

Virological evaluation of the Lithuanian dahlia (*Dahlia* Cav.), gladiolus (*Gladiolus* L.), iris (*Iris* L.) and peony (*Paeonia* L.) cultivars and hybrids

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According to the Lithuanian State Program “Genefund”, the virological evaluation of dahlia (*Dahlia* Cav.), gladiolus (*Gladiolus* L.), iris (*Iris* L.) and peony (*Paeonia* L.) cultivars and hybrids created by Lithuanian breeders was carried out in 1998–2002. The visual symptoms of virus diseases were determined and described. The viruses affecting these crops were identified by the methods of test-plants, electron microscopy, DAS-ELISA. The main identified viruses affecting these flowers were: dahlia mosaic caulimovirus, tomato spotted wilt tospovirus, bean yellow mosaic potyvirus, tomato ringspot nepovirus, tobacco rattle tobtravirus, iris mild mosaic potyvirus. More than 100 cultivars of dahlia and gladiolus, 100 hybrids of iris, 3 cultivars and 80 hybrids of peony were inspected and virologically evaluated.

Key words: dahlia, gladiolus, iris, paeonia, dahlia mosaic caulimovirus, tomato spotted wilt tospovirus, bean yellow mosaic potyvirus, tomato ringspot nepovirus, tobacco rattle tobtravirus, iris mild mosaic potyvirus

INTRODUCTION

Dahlia, gladiolus, iris and peony, having not only the ornamental value but also an economic potential, are important and perspective crops in Lithuanian floriculture. Lithuanian breeders have created many valuable cultivars of these flowers. The process of creating new flower cultivars and hybrids 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 cultivars and hybrids, because they constitute part of the national treasure and ethnic culture. The problem exists that breeders did not pay due attention to virus infections of starting material. New lines resulting from crosses were propagated vegetatively without testing for virus presence. The result of this carelessness is a high level of virus infection in Lithuanian cultivars and hybrids. Our many-year experience shows that the damage caused by virus diseases is extremely high. Virus diseases retard plant growth, damage any or all parts of a plant, distort its standard properties, reduce the aesthetic quality and marketability of ornamental plants. Virus-infected plants are more susceptible to fungal and bacterial pathogens, which lead to premature death. As a result of our investigations

of viral diseases of these crops it was established that dahlia was affected by dahlia mosaic caulimovirus (DaMV), cucumber mosaic cucumovirus (CMV), tomato spotted wilt tospovirus (TSWV), tomato ringspot nepovirus (ToRSV) [1, 2], gladiolus by bean yellow mosaic potyvirus (BYMV), CMV, tobacco rattle tobtravirus (TRV) [1, 3], iris by iris mild mosaic potyvirus (IMMV), CMV, tobacco mosaic tobamovirus (TMV), BYMV, ToRSV [1, 4], and peony by TRV [1, 5].

The aim of the present work was to inspect and virologically evaluate Lithuanian dahlia, gladiolus, iris and peony cultivars and hybrids and to identify the most common viruses infecting these crops.

MATERIALS AND METHODS

The material for investigation was collected in Botanical Garden of Vilnius University where the collections of Lithuanian cultivars and hybrids of dahlia, gladiolus, iris and peony are accumulated and grown. Visual inspection of flower collections for virus symptoms and registration of affected plants were 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 Laboratory of Plant viruses of Institute of

