host range. Some differences between ToRSV isolates from tomato, zucchini and cucumber were revealed in test-plants reaction. ToRSV isolate from tomato is did not induce reaction in *Cucumis sativus* and *Phaseolus vulgaris* cv. 'Bataaf', isolate from cucumber – in *Datura stramonium, Nicotiana glutinosa, N. physalodes*. In EM preparations of naturally affected vegetable and inoculated test-plants virions of 28 nm in diameter were revealed (Fig. 6).

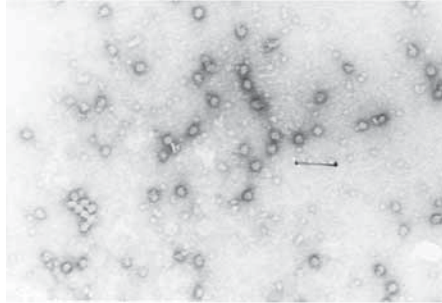

Fig. 6. Electronomicrograph of ToRSV particles. Bar represents 100 nm
6 pav. ToRSV dalelių elektronomikrografija. Brūkšnelis – 100 nm

The virus particles morphologically corresponded to ToRSV. The virus isolated from investigated vegetable samples showing deformation and yellow ring spots on leaves or fruits was identified as ToRSV by its morphological and host range properties [Stace-Smith, 1984]. This virus is wide spread in America, Australia and locally in Europe, and was reported in Russia and Japan [Brunt et al., 1996].The samples positive for ArMV and ToRSV in biological tests were tested by RT-PCR technique using previously described specific primers. Specific bands were observed in gel analysis at the position corresponding to the expected sizes of the amplification products of DNA from symptomatic samples in addition to specific band yielded also smaller PCR products. The PCRs resulted in the specifically amplification of 420 bp and 499 bp genome fragments of ArMV and ToRSV RNA (respectively) (Fig. 7, 8).

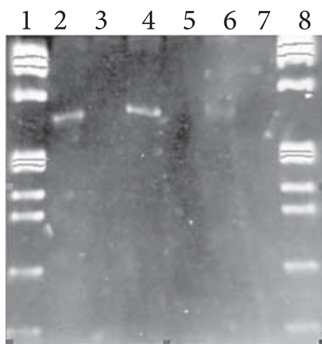

Fig. 7. Gel electrophoresis of PCR product of amplified ArMV from tomato samples. Lanes 1 and 8, DNA Ladder; Lane 2, *N. glutinosa*; Lane 3, *G. globosa*, Lane 4, *N. debneyi*; Lane 5, healthy *N. debneyi* tissue; Lane 6, *Potato X virus*; Lane 7, control

7 pav. Pomidoruose aptikto ArMV PCR produktų elektroforegrama: 1 ir 8 takeliai – DNR dydžio standartas; 2 takelis – *N. glutinosa*; 3 takelis – *G. globosa;* 4 takelis – *N. debneyi;* 5 takelis – sveiki *N. debneyi* audiniai; 6 takelis – *Potato X virus;* 7 takelis – kontrolė

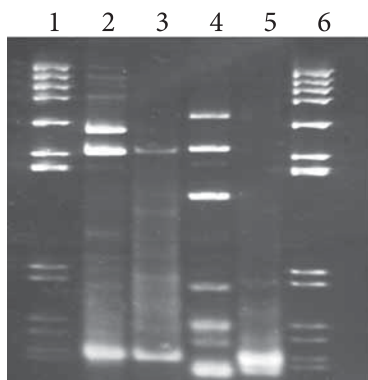

Fig. 8. Gel electroforesis of PCR product of amplified ToRSV from cucumber and tomato samples; Lanes 1 and 6, DNA Ladder; Lane 2, cucumber sample; Lane 3, tomato sample; Lane 4, iris sample; Lane 5, water control

8 pav. Agurkuose ir pomidoruose nustatyto ToRSV PCR produktų elektroforegrama: 1 ir 6 takeliai – DNR dydžio standartas; 2 takelis – agurkų pavyzdys; 3 takelis – pomidorų pavyzdys; 4 takelis – vilkdalgių pavyzdys; 5 takelis – kontrolė

No product was amplified from the negative (healthy plant tissues) and water controls. The primers designed on the basis of published sequences successfully amplified ArMV and ToRSV cDNA templates in RT-PCR of ArMV and ToRSV isolates from vegetable plants. The results of biological, morphological and partial molecular characterization of ArMV and ToRSV support that these viruses are nepoviruses sufficiently distinct from other viruses isolated from cucumber and tomato plants. These data confirmed the results of identification of ToRSV obtained by investigating another methods.

Our study was conducted to provide information on the response of vegetable crops to ArMV and ToRSV infections. These nematode-transmitted viruses have a wide host range in nature including a number of important crop plants. They are distributed worldwide. Vegetatively propagated plant material is the most effective mean of nepoviruses spread. Seed transmission was found in many species of plants. Some evidence exists for pollen transmission of nepoviruses as phytosanitary risk viruses for vegetables.

CONCLUSIONS

1. Results of this study revealed that nematode-transmissible viruses affecting vegetable crops in Lithuania were: *Arabis mosaic nepovirus* (ArMV) and *Tomato ringspot nepovirus.*

2. The identification of viruses was based on biological properties (wide host range of infected test-plants and specific symptoms in them) of the viruses affected cucumber, tomato and zucchini plants, and the morphology of virus particles.

3. The primer pairs designed on bases of published sequences successfully amplified ArMV and ToRSV cDNA templates in RT-PCR and confirm nepovirus infections in investigated samples of vegetables.

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KAI KURIOSE DARŽOVĖSE APTIKTI NEMATODŲ PERNEŠAMI VIRUSINIŲ LIGŲ SUKĖLĖJAI

I. Zitikaitė

Santrauka

Vizualiai tyrinėjant įvairių daržovių virusinių ligų pobūdį buvo pastebėta agurkų, pomidorų ir cukinijų, kurių lapuose ar vaisiuose buvo žiediškoji dėmėtligė, chlorotinis margumas ir įvairios lapų ir augalų viršūnių deformacijos. Užsikrėtusių daržovių pavyzdžiai tyrimams buvo paimti privačiuose daržuose, lauke ir šiltnamiuose Vilniaus, Kauno, Kėdainių rajonuose. Tiriant minėtų daržovių lapų ekstraktus, peršviečiamosios elektroninės mikroskopijos metodu aptikti izometriniai apie 28 nm skersmens virionai, pagal morfologiją būdingi nepovirusams, pernešamiems dirvožemio nematodų ir pažeidžiantiems daug ekonomiškai reikšmingų augalų pasaulyje. Tiriamų augalų inokuliacija, pažeidimų simptomų vystymosi ypatumai ir virusais užsikrėtusių augalų platūs spektrai atitiko nepovirusų biologines savybes. Klasikiniais metodais gautiems rezultatams patvirtinti buvo panaudota molekulinė diagnostika. Atvirkštinės transkripcijos-polimerazės ciklinėse reakcijose (AT-PCR) gauti tiriamų virusų specifinių cDNR fragmentų amplifikacijų produktai parodė, kad iš pomidorų buvo išskirti ir identifikuoti du nepovirusai: vaistučio mozaikos virusas (*Arabis mosaic virus*) ir pomidorų žiediškosios dėmėtligės virusas (*Tomato ringspot virus*, ToRSV). Lietuvoje aptiktuose užkrėstų agurkų ir cukinijų pavyzdžiuose buvo nustatytas tik ToRSV.