Nepoviruses and their influence on field floriculture

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Investigation of ornamental plants with respect to virus diseases revealed a high incidence of *tomato ringspot nepovirus* (ToRSV) and *tobacco ringspot nepovirus* (TRSV) infection in perennial ornamental plants. ToRSV was isolated and identified from 39 flower species belonging to 20 botanical families. TRSV was isolated and identified in four flower plant species belonging to three botanical families. For the first time 36 flower species were ascertained as host plants of ToRSV and *Dicentra spectabilis* was found to host TRSV. Viruses were identified by the methods of test-plants and electron microscopy. The optimum technique for ToRSV purification was selected and the purified preparations of *Gypsophila paniculata, Iris germanica* and *Penstemon murrayana* isolates were obtained.

Key words: *tomato ringspot nepovirus, tobacco ringspot nepovirus,* perennial ornamental plants

INTRODUCTION

To the genus of nepoviruses more than 30 viruses are ascribed. They cause diseases of economical importance in a wide range of cultivated annual, perennial and wood plants. The effects of nepoviruses range from symptomless infection to the production of prominent foliar symptoms, necrosis, stunting and death. All members of the genus are transmitted through soil by free-living nematodes, principally of *Longidorus* or *Xiphinema* species, feeding on roots. They are also transmitted and disseminated through seed and pollen. It is a combination of a mode of transmission (*ne*matode) and virus morphology (*po*lyhedral) that provides the name of the genus. Nepoviruses occur in all parts of infected plants. The particles are isometric, polyhedral, 28 nm in diameter, sedimenting as three components and containing an RNA bipartite genome [1]. Nepoviruses that commonly occur in ornamental plants are *Arabis mosaic, hibiscus latent ringspot, tobacco ringspot, tomato black ring, tomato ringspot, raspberry ringspot* and *strawberry ringspot* [2].

MATERIALS AND METHODS

Material for investigation was collected in different floriculture farms of Lithuania: Botanical Gardens of Vilnius University and Vytautas Magnus University, Experimental Station of Field Floriculture, flower collections of the institutes of Horticulture and Agriculture and in private gardens of flower growers. The experimental work was carried out at the greenhouse and Plant Virus Laboratory of the Institute of Botany. Virus has been identified by an electron microscopy negative staining technique [3, 4], test-plant method, DAS ELISA [5]. The following test-plants were inoculated: *Amaranthus caudatus* L., *A. paniculatus* L., *Atriplex hortensis* L., *Celosia argentea f. cristata* (L.) Kuntze, *Chenopodium amaranticolor* Coste et Reyn, *C. murale* L., *C. quinoa* Willd., *C. urbicum* L., *Cucumis sativus* L., *Datura stramonium* L., *Gomphrena globosa* L., *Lycopersicon esculentum* Mill., *Nicandra physalodes* (L.) Gaertn., *Nicotiana debneyi* Domin., *Nicotiana glutinosa* L., *N. tabacum* L. 'Samsun', 'White Burley', 'Xanthi', *N. rustica* L., *Petunia hybrida* Vilm., *Phaseolus vulgaris* L., 'Baltija', 'Prince', *Physalis floridana* Rybd., *Tetragonia expansa* Murr. The inoculum for mechanical inoculation was prepared by homogenizing infected leaves with 0.1 M phosphate buffer (pH 7,0) containing 0.2% 2-mercaptoethanol or 0.01 M sodium diethyldithiocarbamate. ToRSV isolates were purified according to a modified method [6]. Cooled leaves were grinded in 0.1 M borate buffer (pH 7.4) containing 0.1% 2-mercaptoethanol. The extract was clarified by blending with an 8% n-butanol and 5% chloroform mixture. Virus particles were precipitated with w/v 10% polyethylene glycol Mw 6000 and 1.5% NaCl. Pellets were resuspended in 0.1 M borate buffer (pH 7.4). Virus was purified by two cycles of differential centrifugation. Final purification was accomplished by sedimentation through 20% sucrose cushion. The concentration and purity of virus preparations were estimated by electron microscopy and spectrophotometrically.

RESULTS AND DISCUSSION

Tomato ringspot nepovirus **(ToRSV)** was identified in 39 ornamental plant species belonging to 20 botanical families. Virus was isolated and identified from following naturally infected ornamental plants showing the main symptoms such as plant stunting, malformation of leaves and flowers, chlorotic and necrotic spots and streaks, ringspots on leaves, vein necrosis and shortening inducing leaf crinkling. Severe infection leads to the premature death of a plant. These symptoms may be not specific only to ToRSV because of a mixed infection in most of naturally infected ornamental plants.

