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Arabis mosaic virus on ornamental plants

M. Samuitienė[1]*,

M. Navalinskienė[1],

E. Jackevičienė[2]

[1] Plant Virus Laboratory, Institute of Botany, Žaliųjų Ežerų 49, LT-08406 Vilnius, Lithuania

[2] Phytosanitary Research Laboratory of Lithuanian Plant Protection Service, Sukilėlių 9a, LT-11351 Vilnius, Lithuania

Arabis mosaic virus (ArMV) is pathogenic to a wide variety of plant species including ornamentals. Using the methods of test-plants, electron microscopy, and DAS-ELISA, ArMV was identified in ornamental plant species of the genera *Arum* L., *Camassia* Lindl., *Crocus* L., *Dahlia* Cav., *Dicentra* Bernh., *Dieffenbachia* sp., *Eryngium* L., *Liatris* Gaertn. Ex Schreb., *Lychnis* L., *Muscari* Mill., *Iris* L. and *Phlox* L., representing eight families. In naturally infected host plants, the virus was found in mixed infections with other viruses. Virus idenstity in five ornamental species was confirmed by RT-PCR.

Key words: Arabis mosaic virus, ornamental plants, identification, DAS-ELISA, RT-PCR

INTRODUCTION

Recently, much attention has been given to the development of field floriculture in Lithuania. For small farmers, field floriculture is promising as family business. Farmers grow seedlings of perennial ornamental plants not only for Lithuanian domestic market, but also for neighbouring countries. Like other segments of agriculture, this sector is threatened by plant diseases. The quality and quantity of ornamental plants are affected by viral diseases. Since viral infections are systemic in diseased plants, propagation by division may contribute to the geographical spread of the pathogens. The scientific workers of Plant Virus Laboratory of Institute of Botany carry out a regular survey controlling the phytosanitary state of ornamental plants grown at Botanical Gardens of the Vilnius, Kaunas Vytautas Magnus, Klaipėda universities, the Experimental Station of Field Floriculture and according to requests in other floricultural farms and cities' parterres in order to help the growers to control plant diseases and also to collect plant samples for investigation. As a result of this investigation, arabis mosaic virus (ArMV) has been identified affecting ornamental plants as a component of mixed infections.

ArMV is a member of the genus *Nepovirus* in the *Comoviridae* family. It is an RNA-containing virus which has isometric particles about 30 nm in diameter and spreads worldwide [1]. ArMV is readily transmitted mechanically; it infected 93 species in 28 dicotyledonous families when transmitted by mechanical inoculation. In nature, the virus is transmitted by nematodes belonging to the genera *Longidorus* and *Xiphinema*, and through seed and pollen [2–4]. The virus occurs naturally in many species of wild and cultivated monocotyledonous and dicotyledonous plants. ArMV has been reported from numerous vegetable crops, sugar beet, strawberry, grapevine, olive, hop, cherry, black currant [2, 5]. The effects of virus infection on field-infected plants ranged from symptomless to prominent foliar symptoms, necrosis, stunting and death. The virus was reported in ornamental species, including *Alstroemeria, Begonia, Dianthus, Crocus, Gladiolus, Hyacinthus, Lilium, Narcissus, Nerine, Roses, Tulip*, mostly in mixed infection with other viruses [6]. Reports on ArMV in Lithuania have appeared recently. Reliable detection and identification of viruses affecting plants in mixed infections became possible due to applying modern methods of virus identification, such as DAS-ELISA and RT-PCR. The ArMV virus was described as an agent of virus disease in *Hyacinthus* and *Lycopersicon esculentum* [7, 8].

The aim of this study was to present data on the occurrence and identification of ArMV on ornamental plant species, based on data about test-plant reaction and the morphology of virus particles obtained by sensitive methods of specific virus detection (DAS-ELISA and RT-PCR).

