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Identification of viruses and phytoplasma infecting scarlet lychnis (*Lychnis chalcedonica* L.) plants

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Plants of scarlet lychnis (*Lychnis chalcedonica* L.) exhibiting symptoms characteristic of viral and phytoplasmal diseases were found at Experimental Station of Field Floriculture and Botanical Garden of Klaipėda University. The causal agents of virus disease were isolated and identified by the methods of test-plants and electron microscopy as *Tomato ringspot nepovirus* (ToRSV) and *Tobacco rattle tobravirus* (TRV). Amplification of phytoplasmal 16S rDNA in polymerase chain reactions (PCRs), confirmed phytoplasma infection. Restriction fragment length polymorphism (RFLP) analysis of amplified 16S rDNA from scarlet lychnis revealed that the plants were infected by a phytoplasma belonging to group 16SrI (aster yellows phytoplasma group) subgroup B (I-B, aster yellows subgroup).

Key words: *Lychnis,* identification, virus, phytoplasma, PCR, RFLP

INTRODUCTION

The genus *Lychnis* L. is a member of *Caryophyllaceae* Juss. family and includes numerous species of herbaceous plants which spontaneously grow in Europe and Asia. The most decorative species are grown as garden and cut flowers. The most widespread scarlet lychnis (*Lychnis chalcedonica* L.) has been grown since ancient times and is a popular flower in our country.

Little is known about the diseases of *Lychnis*, caused by viruses or phytoplasmas. M. Klinkowski [1] mentioned that *Lychnis* could be the host plants of *Cucumber mosaic cucumovirus. Tomato spotted wilt tospovirus* was isolated from *L. chalcedonica* in Pennsylvania [2].

Diseases atributed to phytoplasmas have been reported in plant species belonging to more than 90 families worldwide. Molecular methods have been applied to detect them in plants and insect vectors and to construct a system for phytoplasma identification and classification. On the basis of analyses of 16S rDNA, phytoplasmas have been classified into at least 15 groups and over 38 subgroups [3]. Phytoplasmas belonging to six major 16S rRNA gene groups (16SrI, 16SrIII, 16SrV, 16SrX, 16SrXI and 16SrXII) have been reported in Europe [4–6]. 16SrI (aster yellows) is the largest, most diverse and widespread phytoplasma group [5, 7]. Molecular investigation of phytoplasmas in Lithuania have begun recently, and knowledge concerning the genetic and biological diversity is emerging. Phytoplasmas belonging to 16SrI, 16SrIII and 16SrV major phytoplasma groups and 11 subgroups have been detected [6, 8–10]. A phytoplasma belonging to subgroup 16SrI-M has been detected in yellows disease affected scarlet lychnis plants collected in Širvintos region [10].

The objective of this study was to determine a possible association of a virus and phytoplasma with diseases in scarlet lychnis and to identify their causal agents.

MATERIALS AND METHODS

The plant material for investigation was collected at Experimental Station of Field Floriculture and Botanical Garden of Klaipėda University. The experimental work was carried out at the Laboratory of Plant Viruses of the Institute of Botany. Viruses were identified by electron microscopy (EM) negative staining technique [11] and test-plant method [12–14].

Phytoplasma was detected in polymerase chain reactions (PCRs). Nucleic acid for the use as a template in PCR was extracted from the frozen tissue using the Genomic DNA Purification Kit (MBI Fermentas). Ribosomal (r) DNA was amplified in nested PCRs using the primer pair P1/P7 [15] and the phytoplasma-specific primer pair R16F2n/R6R2 [16] as described by Jomantiene et al. [17].

Products from nested PCR primed by R16F2n/R6R2, were analysed by single enzyme digestion according to manufacturer's instructions employing restriction endonucleases: AluI, MseI, KpnI, HhaI, HaeIII, HpaII, RsaI, HinfI, and TaqI (MBI Fermentas). The RFLP profiles of digested DNA were analysed by electrophoresis through 5% polyacrilamide gel stained with ethidium bromide, and visualised using a UV transilluminator. RFLP patterns were compared with previously published ones [3, 5, 7, 17, 18].

RESULTS AND DISCUSSION

Tomato ringspot nepovirus (ToRSV)

Infected scarlet lychnis plants bearing viral symptoms of leaf distortion, mosaic on leaves were collected at the Experimental Station of Field Floriculture. The test-plants were inoculated by mechanical sap inoculation. Results of test-plant reactions are presented in Table.