Botany. Viruses were identified by the methods of electron microscopy (EM, electron microscope JEM-100S, magnification $\times 25000$) [6] and test-plants [7–12]. The DAS-ELISA test was carried out at State Plant Protection Service of Lithuania using the ELISA kit and BIOREBA AG according to a standard protocol [13]. The following test-plants were used: *Amaranthus caudatus* L., *A. paniculatus* L., *Atriplex hortensis* L., *Calendula officinalis* L., *Celosia argentea f. cristata* (L.) Kuntze, *Chenopodium amaranticolor* Coste et Reyn, *C. ambrosioides* L., *C. foetidum* L., *C. murale* L., *C. urbicum* L., *C. quinoa* Willd., *Cucumis sativus* L. 'Delikates', 'Libelle', *Dahlia coccinea* Cav., *D. stramonium* L., *Gomphrena globosa* L., *Lycopersicon esculentum* Mill., *Nicandra physalodes* (L.) Gaertn., *Nicotiana glutinosa* L., *N. tabacum* L. 'Samsun', 'Xanthi', 'White Burley', *N. rustica* L., *Petunia hybrida* Vilm., *Phaseolus vulgaris* L. 'Bataaf', 'Red Kidney', *Physalis floridana* Rybd., *Pisum sativum* L. 'Greitukai', *Tetragonia expansa* Murr., *Verbesina encelioides* Benth. et Hook., *Vicia faba* L. 'Aušra', *Zinnia elegans* Jacq. Test-plants were inoculated by mechanical sap inoculation. The inoculum was prepared by homogenizing infected leaves with 0.1 M phosphate buffer (pH 7.0) containing stabilizing agents 0.2% mercaptoethanol, 0.1% thioglycolic acid or 0.01 M sodium diethyldithiocarbamate.

RESULTS AND DISCUSSION

Dahlia (*Dahlia* Cav.)

Samples for virus identification were collected from plants expressing the main types of symptoms: plant stunting, leaf chlorosis, narrowing, distortion and rugosity, mosaic oakleaf pattern, ringspot, vein banding and clearing (Fig. 1), flower distortion and breaking (Fig. 2).

EM of naturally affected dahlia plants revealed the isometric particles 30, 50 and 85–110 nm in



Fig. 1. Virus disease symptoms in naturally infected dahlia leaflets



Fig. 2. Virus disease symptoms in naturally infected dahlia flower

diameter. Viruses were found mostly in mixed infection. Three viruses were identified to affect Lithuanian dahlia cultivars: dahlia mosaic caulimovirus (DaMV), tomato ringspot nepovirus (ToRSV) and tomato spotted wilt tospovirus (TSWV). Cucumber mosaic cucumovirus and Tobacco streak ilarvirus described in literature infecting dahlias were not identified in Lithuanian dahlia cultivars [14, 15].

DaMV was identified by the methods of test-plant and EM. The symptoms on *Calendula officinalis*, *Dahlia coccinea*, *Verbesina encelioides*, *Zinnia elegans* (mosaic, vein chlorosis, vein clearing, ringspot) became pronounced only in autumn and were inconspicuous (Table). Other inoculated test-plants were symptomless. The virus has isometric particles 50 nm in diameter. According to literature, DaMV is generally considered to be the most prevalent and harmful of the viruses that infect dahlias. It has been found all over the world and probably occurs wherever dahlias are grown. Although it is geographically widespread, it is very limited in its natural host range; natural infection is found only in dahlia species. DaMV belongs to the Caulimovirus group. Caulimoviruses have isometric particles 50 nm in diameter. Virions contain a single molecule of circular double-strained DNA, are transmitted by mechanical inoculation with sap and by aphids in non-persistent or semi-persistent manner [7, 14, 15].

The second virus isolated from dahlia and identified by test-plant reaction was tomato ringspot nepovirus (ToRSV). This virus infected a great number of test-plants which developed local (chlorotic and necrotic lesions) and systemic (leaf distortion, vein clearing, plant top necrosis and deformation) reactions (Table). EM examination of preparations made from infected test-plants revealed isometric virions 28 nm in diameter.

ToRSV is a type member of the Nepovirus group and causes economically important diseases in a range of crops. The virus has isometric particles about

28 nm in diameter, sedimenting as three components and containing RNA as bipartite genome. It is readily transmitted by the nematode *Xiphinema* spp. Seed transmission has been reported in several crops. The virus is also transmitted by vegetative propagation and pollen. ToRSV occurs in nature mostly in perennial crops [15]. In Lithuania, ToRSV has been found to affect 43 ornamental species belonging to 13 botanical families, dahlia being one of them [16, 17].