MATERIALS AND METHODS

The plant material was collected in the Botanical Gardens of Vilnius, Kaunas Vytautas Magnus, Klaipėda universities and at the Experimental Station of Field Floriculture.

The virus was identified by the methods of test-plant [1, 2], electron microscopy (EM) [9, 10], DAS-ELISA [11] and RT-PCR [5, 12].

The test-plants were inoculated in early stages of growth by mechanical sap transmission, applying carborundum as an

* Corresponding author. E-mail: marija.samuitiene@botanika.lt

abrasive. The inocula were prepared by homogenizing infected plant tissue in 0.1 M phosphate buffer pH 7.0, containing 0.2% 2-mercaptoethanol or 0.01 M sodium diethyldithiocarbamate as virus-stabilizing additives.

Virus particles were examined in leaf dip preparations negatively stained with 3% uranyl acetate EM using a JEM-100S electron microscope, magnification 25000.

Double-antibody sandwich enzyme-linked immunosorbent assays (DAS-ELISA) were carried out at the Phytosanitary Research Laboratory of Lithuanian Plant Protection Service and at Plant Virus laboratory of Institute of Botany using commercial kits (Bioreba, Switzerland and DSMZ Plant Virus Collection, Germany), according to standard procedures. ArMV IgG was used at a 1 / 1000 dilution and alkaline phosphatase conjugate at a 1/1000 dilution. 50 mg of sample was extracted in 1 ml of sample buffer. 0.1% p-nitrophenyl phosphate was used as a substrate. The reactions were measured after 90 min of incubation with substrate photometrically at 405 nm (Labsystems Multiskan RC).

Reverse transcription polymerase chain reaction (RT-PCR) was accomplished using primers designed for ArMV coat protein gene. The upstream primer AP1[2]: 5-AAT ACC CCG GGT GTT ACA TCG-3' and the downstream primer AP2[2]: 5'-CAT TAA CTT AAG ATC AAG GAT TC-3' were selected, resulting in 421 bp amplification product [5].

Total RNA was extracted from symptomatic test-plant material stored frozen at –20 °C using QuickPrep™ Total RNA Extraction Kit (Amersham Biosciences UK). The extraction procedure was carried out according to the manufacturer's instructions. Total RNA was dissolved in PCR water-containing RNAse inhibitor and was stored at –20 °C.

All PCR procedures were carried out in an Eppendorf Master Cycler Personal.

For reverse transcription (RT, cDNA synthesis), the reaction mixture contained (for one sample): 2 µl downstream primer AP2[2], 2 µl 10 × PCR buffer; 4 µl 25 mM $MgCl_2$, 1 µl RNAse inhibitor; 2 µl 4 mM dNTPs; 1 µl 2.5 U RevertAid™ M-MulRev Transcriptase (MBI Fermentas) and 6 µl of RNA solution. The reaction was performed incubating at 42 °C for 15 min, at 99 °C for 5 min and at 5 °C for 5 min.

The DNA amplification mixture contained (for one sample): 7 µl c DNA, 58 µl PCR water, 4 µl 2 mM dNTPmix, 1 µl upstream primer, 8 µl 10 × PCR buffer, 4 µl $MgCl_2$, 0.5 µl Taq DNA polymerase (MBI Fermentas). Reaction mixtures were incubated at 94 °C for 1.5 min (for the first step), 23 cycles of 55 °C for 2 min, 72 °C for 3 min, 94 °C for 1 min, the final with annealing and extension at 55 °C for 2 min and at 72 °C for 10 min.

The resulting PCR products were analysed by electrophoresis through 5% polyacrylamide gel, stained with ethidium bromide, and DNA bands were visualized using a UV transilluminator. PhiX174 RFI DNA *Hae*III digest (fragment sizes from top to bottom: 1353, 1078, 872, 603, 310, 281, 271, 234, 194, 118, 72 bp) was used as a DNA fragment size standard.

