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Identification and subgrouping of cucumber mosaic virus isolate infecting New Guinea impatiens (*Impatiens hawkeri* Bull) in Serbia

Identifikacija i svrstavanje u podgrupe izolata virusa mozaika krastavca infektivnog za New Guinea impatiens (*Impatiens hawkeri* Bull) u Srbiji

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ABSTRACT

New Guinea impatiens (*Impatiens hawkeri*) is a species of flowering plant in the family Balsaminaceae that is popular as a bedding and pot plant. In May 2020, impatiens plants showing chlorotic and necrotic concentric rings, mosaic, leaf malformation and filiformism were noticed in a greenhouse in Rača Kragujevačka, Šumadija District, Serbia. Collected leaves were serologically tested for impatiens necrotic spot orthotospovirus (INSV, *Orthotospovirus impatiensnecromaculae*), tomato spotted wilt orthotospovirus (TSWV, *Orthotospovirus tomatomaculae*), cucumber mosaic virus (CMV), turnip mosaic virus (TuMV), and tobacco mosaic virus (TMV). Most of the tested samples with chlorotic and necrotic concentric rings (81.81%) were positive for TSWV, while CMV was detected in four samples with mosaic, leaf malformation and filiformism. CMV ELISA-positive sample (106-20) was mechanically inoculated to five plants of *Chenopodium quinoa*, *Nicotiana debneyii*, and *N. glutinosa*. Local chlorotic lesions on *C. quinoa* and severe mosaic and leaf malformation on *N. debneyii* and *N. glutinosa* were observed 5 and 13 days post-inoculation, respectively. PCR fragments of all five genes were digested by following restriction enzymes: *Hind*III, *Sac*II (1a gene), *Mlu*I (2a gene), *Stu*I, *Sal*I (2b gene), *Bae*I (MP gene), *Sfc*I and *Hae*III (CP gene). Differentiation of CMV subgroups was conducted based on the restriction patterns obtained by *in situ* RT-PCR-RFLP analyses and isolate 106-20 was classified into subgroup IA with haplotype IA; IA, IA; IA, IA. This study reports for the first time the presence of CMV on impatiens in Serbia and its occurrence on this widely cultivated ornamental in our country, which may have a destructive impact on its production.

Keywords: New Guinea impatiens, CMV, bioassay, serology, RT-PCR-RFLP

SAŽETAK

New Guinea impatiens (*Impatiens hawkeri*) ie veoma popularna ukrasna baštenska i saksijska biljka iz familije Balsaminaceae. U maju 2020. godine u jednom stakleniku na lokalitetu Rača Kragujevačka (Šumadijski okrug, Srbija) primećene su biljke impatiensa sa simptomima u vidu hlorotičnih i nekrotičnih koncentričnih prstenova, mozaika, deformacija listova i nitavosti. Sakupljeno lišće je serološki testirano na prisustvo virusa nekrotične pegavosti impatiensa (impatiens necrotic spot orthotospovirus, INSV, *Orthotospovirus impatiensnecromaculae*), virusa bronzavosti paradajza (tomato spotted wilt orthotospovirus, TSWV, *Orthotospovirus tomatomaculae*), virusa mozaika krastavca (cucumber mosaic virus, CMV), virusa mozaika postrne repe (turnip mosaic virus, TuMV) i virusa mozaika duvana (tobacco mosaic virus, TMV). U većini testiranih uzoraka (81.81%) sa simptomima hlorotičnih i nekrotičnih koncentričnih prstenova dokazano je prisustvo TSWV, dok je CMV dokazan u četiri uzorka sa simptomima mozaika, deformacija i nitavosti lista. CMV ELISA-pozitivan uzorak (106-20) je mehanički inokulisan na po pet biljaka *Chenopodium quinoa*, *Nicotiana debneyii* i *N. glutinosa*. Pojava lokalnih hlorotičnih pega na *C. quinoa* i mozaika i deformacije lišća na *N. debneyii* i *N. glutinosa* zabeležena je 5, odnosno 13 dana nakon inokulacije. Digestija amplifikovanih PCR fragmenta svih pet gena obavljena je primenom restrikcionih enzima: *Hind*III, *Sac*II (1a gen), *Mlu*I (2a gen), *Stu*I, *Sal*I (2b gen), *Bae*I (MP gen), *Sfc*I i *Hae*III (CP gen). Na osnovu dobijenih restrikcionih profila primenom *in situ* RT-PCR-RFLP analize, izolat 106-20 pripada podgrupi IA sa haplotipom IA; IA, IA; IA, IA. U ovom radu prvi put je dokazano prisustvo CMV na impatiensu u Srbiji čija pojava može imati destruktivne posledice na proizvodnju ove veoma popularne ukrasne biljke u našoj zemlji.