The third virus which has been found to infect dahlia, tomato spotted wilt tospovirus (TSWV), was identified according to particle morphology and by DAS-ELISA test. EM investigation revealed isometric, enveloped irregular-shaped particles 85–110 nm in diameter, specific for tospoviruses. TSWV has been recorded in many different countries for many years, particularly in the last decade. The high incidence of TSWV in dahlia cultures is correlated with development of the efficient vector thrips *Frankli-*

Table. Test plant response to mechanical inoculation of viruses

Test plant	Virus and reaction on the test plant				
	DaMV	ToRSV	BYMV	TRV	IMMV
<i>Amaranthus caudatus</i>	0	L: NLL; S:SpLeDis	0	L: LLBr	–
<i>A. paniculatus</i>	0	L: LLNSp; S: Sp, LeDis	–	L: LLBr	–
<i>Atriplex hortensis</i>	0	–	0	L: LL	0
<i>Calendula officinalis</i>	S: Mo	–	–	–	0
<i>Celosia argentea</i>	0	L: DRi; S: VC, LeDis, SpStr	0	0	0
<i>Chenopodium amaranticolor</i>	0	L: CILL; S: YDot, LeRu, TR, NT	L:CILLN	L: NLL	0
<i>C. ambrosioides</i>	–	L: NLL	0	L: NLL	–
<i>C. foetidum</i>	–	–	–	L: LL	–
<i>C. murale</i>	–	L: CIRiLL; S: VCl, NSp, LeDis	–	L: LLCl	–
<i>C. urbicum</i>	–	L: LLCISp; S: VCl, LeDis, TN	0	L: LL	–
<i>C. quinoa</i>	0	L: CILL; S: CIDot, ApN	L:CILLN	L: Cl, NSp	L:NLL
<i>Cucumis sativus</i>	0	L: N or CILL; S: Mo	0	L: CINLL	0
<i>Dahlia coccinea</i>	S:Mo	–	–	–	–
<i>Datura stramonium</i>	–	0	0	0	–
<i>Gomphrena globosa</i>	0	L: LLNSp; S: Mo, Dis	L:CILLN	L: NSp	0
<i>Lycopersicon esculentum</i>	–	L: NSp; S: MoN	0	–	0
<i>Nicandra physalodes</i>	–	–	0	L: Cl, NSp, NEt	0
<i>Nicotiana glutinosa</i>	0	L: LLNSp	0	L: GNRiPat	0
<i>N. tabacum</i>	–	L: NLL or RiSp; S: Ri or LnPat	0	L: NSp, NRiPat	0
<i>N. rustica</i>	–	L: LLNdotSp	0	L: RiSp	0
<i>Petunia hybrida</i>	–	L: GN; S: ClSp, NSp, TN	–	–	–
<i>Phaseolus vulgaris</i>	–	L: CILL; S: LeRuTN	S: ClMo, LeDis	L: SmNLL	0
<i>Physalis floridana</i>	–	L: LLNdotSp	–	–	–
<i>Pisum sativum</i>	–	0	S: Mo	–	–
<i>Tetragonia expansa</i>	0	L: LDifClSp; S: CIDot, LeDis	0	–	–
<i>Verbesina encelioides</i>	S: VC, LeDis, LeRu	0	0	0	0
<i>Vicia faba</i>	–	–	S: M	0	–
<i>Zinnia elegans</i>	S: Cl, Mo, Stu	0	0	0–	0

Abbreviations: L – local reaction, S – systemic reaction, LL – local lesions, Cl – chlorosis, V – vein, Stu – stunting, T – plant top, LeDis – leaf distortion, Sm – small, Sp – spots, Ri – rings, Pat – pattern, Ln – line, Ru – rugosity, Dif – diffuse, Dot – dots, G – gray, N – necrosis, Str – stripes, Et – etched, Mo – mottle, M – mosaic, D – dark, Br – brown, Y – yellow, 0 – no reaction, ‘–’ – no inoculation.

niella occidentalis and the presence of many others infected crops [14].

