

Identification of viruses affecting primroses (*Primula L.*) and virological evaluation of the Lithuanian hybrids

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This work presents data on investigation of virus diseases affecting primrose (*Primula L.*) in Lithuania and identification of the causal agents. The symptoms for virus diseases visual recognition were determined and described. Three viruses, *Cucumber mosaic cucumovirus* (CMV), *Tobacco necrosis necrovirus* (TNV), *Tobacco rattle tobnavirus* (TRV), causing diseases in this crop were identified by the methods of test-plants and electron microscopy. According to Lithuanian State Program “Genefund” the virological evaluation of primrose hybrids created by Lithuanian breeders was carried out. Twenty five hybrids of primrose were surveyed their virological state evaluated and the causal agent CMV was identified. The most resistant to virus diseases primula hybrids were selected for propagation.

Key words: *Primula*, *Lithuanian hybrids*, *Cucumber mosaic cucumovirus*, *Tobacco necrosis necrovirus*, *Tobacco rattle tobnavirus*.

INTRODUCTION

Primrose (*Primula L.*) a perennial plant belonging to *Primulaceae* Vent. family, is one of the most popular spring flowers worldwide. More than 30 species are grown as garden and greenhouse ornamental plants. The most popular in our country are species *P. vulgaris* Huds., *P. denticulata* Sm., *P. juliae* Kusn., *P. elatior* (L.) Hill., *P. malacoides* Franch., *P. obconica* Hance. Every year the breeders create new cultivars and hybrids. Primrose growers have active international association, publish the journal “Primroses”. Lithuanian breeders have also created many valuable hybrids of this flower. The process of creating new flower cultivars and hybrids is one that requires considerable skill, patience, is work- and time-consuming. It is very important to maintain cultivars, to keep their standard properties stable and unchanged, and to accumulate the genefund of the Lithuanian flower cul-

tivars and hybrids because they constitute a part of the national treasure and ethnic culture. The problem exists, that breeders did not pay needful attention to virus infection of starting material. New lines resulting from crosses were propagated vegetatively without testing for virus presence. The result of this carelessness is the great level of virus infection in Lithuanian cultivars and hybrids [1]. The damage of virus diseases is extremely serious. Virus diseases retard plant growth, damage any or all parts of a plant, distort standard properties, reduce the aesthetic quality and marketability of ornamental plants. The plants are commonly propagated vegetatively and this practise allows the viruses once established in a cultivar, to be perpetuated. Virus infected plants are more susceptible to fungal and bacterial pathogens which lead to premature death [2].

The primroses are susceptible to different virus infections. Several viruses have been described on primroses in literature: *Alfalfa mosaic alfamovirus*, *Cucumber mosaic cucumovirus*, *Tobacco necrosis necrovirus*, *Primula mosaic potyvirus*, *Primula mottle potyvirus*, *Tomato spotted wilt tospovirus*, *Tomato bushy stunt tombusvirus*, *Tobacco ringspot nepovirus* [3 – 6].

The aim of this work was to investigate virus diseases affecting primrose in Lithuania, to identify their causal agents and to evaluate the virological state of primrose hybrids, created by Lithuanian breeders.

MATERIALS AND METHODS

The material for investigation was collected at Botanical Gardens of Vilnius and Kaunas Vytautas Magnus universities, Experimental Station of Field Floriculture and private gardens in Lithuania. The collections of Lithuanian primrose hybrids are accumulated and grown at Botanical Garden of Vilnius University. Visual inspection of collections for virus symptoms and registration of affected plants has been done twice a year before and during flowering. The samples for virus identification were collected from plants bearing the most characteristic virus disease symptoms. Experimental work was carried out at the Plant Virus laboratory of Institute of Botany. The viruses have been identified by the methods of electron microscopy (EM, electron microscope JEM-100S, magnification $\times 25000$) and test-plants [6 – 10]. The inoculum for mechanical sap inoculation was prepared by homogenizing infected leaves with 0.1 M phosphate buffer (pH 7.0), containing the stabilizing agents 0.2 % mercaptoethanol, 0.1 % thioglycolic acid or 0.01 M sodium diethyldithiocarbamate.

RESULTS AND DISCUSSION

Cucumber mosaic cucumovirus (CMV) was isolated from primroses expressing following symptoms: stunting, general chlorosis, leaf rugosity, blisterity, vein chlorosis, colour breaking of petals. The edges of petals were uneven, look like cut out.