RESULTS AND DISCUSSION

ArMV was detected in species of 12 ornamental plant genera representing eight families. The virus was isolated and identified from naturally infected ornamental plants showing definite symptoms. These symptoms may be not specific only to ArMV, because many ornamental plants were naturally infected by a mixed viral infection.

Apiaceae Lindl.

Eryngium L. Infected plants were stunted, leaves slightly distorted, showing light green spots. Previously, two viruses, tobacco rattle virus (TRV) and tomato ringspot virus (ToRSV) had been identified in this plant [13].

Araceae Juss.

Arum L. Infected plants were smaller than normal. Leaves showed yellow spots and streaks.

Dieffenbachia sp. Infected plants showed a slight leaf distortion, mosaic of irregular spots.

Asteraceae Dumort.

Dahlia Cav. Leaves of infected plants were slightly distorted, with symptoms of vein clearing. Flowers were smaller than normal. Dahlia mosaic virus (DaMV) and tobacco necrosis virus (TNV) had earlier been identified from this dahlia isolate [13].

Liatris Gaertn. Ex Schreb. Leaves of infected plants showed light green spots, mosaic. Previously ToRSV had been identified [13].

Caryophylaceae Juss.

Lychnis L. Symptoms of infected L. *chalcedonica* plants were expressed by plant stunting and chlorosis, light mottling on leaves. Flowers were poorly developed, smaller than normal. Plants were infected also with ToRSV and TRV [14].

Fumariaceae DC

Dicentra Bernh. Virus was isolated from *D. formosa* plants showing symptoms of plant chlorosis and stunting, yellow ringspots on leaves. The host plant had was infected with ToRSV, tomato spotted wilt virus (TSWV) and TRV [13].

Hyacinthaceae Batsch ex Borkh.

Camassia Lindl. Virus was isolated from *C. cusickii* and *C. quamash* exhibiting light green streaks and pinpoint necrotic lesions on leaves.

Muscari Mill. Plants were smaller than normal, leaves were distorted, with chlorotic spots and streaks which later in season turned to necrosis. Virus was present in mixed infection with muscari mosaic virus (MMV), TSWV and TRV [13].

Iridaceae Juss.

Crocus L. Infected *C. chrysanthus* and *C. vernus* plants were smaller than normal, leaves distorted, having indented margins, displaying chlorotic streaks and necrotic spots. Mixed infection with TRV, TSWV, *Tobacco necrosis necrovirus* (TNV) was established [13].

Iris L. Virus was isolated from infected *I. hollandica* plants. Leaf symptoms appeared as pinpoint spots on tips and a light green mosaic pattern which may be more conspicuous on the flower bud sheaths. Virus was present in mixed infection with IMMV and ToRSV [15].

Polemoniaceae Juss.

Phlox L. Leaves of infected *P. paniculata* plants were variegated with light green ringspots. Later vein clearing appeared and formed netting-like mosaic. Virus was present in mixed infection with cucumber mosaic virus (CMV), alfalfa mosaic virus (AMV), TSWV [16].

Using mechanical inoculation, a group of test-plants consisting of 12 species representing five families was inoculated

with virus isolates from naturally infected ornamental plants. Test-plants and the results of their reaction to inoculation are presented in Table. Virus isolates induced local reactions (chlorotic, necrotic local lesions, spots) in test-plant representatives of the *Amaranthaceae*, *Asteraceae*, *Fabaceae*, *Chenopodiaceae, Cucurbitaceae* families; local necrotic ringspots in *Nicotiana* species and no local reaction in *L. esculentum*. Various systemic symptoms were induced in all inoculated test-plants (Fig. 1).