Visual inspection of dahlia collections for virus symptoms and the registration of affected plants were done 2 times a year (before and during flowering). In the first year, according to the number of affected plants in each cultivar or hybrid, they were classified into 3 conditional groups. The first group contained healthy cultivars, plants expressed no visual symptoms; in the second group, named partially infected, there were both healthy and infected plants, and to the third group were classified cultivars in which all plants were infected. Subsequent indexing of dahlia cultivars and hybrids for virus infection showed that their virological state had changed during five years. The cultivars Arnoldas, Auksis, Gintaras, Morkus, Naktis, Obelėlė, Pastelė, Pirmokas, Raibuolis, Ugnelė, Vilniaus Universitetas, Violeta, which have been classified into the first group, remained healthy during the following growing seasons. The cultivars of this group (Aranžuotoja Danutė, M. Lukaitienė, Pumpurėlis, Sabonis, Vakaras, Vitalija, Žalgiris, Žiemos Pasaka) became infected. A great number of cultivars classified into the third group were lost. It is obvious that virus infected plants are more susceptible to fungal and bacterial diseases and do not survive during storage in the winter period. The infected plants in cultivars Baltukas, Baltuolis, Bičkoris, Debesėlis, Gintarėlis, Juodulyš, Kaprizas, Kaukių Balius, Kokeitė, Kudlius, Margutis, Rudenėlis, Svajonė, Viltis, Žirafa survived but remained infected. The number of infected plants in cultivars classified into the second group increased every year, and the infectivity of some cultivars (Gedimino Pilis, Laisvė, Lietuvos Knygnešiams, J. Miltinis, Pergalė, Saulėlydis, Trakai) has reached 100%.

Gladiolus (*Gladiolus* L.)

The mixed virus infection is very common in gladiolus, so symptom expression was very various. The most characteristic symptoms were chlorotic stripes and spots on leaves (Fig. 3), sometimes necrosis; distortion of flower stems; colour-breaking pattern expressed by intensification or disappearance of pigment in flowers occurring in some cultivars (Fig. 4); yellowish brown streaks along the leaf veins and margins turning to leaf notching later. Severely affected plants mostly did not form flowers. Roots were abnormally ramified and poorly developed. Infected plants retarded in growth and development. Their corms lost their vigour and did not survive during storage.

Viruses have been identified by the methods of test-plants (Table) and EM. EM of negatively stained preparations from leaves of naturally in-



Fig. 3. Virus disease symptoms in naturally infected gladiolus leaves



Fig. 4. Virus disease symptoms in naturally infected gladiolus flowers

fectured gladiolus revealed filamentous and rod-shaped virions.

The virus, with filamentous virions on the basis of test-plant reaction, particle morphology (slightly flexuous virions with a normal length of 720 nm) and according to literature data [15, 18], was identified as bean yellow mosaic potyvirus (BYMV). BYMV is readily transmitted mechanically by sap inoculation and by numerous aphid species in non-persistent manner. BYMV is widespread in Lithuania and affects almost all gladiolus cultivars grown in our country. The virus is usually found in mixed infections with other viruses affecting gladiolus. A. Stein also indicated that BYMV is ubiquitous in gladiolus and all tested commercial stocks of many cultivars were found almost 100% infected. BYMV causes only mild symptoms in gladioli, and in most plants there are no visual symptoms at all. The effects of BYMV infection on the etiology and symptom expression in the presence of other viruses have not yet been studied, since almost no BYMV-free gladioli are available [19].

Another virus with rod-shaped particles of two modal lengths, 45–115 (short particles) and 190 nm

(long particles), according to test-plant reaction and literature data [15, 17, 18], was identified as tobacco rattle tobnavirus (TRV). TRV is transmitted by mechanical sap inoculation and by nematodes of the *Trichodoridae* family. This virus occurs on gladiolus in our country not so frequently as BYMV. Sporadic occurrence of the virus in gladiolus was reported from Holland, Israel, Egypt and Poland, but in general the damage to gladioli is minimal [19].

All viruses infecting gladiolus survive in infected corms and spread due to vegetative propagation.