EM investigation of preparations made from infected scarlet lychnis and inoculated test-plants revealed the presence of isometric particles 28 nm in diameter (Fig. 1).

On the basis of the results of test-plant reactions, morphology of virus particles and literature data [12, 14] we concluded that plants were infected by ToRSV.

ToRSV is a type member of the genus *Nepovirus* and causes economically important diseases in a range of crops. The virus has isometric particles 28 nm in diameter, sedimenting as three components and containing RNA as a bipartite genome. It is readily transmissible by inoculation of sap and has a wide host range including both woody and herbaceous plants. It is transmitted by the nematode *Xiphinema* spp. Seed transmission has

Table. **Reactions of test-plants inoculated by viruses isolated from scarlet lychnis**

Test–plant	ToRSV	TRV
Amaranthus caudatus	L: NLL; S:Sp,Dis	
A. paniculatus	L:NSp; S:Sp,LeDis	L: BrLL
Atriplex hortensis		L: LL
Celosia argentea	L: DBrRi; S:VCl, LeDis, BrSp, Ln	
Chenopodium amaranticolor	L: ClLL; S: VStu,TR,NT	L: NLL
C. ambrosioides		L: NLL
C. foetidum		L: LL
C. murale	L: ClSp; S: VCl,Mo,N,LeDis	L: ClLL
C. urbicum	L: ClSp; S: VCl,LeDis	L: LL
C. quinoa	L: ClSp,N,Dis; S: Cl,N,LeDis,ApN	L: Cl and NSp
Cucumis sativus	L: N or ClLL; S: Mo	L: Cl or NLL
Galinsoga parviflora		L: NStr, Ri
Gomphrena globosa	L: NSp; S: Dis, Mo	L: NSp
Lycopersicon esculentum	L: NSp; S: Mo,N	
Nicandra physalodes		L: ClNSp,NEt
Nicotiana debneyi		L: NSp; S: NSp,LeDis
N. glutinosa	L: NSp	L: GNRi; S: NSp,NRi,Stu
N. rustica	L: NRiSp	
N. tabacum	L: NSp; S: NSp,Str	L: NSp; S: NRiPat,Dis
Petunia hybrida	L: GNRi; S: LeDis,ClSp,NSp	
Physalis floridana	L: NLL,LeRu	L: SmNLL
Tetragonia expansa	L: DifClSp; S: LeDis,ClDot	

Abbreviations: L – local reaction, S – systemic reaction, LL – local lesions, Cl – chlorotic, chlorosis, N – necrotic, necrosis, V – vein, Stu – stunting, T – plant top, D – dots, Le – leaves, Dis –distortion, Sm –small, Sp – spots, Br – brown, G – gray, Ri – rings, M – mosaic, Mo – mottling; Str – streaks; L – line, R – rolling, Ru – rugosity, Dif – diffusive, Ap – apical; Et – etching; Pat – pattern; D – dark.