Table. **Reactions of test-plants inoculated with virus isolated from ornamental plants**

Test-plant	Symptoms
Amaranthaceae Juss.	
Amaranthus caudatus L.	L : NLL; S : Cl, NSp, LeDis
Celosia argentea L.	L : LL; S : LeMo, LeDis
Fabaceae Lindl.	
Phaseolus vulgaris	L : ClSp, NDot; S : MrbPat, FlN
Chenopodiaceae Vent.	
Atriplex hortensis L.	L : LL; S : NDot, VC, LeDis, TDis
Chenopodium amaranticolor Coste et Reyn	L : YDot; S : ClMo with N, LeDis
C. ambrosioides L.	L : SmBrLL; S : LeMo, TDis
C. hybridum	L : LL; S : LeNDot, LeDis
C. quinoa Willd.	L : ClLL S : YDot, LeDis
Cucurbitaceae Juss.	
Cucumis sativus L.	L : ClSp; S : LeMo, TDis
Solanaceae Juss.	
Lycopersicon esculentum Mill.	S : LiGrMo
Nicotiana rustica L.	L : GRiSp; S : YSp, NRi
N. tabacum L. 'Samsun'	L : SmNRi; S : Cl, NRi

Abbreviations: L – local reaction; S – systemic reaction; Br – brown; Cl – chlorosis, chlorotic; Dif – difussional; Dis – distortion; Dot – dots; F – flower, G – grey; Gr – green; LL – local lesions; Le – leaf; Li – light; M – mosaic; Mi – mild; Mo – mottling; MrbPat – marble pattern; N – necrosis, necrotic; R – rolling; Ri – rings; RiSp – ringspot; Sm – small; Sp – spots; Str – streaks; T – top; VC – vein clearing; Y – yellow.