CMV was identified on the basis of symptom expression in mechanically inoculated test-plants. The results are presented in the table (Table). The most characteristic for CMV symptoms were induced on following test-plants: *Atriplex hortensis* (local reaction, L), *Cucumis sativus* (L) and systemic (S), *Nicandra physalodes* (L and S), *Nicotiana glutinosa* (L and S), *N. rustica* (L and S) (Fig. 1). EM revealed isometric virus particles 30 nm in diameter in the preparations made from naturally infected primrose plants and from inoculated test-plants (Fig. 2).

The first natural infection of *P. obconica* by CMV was found in 1935 by K.M. Smith in England and it seems remaining one of the most prevalent agent of virus diseases affecting this crop [3, 4, 11]. CMV has an extremely wide host range, infects more than 190 plant species in 40 families. The virus is transmissible by inoculation with sap, inducing a variety of symptoms depending on virus strain and host cultivar and in non persistent manner by more than 60 species of aphid [6, 9].

Tobacco necrosis necrovirus (TNV) was isolated from primrose plants showing symptoms of stunting and necrotic spotting on the young leaves along the veins. This virus causes considerable losses, because severely infected plants do not form flowers, necrosis leads to premature death of plant. In initial stage of infection primroses can be symptomless carriers and virus is contained in their roots.

TNV was identified on the basis of test-plant reactions (Table). In the most cases TNV on test-plants induces local reaction expressed by various necrotic patterns. EM revealed isometric particles 26 nm in diameter in the preparations made from naturally infected primrose plants and from inoculated test-plants.

TNV is RNA-containing virus with isometric particles about 26 nm in diameter. Readily transmitted by mechanical inoculation to a wide range of plants, but usually does not infect them systemically. Soil borne, transmitted by zoospores of fungus *Olpidium brassicae*, not transmitted by seed and pollen. Virus occurs worldwide, common in irrigated fields, in unsterilized soil of glasshouses [6, 8].

Tobacco rattle tobnavirus (TRV) was isolated from primrose plants expressing symptoms of plant stunting, leaf distortion and mosaic. Petals of flowers were slightly distorted with conspicuous colour breaking symptoms.

TRV was identified on the basis of test-plants reaction (Table). EM revealed tubular particles of two predominant lengths, the longer ones 200

nm and the shorter ones ranging from 50 to 115 nm. TRV on primroses is not widespread, was detected occasionally. We have not found references about primrose as TRV host plant in literature.

TRV has the widest known host range of any plant virus. More than 400 species in more than 50 dicotyledonous and monocotyledonous families can be infected experimentally; in many cases the infection does not become systemic. Seven nematode species in the genera *Paratrichodorus* and *Trichodorus* are natural vectors [6, 10].

According to Lithuanian State Program “Genefund” the virological evaluation of primrose hybrids, created by Lithuanian breeders, was carried out. Six hybrids of *P. vulgaris* and nineteen of *P. elatior*, created by E. Tarvidienė and J. Tarvidas were investigated. Twice in vegetation period all plants were inspected on presence of visual viral symptoms, leaf samples collected and tested for virus presence by EM. Primrose plants of three *P. vulgaris* (Auksė, Greitukė, Mėlynukė) and also of three *P. elatior* hybrids (Laimikė, Retukė, Rusvukė) visually were symptomless and EM revealed no virus particles. Plants of these hybrids were recognized healthy and suitable for propagation. Plants of other hybrids had more or less conspicuous symptoms on leaves and flowers. EM revealed isometric particles 30 nm in diameter. According experimentally inoculated test-plants reactions the virus affecting Lithuanian primrose hybrids was identified as CMV.

Summarizing the results of this investigation, we can assert that the virological state of Lithuanian primrose hybrids is complicated. Only 25 % of investigated plants were recognized being healthy. In order to preserve other primrose hybrids, virus-free material should be produced by the methods of tissue culture, thermotherapy and chemotherapy, either alone or in combination. In future breeders should know that for breeding purposes it is necessary to use only virus-free starting material tested for virus presence. Healthy and newly produced hybrids should be protected from infection by viruses. Indexing for diseases should be repeated several times throughout the season and affected plants should be eliminated. Plants should be grown keeping right to agrotechnical requirements and under strict conditions minimising the risks of reinfection by vectors.