Inspection of Lithuanian gladiolus cultivars and hybrids was accomplished visually on symptom presence on leaves and flowers. The first year data revealed that 63% of cultivars and hybrids had conspicuous symptoms on leaves, 21% on leaves and flowers, other cultivars and hybrids did not have visual symptoms. Visually healthy cultivars were: Birutės Daina, Fėja, Gedimino Stulpai, Geltonkasė, Lėvuo, Lionės Sesuo, Ogni Kasandry, Oranžinis Žaibas, Paparčio Žiedas, Raudonasis Korolas, Saulės Takas, 75-mečiui, Seryj Kardinal, Suvalkietis, Šamanas, Trispalvis, Undinė, Žydruoelis. The cultivars Oranžinis Žaibas, Raudonasis Korolas, Undinė were found infected by viruses in the first year, but the well-timed elimination of infected plants improved the virological state of these cultivars. Conspicuous visual symptoms on leaves were observed in the cultivars Bangelė, Dūmelis, Kardinalas, Grožybė, Laisvės Rytas, Laukagaliukas, Laimės Žiburys and others. The cultivars Poliot Mečty, Vėlius, Morisabel and others had symptoms on leaves and flowers. Several cultivars had not only viral but also fungal infection (Bangelė, Fiji, LDK Algirdas, Jotvingis, Malinovyj Zvon, Morisabel, Na, Gromovai, palauk, Skudurinė Onutė, Šešupė, Švelnumas, Žarija).

Similar field data on the prevalence of virus-diseased gladioli were described in different parts of the world, ranging from 20% to approximately 70% when the plant symptoms were considered; however, when tests on symptomless plants were included, a 100% prevalence was recorded [19]. Gladiolus is thus a highly susceptible crop, which suffers considerable losses if control measures are not taken.

Iris (*Iris L.*)

Leaves of iris plants affected by viruses showed a mosaic pattern consisting of greenish and yellowish prolonged spots and stripes (Fig. 5). Several hybrids had symptoms on flowers and flower-stems. The flower-breaking pattern was expressed by streaks and spots formed by intensification of the main colour pigment (Fig. 6). In some cases flowers were misshapen. Flower-stems had light-green or grey streaks looking like impressions.



Fig. 5. Virus disease symptoms in naturally infected iris leaves

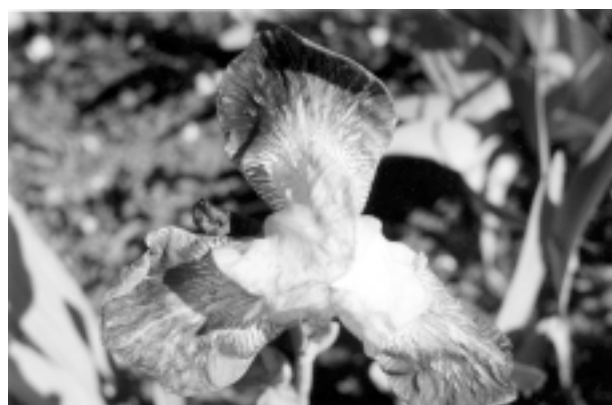


Fig. 6. Virus disease symptoms in naturally infected iris flower

Three viruses, iris mild mosaic potyvirus (IMMV), tomato ringspot nepovirus (ToRSV) and bean yellow mosaic potyvirus (BYMV), were isolated from affected iris plants and identified. Viruses were identified by the methods of test-plants (Table) and EM.

IMMV infected only the diagnostic test-plant *Chenopodium quinoa*, which reacted by local necrotic lesions. EM revealed filamentous flexuous particles 750 nm long. These characteristics coincide with data reported in the literature [10, 21]. IMMV is transmissible in a non-persistent manner by the aphids. It survives in bulbs and rhizomeus of infected plants and spreads due to vegetative propagation. IMMV is widespread on iris and occurs mostly in mixed infection wherever this crop is grown. Commercial iris stocks are almost totally infected with IMMV [21].

ToRSV infected many test-plants which reacted specifically to this virus. Virus particles are isometrical with a diameter of about 28 nm. Its transmission occurs via the nematode *Xiphinema americana* and related species and mechanically with sap. Iris is one of the new host plants described for this virus [17].

BYMV infected many test-plants which reacted specifically to this virus. Its particles are filamentous flexuous, 720 nm in length. The virus is transmitted in non-persistent manner by many aphid species and mechanically with sap. The test-plant reaction and virus morphology data coincided with data described in the literature [15].