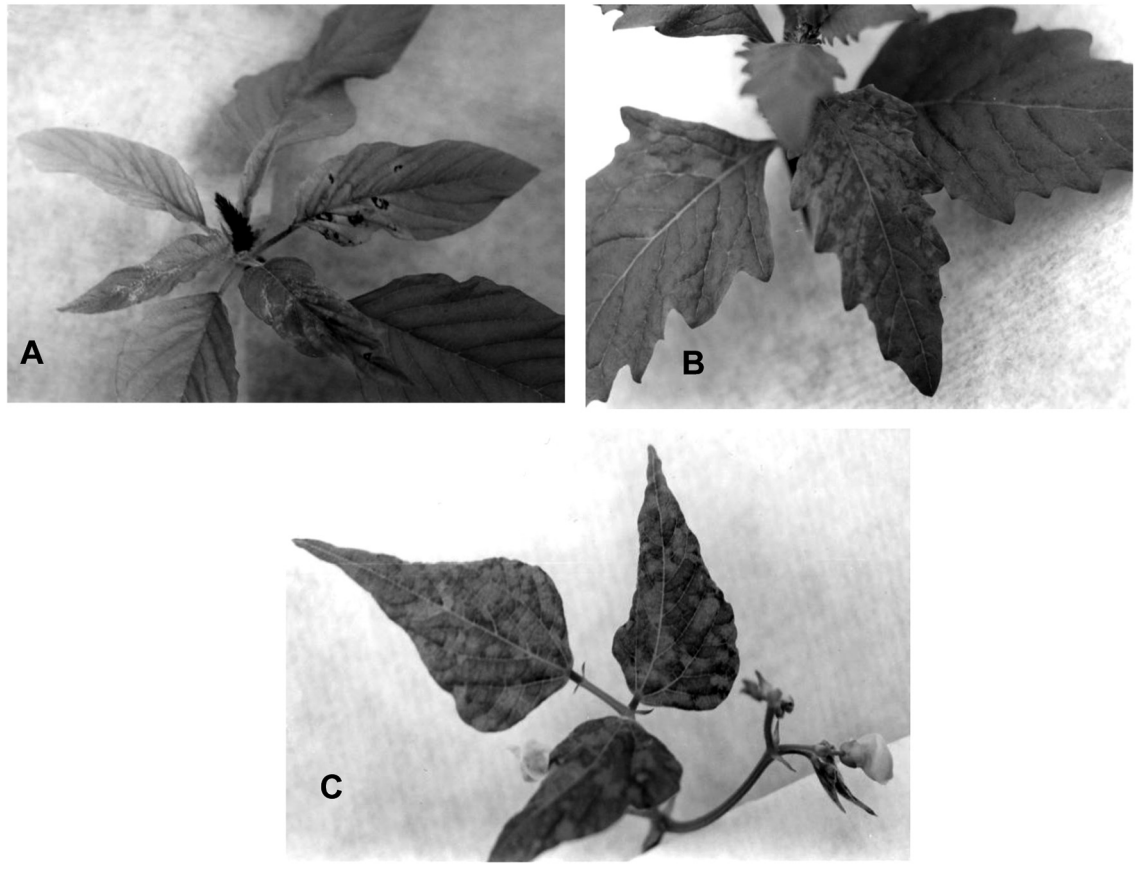

Fig. 1. Systemic symptoms induced by ArMV in test-plants: A – *Amaranthus caudatus*, B – *Chenopodium ambrosioides*, C – *Phaseolus vulgaris*

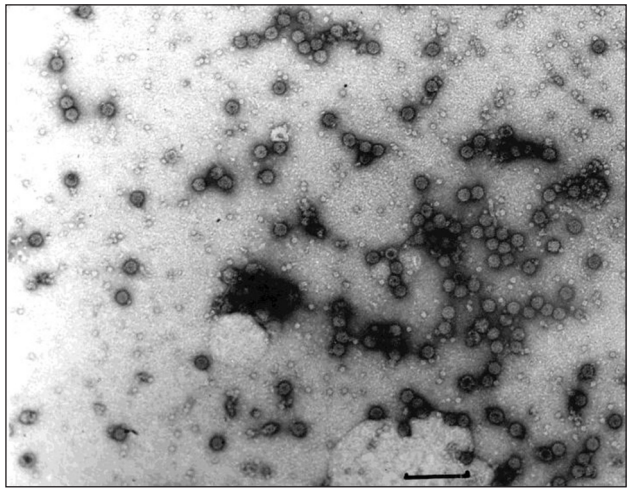

Fig. 2. Electron micrograph of ArMV particles negatively stained with uranyl acetate. Bar represents 100 nm

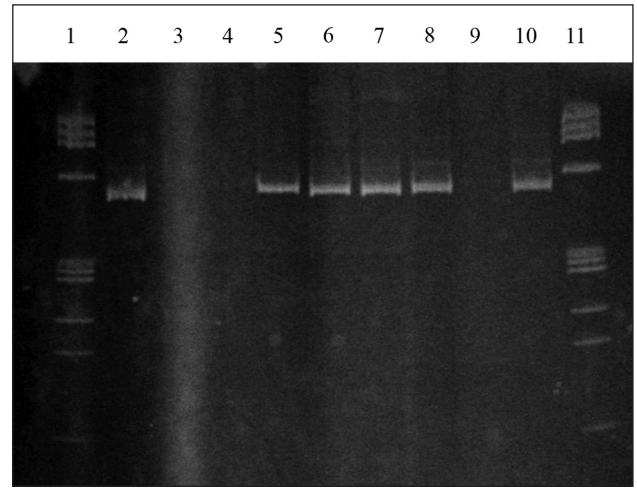

Fig. 3. Electrophoresis of RT-PCR products of amplified DNA fragments from ArMV isolates. Lanes: *1, 11* – DNA size standard PhiX174 RFI DNA *Hae*III digest, fragment sizes (from top to bottom): 1353, 1078, 872, 603, 310, 281, 271, 234, 194, 118, 72 bp., *2, 3, 4* – isolates from *Camassia*, *5* – isolate from *Iris*, *6* – isolate from *Lychnis*, *7* – isolate from *Phlox*, *8* – isolate from *Crocus*, *9* – K^-, *10* – K^+ (isolate from *Hyacinthus*). Size of product – 420 bp

EM examination of naturally infected host plants and inoculated test-plant tissue revealed the presence of isometric particles 30 nm in diameter (Fig. 2). Such morphology of particles is characteristic of ArMV [1–4].