Amaryllidaceae I. St.-Hill. (*Narcissus* sp.); ***Apiaceae*** Lindl. (*Eryngium alpinum* L.); ***Asteraceae*** Dumort. (*Dahlia* Cav., *Echinacea augustifolia* (L.) DC, *E. purpurea* (L.) Moench, *Echinops sphaerocephalus* L., *Helenium autumnale* L., *Liatris spicata* (L.) Willd., *Solidago canadiensis* L.); ***Caryophylaceae*** Juss. (*Gypsophila paniculata* L., *Silene* sp.); ***Commelinaceae*** R. Br. (*Tradescantia Andersoniana* hybr.); ***Fumariaceae*** D. C. (*Dicentra formosa* Walp., *D. spectabilis* (L.) Lem.; ***Hydrangeaceae*** Dumort. (*Hydrangea arborescens* L.); ***Hostaceae*** B. Mathew (*Hosta alba-marginata* (Hook) Hyl., *H. glauca* (Siebold) Stearn., *H. lancifolia* (Thunb.) Engl., *H. plantaginea* (Lam.) Asch., *H. venticosa* (Salisb.) Stearn; ***Iridacea***e Juss. (*Iris* sp.); *Laminaceae* Lindl. (*Monarda didyma* L.); ***Malvaceae*** Juss. (*Alcea rosea* L.); ***Onagraceae*** Juss. (*Oenothera tetragona* Roth.); ***Plumbaginaceae*** Juss. (*Limonium* sp.); ***Polemoniaceae*** Juss. (*Polemonium caeruleum* L.); ***Ranunculaceae*** Juss. (*Anemone hupehensis* Lemoine, *Aquilegia vulgaris* L., *Delphinium* sp., *Helleborus foetidus* L., *Thalictrum aquilegifolium* L., *Trollius* sp.); ***Rutaceae*** Juss. (*Dictamnus alba* L.); ***Saxifragaceae*** Juss. (*Heuchera x brysoides* Lemoine, *H. sanguinea* Eng.); ***Scrophulariaceae*** Juss. (*Digitalis purpurea* L., *Penstemon murrayana* Hook.); ***Solonaceae*** Juss. (*Physalis alkekengi* L.); ***Violaceae*** Batsch (*Viola cornuta* L.). Twenty-two plant species were found to have mixed viral infection.

For virus identification, the test-plants were inoculated. The results are presented in Table 1. The test-plants exhibited local and systemic reaction. Electron microscopy investigation of negatively stained dip preparations from naturally infected plants and inoculated test plants revealed isometric particles 28 nm in diameter. The morphology of particles was characteristic of ToRSV. The identification of ToRSV was confirmed by a positive reaction in DAS ELISA technique, using alkaline phosphatase linked to ToRSV antibodies with glutaraldehyde and nitrophenyl phosphate as a substrate. A great number of investigated species have not been reported as ToRSV host plants earlier: *Alcea rosea, Anemone hupehensis, Aquilegia vulgaris, Dahlia* sp., *Delphinium* sp., *Dicentra formosa, D. spectabilis, Dictamnus alba, Digitalis purpurea, Echinacea augustifolia, E. purpurea, Eryngium alpinum, Gypsophila paniculata, Helenium autumnale, Heleborus foetidus, Heuchera x brisoides, H. sanguinea, Hosta alba-marginata, H. glau-*

Table. **Test-plant reaction to inoculation of nepoviruses**

Test-plant	ToRSV	TRSV
Amaranthus caudatus	L: LLN; S: Sp,DisLe	
A. paniculatus	L:LNSp; S: Sp, DisLe	
Celosia argentea f. cristata	L: DBrRi; S: VChl, LeDis, BrSp, Ln	
Chenopodium amaranticolor	L: ChlLL; S: VStu, TR, NT	L: ChlLL
C. murale	L: LChlSp; S: VChl, Mo, N, LeDis	
C. urbicum	L: LClSp; S: VChl, LeDis	
C. quinoa	L: LChlSp, N,LeDis; S: Chl, N, LeDis, ApN	L: LChlSp, N
Cucumis sativus	L: N or Chl LL; S: Mo	L: LYSp, N; S: Mo,DisT
Datura stramonium		L: RiDifSp; S: Chl,NV
Gomphrena globosa	L: LNSp; S: DisLe, Mo	L: SmGLL, N
Lycopersicon esculentum	L: NSp; S: Mo, N	
Nicandra physalodes		L: NV, RiDBr; S: ChlV, DBrN, DNSp
Nicotiana debneyi		L: DifChlSp; S: ChlMo, NSp
N. glutinosa	L: LNSp	L: DNRi; S: RiMSp, NV
N. rustica	L: LNDotSp	
N. tabacum	L: LNSp; S: NSp, Str	L: RiNSp; S: NV, RiSp
Petunia hybrida	L: GNRi; S: LeDis, ChlSp, NSp	L: DifChlSp
Phaseolus vulgaris		L: NSp; S: RiSp
Tetragonia expansa	L: DifChlSp; S: LeDis, ChlDot	L: Sp, RiChlSp, N