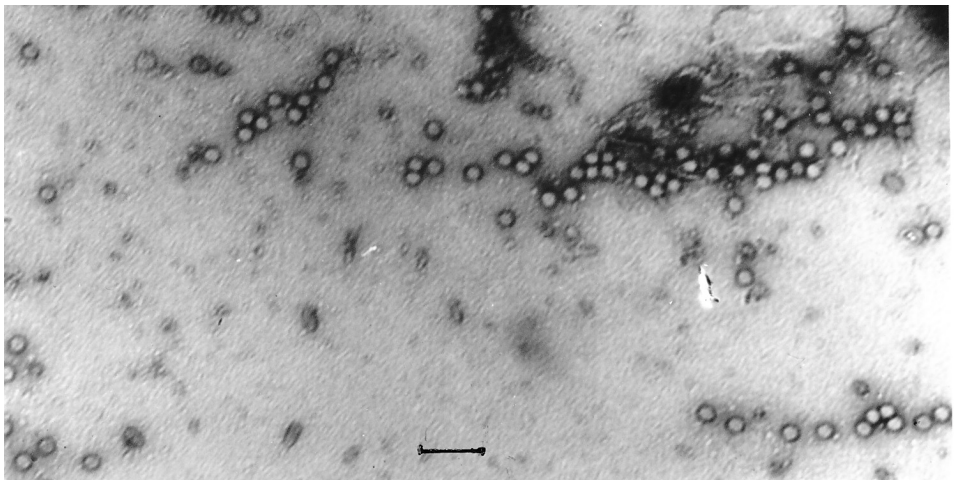

Fig. 1. Particles of *Tomato ringspot nepovirus*. Bar represents 100 nm

been reported in several crops. Most infected plants show distinct symptoms as a shock reaction; chronically infected plants usually exhibit no obvious symptoms but show a general decline in productivity [12, 14]. The virus occurs in nature mostly in perennial crops. Ornamental hosts have been found naturally infected by ToRSV including: *Anemone* L., *Gladiolus* L., *Hydrangea* L., *Iris* L., *Narcissus L.*, *Pelargonium* L'Her., *Petunia* Juss. [19]. Our investigation has enlarged the range of ToRSV host-plants by including 14 species of ornamental plants [20, 21]. Such a wide distribution of ToRSV can be explained by the efficient action of the virus vectors, nematodes and a high soil infestation by them.

***Tobacco rattle tobravirus* (TRV)**

The diseased scarlet lychnis plants exhibiting symptoms of leaf yellowing, severe shortening of internodes (stunting) were found at Botanical Garden of Klaipėda University. Nevertheless, the symptoms were obviously typical of phytoplasmal disease. EM of preparations made from these plants revealed virus particles with a morphology specific of *Tobraviruses*. TRV was isolated and identified by the reactions of experimentally inoculated test-plants (Table). EM investigation of preparations from infected test-plants revealed the presence of rod-shaped particles of two predominant lenghts – 200 nm (long) and 45–115 (short) (Fig. 2).

TRV is the type member of the genus *Tobravirus* and is the only species of the genus to infect ornamental plants. The virus has a very wide natural host range including ornamental plants: *Alstroemeria* L., *Aster* L., *Crocus* L., *Freesia* Eckl. ex Klatt., *Gladiolus*, *Hyacinthus* L., *Iris* L., *Lilium* L., *Narcissus* L., *Nerine* Herb., *Paeonia* L., *Phlox* L., *Tulipa* L. [19]. In Lithuania, TRV was isolated and identified in 15 ornamental plant species belonging to 10 botanical families [21]. It is transmitted by *Trichodorus* and *Paratrichodorus* nematodes. The virus is also seed-borne in some host species [13, 14].

Phytoplasma subgroup 16SrI–B

A phytoplasma strain was isolated from the TRV-infected scarlet lychnis plants, once again confirming the statement that virus-infected plants are more susceptible to other pathogens [22].

Phytoplasma detection was carried out by PCRs. The phytoplasma characteristic 1.2 kbp 16S rDNA was amplified in nested PCR primed by the primer pair R16F2n/R6R2, confirming that the plants were infected by a phytoplasma (data not shown). This phytoplasma was termed lychnis stunting (LyStu).

The 1.2 kbp 16SrDNA product was subjected to single digestions with 9 different restriction endonucleases. The RFLP patterns of LyStu phytoplasma 16SrDNA were indistinguishable from patterns of 16S rDNA from the phytoplasma classified in group 16SrI (aster yellows phytoplasma group) and subgroup 16SrI-B (aster yellows subgroup) (Fig. 3) [5, 7, 8]. Our previous investigation of the phytoplasmal disease of this crop revealed a phytoplasma belonging to 16SrI-M subgroup. Naturally infected plants showing symptoms of general yellows and shoot proliferation were collected in Širvintos district. The molecular characterization of phytoplasma DNAs revealed that scarlet lychnis plants collected from different regions of Lithuania were infected by phytoplasmas belonging to the same group but to different subgroups. The similar results were obtained with the phytoplasma strains infecting *Aquilegia* L. [23]. The species of insect vectors in different regions may influence the geographical distribution and prevalence of the phytoplasma strains belonging to these subgroups.