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RAKTAŽOLES (*PRIMULA L.*) PAŽEIDŽIANČIŲ VIRUSŲ IDENTIFIKAVIMAS IR LIETUVIŠKŲ HIBRIDŲ VIRUSOLOGINĖS BŪKLĖS ĮVERTINIMAS

S a n t r a u k a

Darbe pateikiami duomenys apie raktažoles (*Primula L.*) pažeidžiančias virusines ligas ir jų sukėlėjų identifikavimą. Aprašyti virusinių ligų pažeistų raktažolių požymiai. Augalų indikatorių ir elektroninės mikroskopijos metodais identifikuoti trys virusai: agurkų mozaikos (*Cucumber mosaic cucumovirus*, CMV), tabako nekrozės (*Tobacco necrosis necrovirus*, TNV) ir tabako garbanotosios dryžligės (*Tobacco rattle tobrovirus*, TRV). Pagal Lietuvos valstybinę programą „Genofondas“ buvo atliktas lietuviškų raktažolių hibridų virusologinės būklės patikrinimas ir įvertinimas. Patikrinta ir įvertinta 25 hibridų augalų būklė, atrinkti sveiki, tinkami dauginimui augalai. Nustatyta, kad lietuviškas raktažoles pažeidžia CMV.

Table. Test-plants response to mechanical inoculation of viruses isolated from primrose

Test-plant	Virus and reaction on the test-plant		
	CMV	TNV	TRV
<i>Amaranthus caudatus</i> L.		L: NRi	
<i>A. paniculatus</i> L.		L: NRi	L: BrLL
<i>Atriplex hortensis</i> L.	L: GNLL	L: GNLL	L: LL
<i>Celosia argentea</i> L.		L: ReNRi	
<i>Chenopodium amaranticolor</i> Coste et Reyn	L: CILL;	L: BrGrRi,NSp	L: NLL
<i>C. ambrosioides</i> L.		L: NLL	L: NLL
<i>C. foetidum</i> Schrad.			L: LL
<i>C. hybridum</i> L.		L: NLL	
<i>C. murale</i> L.			L: CILL
<i>C. urbicum</i> L.	L: ClSp;		L: LL
<i>C. quinoa</i> Willd.	L: CILL	L: NRiSp	L: Cl and NSp
<i>Cucumis sativus</i> L.	L: DifSp; S: Mo, YSp	L: NLL	L: Cl or NLL
<i>Galinsoga parviflora</i> L.			L: NStr, Ri
<i>Gomphrena globosa</i> L.	L: NLL	L: NSp	L: NSp
<i>Nicandra physalodes</i> (L.) Gaertn.	L: NLL; S: M, LeDis		L: ClNSp, NEt
<i>Nicotiana alata</i> Link et Otto			L: NRiSp; S: NSp, LeDis
<i>N. debneyi</i> Domin.	L: DifSp; S: LeMo, VC, Dis		L: NSp; S: NSp, LeDis
<i>N. glutinosa</i> L.	S: MiMo, LeDis	L: BINRiSp	L: GNRi; S: NSp, NRi, Stu
<i>N. rustica</i> L.	L: ClSp; S: M	L: NSp	
<i>N. tabacum</i> L.	L: NSp; S: NSp, Str	L: NRiSp	L: NSp; S: NRiPat, Dis
<i>Petunia hybrida</i> Vilm.	L: ClSp; S: M, Mo	L: BINSp	
<i>Phaseolus vulgaris</i> L.		L: BrNRi	
<i>Physalis floridana</i> Rybd.			L: SmNLL
<i>Pisum sativum</i> L.		S: M	
<i>Tetragonia expansa</i> Murr.	L: NSp, LeR	L: GNLL	
<i>Vicia faba</i> L.		S: M	

Abbreviations: L – local reaction, S – systemic reaction; B – black; Br – brown; Cl – chlorosis, chlorotic; Cot – cotyledons; Dif – diffuse; Dis – distortion; Et – etching; G – grey; Y – yellow; LL – local lesions; Le – leaf; M – mosaic; Mi – mild; Mo – mottling; N – necrosis, necrotic; Pat – pattern; R – rolling; Re – red; Ri – rings, RiSp – ringspots; Sm – small; Sp – spots; Str – streaks; Stu – stunting; VC – vein clearing;

Fig. 2. CMV particles. Bar represents 100 nm



A



B



C



D

Fig. 1. Symptoms induced by CMV on *Nicotiana glutinosa*: A – local reaction, B – systemic reaction and on *N. rustica*: C – local reaction, D – systemic reaction