Abbreviations: L – local reaction, S – systemic reaction, LL – local lesions, Chl – chlorotic, N – necrotic, Sp – spots, Dot – dots, Mo – mosaic, Str – striping, V – vein, Dif – diffusial, ApN – apical necrosis, LeDis – leaf distortion, RiSp – ringspot, Sm – small, Y – yellow, Br – brown, D – dark, G – grey, T – top, Stu – stunting.

ca, H. lancifolia, H. plantaginea, H. ventricosa, Iris sp., *Liatris spicata, Limonium latifolium, Monarda didyma, Oenothera tetragona, Penstemon murrayana, Physalis alkekengi, Polemonium caeruleum, Silene* sp., *Solidago canadiensis, Thalictrum aquilegifolium, Tradescantia Andersoniana, Trollius* sp.

ToRSV isolates from *Gypsophila paniculata, Iris* sp. and *Penstemon murrayana* were purified. For purification, ToRSV isolates were propagated in *Chenopodium quinoa* or *Datura stramonium*. Purified virus preparations had A_{max} at 260 nm and A_{min} at 240 nm, the $A_{260/280}$ ratio being 1.2. The yield of the purified virus, taking into acount the ToRSV specific absorbance $A^{0.1\%}_{260nm}$ being 10.0 [8], was calculated to be 74 mg (*Gypsophila paniculata* isolate), 90 mg (*Iris* sp. isolate) and 50 mg (*Penstemon murrayana* isolate) from 1 kg infected plant tissue.

Tobacco ringspot nepovirus* (TRSV).** This virus has been reported infecting 13 ornamental plant species [2]. We have isolated and identified this virus in four ornamental plant species belonging to three botanical families: ***Fumariaceae D. C. (*Dicentra spectabilis* (L.) Lem.; ***Iridaceae*** Juss. (*Gladiolus* L., *Iris* L.); ***Liliaceae*** Juss. (*Tulipa* L.). Main symptoms on naturally infected plants were chlorotic and necrotic spots and streaks, ringspot and oak leaf pattern mosaics on leaves. Sometimes, particularly in late stages, plants were symptomless. First reports of this virus in Lithuania were presented in 1977 [9].

For virus identification the test-plants were inoculated. Results are presented in Table 1. Local reaction was observed on *Chenopodium amaranticolor, C. quinoa, Gomphrena globosa, Petunia hybrida* and *Tetragonia expansa.* Others test plants showed a local and systemic response. Electron microscopic examination of negatively stained leaf dip preparations made from infected test plant leaves revealed isometric particles 28 nm in diameter.

According to the test-plant reaction, electron microscopy, and literature data [2, 7, 10] the isolated virus was identified as TRSV. TRSV has been found affecting *Dicentra spectabilis* for the first time.

Both nepoviruses are widespread in field perennial flowers due to easy transmission by nematodes, vegetative propagation, dissemination through seeds and pollen in some plants. Viral infection has a negative influence on plant growth, yield, reduces the aesthetic quality of ornamental plants. Perennial ornamental plants are reservoirs for viruses and a source of infection for other crops. Methods of controlling viral diseases consist in the growing and propagation of selected healthy planting material, inspection of plants during vegetation for the symptoms, and elimination of affected plants. Losses caused by nematode-transmitted viruses can be reduced by soil fumigation with fumigant nematicides to control nematodes. The disease problems can sometimes be minimised by employing crop rotations that diminish nematode populations. Virus-free stocks of valuable cultivars can be produced by heat treatment and meristem culture.

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NEPOVIRUSAI IR JŲ REIKŠMĖ LAUKO GĖLININKYSTĖJE

S a n t r a u k a

Tyrimo duomenys parodė, kad daugiametės gėlės yra stipriai pažeistos pomidorų žiediškosios dėmėtligės (ToRSV) ir tabako žiediškosios dėmėtligės (TRSV) nepovirusų. ToRSV išskirtas ir identifikuotas 39 gėlių rūšyse, priklausančiose 20 botaninių šeimų. TRSV identifikuotas 4 gėlių rūšyse, priklausančiose 3 botaninėms šeimoms. 36 augalų rūšys, kaip ToRSV augalai-šeimininkai ir *Dicentra spectabilis,* kaip TRSV šeimininkas aprašyti pirmą kartą. Virusai identifikuoti augalų-indikatorių, elektroninės mikroskopijos ir DAS-ELISA metodais. Išgryninti trys ToRSV izoliatai, išskirti iš *Gypsophila paniculata, Iris* sp., *Penstemon murrayana.*