Symptomatic host plants and inoculated test-plants were tested for ArMV infection by DAS-ELISA. The reaction was considered positive when absorbance at 405 nm was more than twice the mean of healthy (negative) controls. A positive reaction confirming ArMV infection was obtained with isolates (is.) from ornamental plants: *Camassia* (six is.), *Crocus* (four is.), *Dicentra* (two is.), *Arum*, *Dahlia, Dieffenbachia*, *Eryngium*, *Liatris*, *Lychnis*, *Muscari*, *Iris*, *Phlox* (one is. each) (data not shown).

The identification of test-plant reactions, EM and DAS-ELISA results was verified in RT-PCR using virus isolates from *Camassia*, *Iris*, *Lychnis*, *Phlox*, *Crocus* as samples. Total RNA was extracted from frozen leaf tissue of infected test-plants. Leaf tissue from a healthy *N. rustica* plant was used as a negative control (K^-). ArMV from *Hyacinthus* was used as a positive control (K^+). Products specific for ArMV PCR were obtained with isolates from *Camassia, Iris, Lychnis, Phlox, Crocus* and the positive control, but not with the negative control and two isolates from *Camassia*. Specific bands in polyacrylamide gel of the analysed products after electrophoresis at a position corresponding to the expected size of amplification product of 420 bp were obtained, confirming ArMV identity (Fig. 3).

Based on the data of test-plant reactions, particle morphology, positive reaction with ArMV-specific antiserum in the DAS-ELISA test, RT-PCR results it was ascertained that one representative of mixed viral infections causing diseases in ornamental plants was ArMV. Application of modern virological methods (DAS-ELISA and RT-PCR), together with classical biological methods, increased the reliability of virus identification in complex infections. Recently, the ArMV virus has been described as an agent of viral disease in *Hyacinthus* and *Lycopersicon esculentum* in Lithuania [7, 8].

This study, presenting data on ArMV identification, extended the natural host range of this virus, reported from different countries [1, 2, 6], revealing new for ArMV ornamental host plants: *Arum*, *Camassia*, *Dahlia*, *Dicentra*, *Dieffenbachia*, *Eryngium, Iris*, *Liatris*, *Lychnis*, *Muscari*, and *Phlox*. The above results confirm that ArMV is widespread on perennial, bulb and corm ornamental plants and, mostly being present in mixed infections with other viruses, causes harmful diseases, retards plant growth, damages some or all parts of a plant, distorts its standard properties, reduces the aesthetic quality and marketability. Virus-infected plants are more susceptible to fungal and bacterial pathogens which lead to premature death.

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VAISTUČIO MOZAIKOS VIRUSAS DEKORATYVINIUOSE AUGALUOSE

Santrauka

Vaistučio mozaikos virusas (*arabis mosaic virus*, ArMV) pažeidžia platų augalų spektrą, tarp jų ir dekoratyvinius augalus. Pritaikius augalų indikatorių, elektroninės mikroskopijos, DAS-ELISA metodus ArMV buvo rastas dekoratyviniuose *Arum* L., *Camassia* Lindl., *Crocus* L., *Dahlia* Cav., *Dicentra* Bernh., *Dieffenbachia* sp., *Eryngium* L., *Liatris* Gaertn. Ex Schreb., *Lychnis* L., *Muscari* Mill., *Iris* L. and *Phlox* L. genčių, priklausančių aštuonioms botaninėms šeimoms, augaluose. Natūraliai užsikrėtusiuose augaluose šis virusas buvo tarp kitų mišrios infekcijos virusų. ArMV identifikacija 5 rūšių dekoratyviniuose augaluose buvo patvirtinta AT-PGR metodu.