One-hundred iris hybrids were visually tested on the presence of virus symptoms. All tested hybrids had visual viral symptoms on leaves. Several iris hybrids (VT-60,109, 113a, 113b, 143, 147, 204, 231, 232, 264) had symptoms on flowers and others (VT-60, 90, 106, 221, 246, 248, 252) on flower stems. It was established that irises infected by viral diseases were more susceptible to fungal and bacterial infection and did not survive. Several hybrids (VT-52, 53, 54, 58, 90, 104, 104, 141, 149, 156, 206, 207, 214, 250, 258, 259) grew luxuriantly in spite of virus infection. Symptoms on these plants were inconspicuous.

Peony (*Paeonia* L.)

Tobacco rattle tobnavirus (TRV) infects peony plants causing peony ringspot disease [5] (Fig. 7). At the early stage of disease the interveinal leaf tissue develops light green irregular spots, which later become yellowish. Around them and also around green patches yellow rings appear. The pattern looks like concentric irregular rings and semi-rings. This pattern covers all leaf lamina. Symptoms make progress later in season and become particularly visible in autumn. A considerable variation in symptom expression can be noticed depending on cultivar, growing conditions and season. TRV was identified by the morphology of particles. EM investigation of negatively stained dip preparations revealed rod-shaped virions of two modal lengths, 55–115 (short particles) and 190 nm (long particles), characteristic of



Fig. 7. Ringspot disease symptoms in naturally infected peony leaf

TRV [15]. The virus is transmitted by nematodes and is mechanically with sap, but from peony with difficulty. TRV retains in roots of affected plants and spreads due to peony vegetative propagation.

Three Lithuanian peony cultivars (Garbė Motinai, Prof. Grybauskas and Virgilijus) and 80 hybrids were tested for visual viral symptoms. Plants were found to be healthy, except one plant in the cultivar Garbė Motinai and solitary instances in some hybrids which showed symptoms of ringspot disease.

Summarizing the results of this investigation, we can assert that the virological state of Lithuanian flowers is complicated. Many cultivars and hybrids were lost and will be lost due to viral diseases. In future, breeders should know that for breeding purposes it is necessary to use only virus-free starting material tested for virus presence. New hybrids should be protected from reinfection 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 each crop agrotechnical requirements and under strict conditions minimising the risks of reinfection by vectors. No practical treatments to cure virus-infected plants in fields are available. Virus-free material can be produced by tissue culture methods, thermotherapy and chemotherapy, either alone or in combination.

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LIETUVIŠKOS SELEKCIJOS JURGINŲ (*DAHLIA CAV.*), KARDELIŲ (*GLADIOLUS L.*), VILKDALGIŲ (*IRIS L.*) IR BIJŪNŲ (*PAEONIA L.*) VEISLIŲ BEI HIBRIDŲ VIRUSOLOGINIS ĮVERTINIMAS

S a n t r a u k a

1998–2002 m. pagal Lietuvos valstybinę programą „Genofondas“ buvo atliktas lietuviškos selekcijos jurginų, kardelių, vilkdalgių ir bijūnų veislių bei hibridų (selekcinių numerių) virusologinis patikrinimas ir įvertinimas. Nustatyti simptomai bei parengta metodika kaip vizualiai atpažinti virusines ligas. Elektroninės mikroskopijos, augalų-indikatorių ir DAS-ELISA metodais nustatyti šias gėles pažeidžiantys virusai: jurginų mozaikos (*Dahlia mosaic caulimovirus*), pomidorų dėmėtasis vytulys (*Tomato spotted wilt tospovirus*), pupelių geltonoji mozaika (*Bean yellow mosaic potyvirus*), pomidorų žiediškoji dėmėtligė (*Tomato ringspot nepovirus*), tabako garbanotoji dryžligė (*Tobacco rattle tobravirus*) ir vilkdalgių silpnoji mozaika (*Iris mild mosaic potyvirus*). Patikrinta per 100 veislių jurginų, tiek pat kardelių, 100 vilkdalgių hibridų (selekcinių numerių), 3 bijūnų veislės ir 80 hibridų. Pateiktos rekomendacijos selekcininkams ir augintojams.