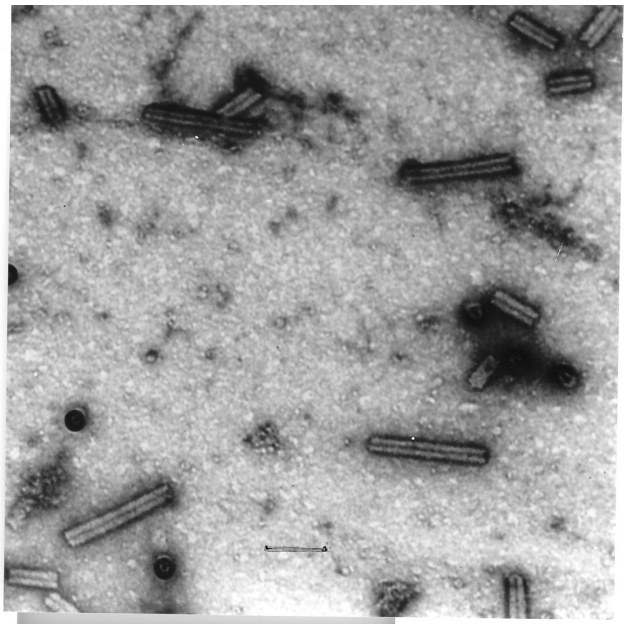

Fig. 2. Particles of *Tobacco rattle tobravirus*. Bar represents 100 nm

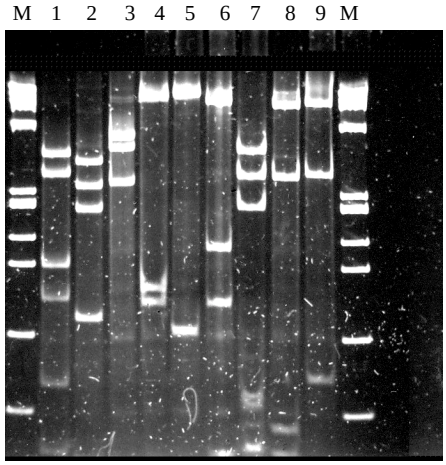

Fig. 3. RFLP analysis of LyStu phytoplasma 16S rDNA, amplified in n-PCR. Lanes M, PhiX174 DNA *Hae*III digest, fragment sizes (bp) from top to bottom: 1353, 1078, 872, 603, 310, 281, 271, 234, 194, 118, 72. 1 – AluI, 2 – MseI, 3 – KpnI, 4 –HhaI, 5- HaeIII, 6 – HpaII, 7 – RsaI, 8 – HinfI, 9 – TaqI

Phytoplasmas belonging to subgroup 16SrI-B are widespread worldwide, mostly in herbaceous plants, but have also been reported in woody plants [5, 7, 24, 25]. In Lithuania, this phytoplasma subgroup hase been detected in woody plants *Salix* L., *Pyrus communis* L. and herbaceous plants *Valeriana* L. [8], *Delphinium* L. [26], *Hordeum vulgare* L. and *Triticosecale* Wittm. ex Camus [9]. Identification of subgroup I-B phytoplasmal infection in scarlet lychnis together with previous findings of subgroup I-B in other plant species emphasises the broad host plant range of subgroup I-B phytoplasma strains in Lithuania.

The results of this work provide more information on the biodiversity, variability and distribution of plant pathogens in Lithuania. The work revealed the new host-plant of ToRSV, TRV and subgroup 16Sr I-B of phytoplasmas.

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VIRUSŲ IR FITOPLAZMOS, PAŽEIDŽIANČIŲ GOŠTAUTINĘ GAISRENĄ (*LYCHNIS CHALCEDONICA* L.), IDENTIFIKAVIMAS

Santrauka

Serganti goštautinė gaisrena su būdingais virusinio ir fitoplazminio pažeidimo požymiais buvo rasta Lauko gėlininkystės bandymų stoties ir Klaipėdos universiteto botanikos sodo gėlių kolekcijose. Augalų indikatoriais ir elektroninės mikroskopijos metodais išskirti bei identifikuoti pomidorų žiediškosios dėmėtligės (*Tomato ringspot nepovirus*) ir tabako garbanotosios dryžligės (*Tobacco rattle tobravirus*) virusai. Fitoplazmos 16S rRNR geno sekos amplifikacija polimerazės grandininės reakcijos (PGR) metodu, naudojant universalią pradmenų porą R16F2n/R6R2 ir iš pažeistos gaisrenos išskirtą visą DNR, patvirtino fitoplazminę infekciją. 16S rDNR produkto fragmentų ilgio polimorfizmo analizė rodo, kad gaisrenos fitoplazma priklauso 16SrI (astrų geltos) fitoplazmų grupei ir 16SrI-B (astrų geltos) fitoplazmų pogrupiui.