Ključne reči: New Guinea impatiens, CMV, biotest, serologija, RT-PCR-RFLP

INTRODUCTION

The ornamental horticulture industry is a profitable sector of plant production that is constantly expanding. In 2021, the value of flower trade at the largest flower exchange in the world, Royal FloraHolland, reached 5.6 billion EUR (Diningsih et al., 2020). Ornamental plants are also very popular in Serbia, and their cultivation has increased significantly in recent years. Overall, the Serbian ornamental plant trade value in 2022 was estimated at 31.3 million EUR, of which exports were 6.61 million EUR and imports were about 24.7 million EUR (www.pks.rs). Impatiens is a genus of more than 1000 species of flowering plants in the family Balsaminaceae, which are among the most important ornamental plants and are very popular as bedding and pot plants. It is well-known that many diseases cause problems in the cultivation of impatiens, but plant viruses are the greatest obstacle to global production. Impatiens may be hosts for quite a lot of viruses including tomato spotted wilt orthotospovirus (TSWV) and impatiens necrotic spot orthotospovirus (INSV) of the species *Orthotospovirus tomatomaculae* and *Orthotospovirus impatiensnecromaculae* respectevely, *Cucumber mosaic virus* (CMV), turnip mosaic virus (TuMV), tobacco mosaic virus (TMV), helenium virus S (HVS), tobacco streak virus (TSV), and tobacco ringspot virus (TRSV), of which INSV and TSWV are the main constraints to its production in various parts of the world (de Ávila et al., 1992; Daughtrey, 1996; Elliott et al., 2009; Diningsih et al., 2020).

However, recent reports of impatiens viral diseases indicate an increasing presence of aphid-borne viruses. Many authors state that CMV causes significant damage in nurseries during the seedling production season (Hu and Chang, 2006; Choi et al., 2015; Diningsih et al., 2020, 2022).

CMV, a type member of the genus *Cucumovirus* (family *Bromoviridae*), is one of the most important viruses of many vegetables and horticultural crops causing significant economic losses. It has a worldwide distribution and a very wide host range including more than 1,300 species (García-Arenal and Palukaitis, 2008). The virus is primarily transmitted by about 80 aphid species in a non-persistent manner. CMV is also transmitted through the seed of some hosts, mechanically or by *Cuscuta* plants (Palukaitis et al., 1992; García-Arenal and Palukaitis, 2008).

CMV has icosahedral particles of 28-30 nm in diameter, containing a single-stranded, positive-sense RNA. The genome consists of three genomic RNAs (RNA1-RNA3) and two subgenomic, RNA4 and RNA4A. RNAs 1 and 2 are encapsulated separately, whereas RNA-3 and subgenomic RNA-4 are encapsidated within the same particle. RNA1 and RNA2 encode proteins 1a and 2a, respectively, which are involved in virus replication. RNA2 also encodes protein 2b, an inhibitor of plant defence responses that is also involved in virus movement and symptom severity. Bicistronic RNA3 encodes 3a and 3b proteins corresponding to the movement protein (MP) and coat protein (CP), respectively. MP is responsible for the cell-to-cell and vascular movement of the virus and in aphid-mediated transmission, whereas CP is involved in encapsidation, systemic movement, host range and transmission by aphids (Palukaitis et al., 1992; Roossinck, 2002; Palukaitis and García-Arenal, 2003; Jacquemond, 2012).

The symptoms caused by CMV vary depending on the host, time of infection, environmental conditions and aggressiveness of the isolate. Most CMV strains cause systemic infections, but in some cases, they can be asymptomatic (Palukaitis and García-Arenal, 2003; Jacquemond, 2012). However, common symptoms of CMV in ornamental plants include mosaic, mottling, necrosis, leaf distortion, stunting, color breakage, and flower malformation (Duarte et al., 2013; Ashfaq et al., 2017).

CMV isolates are divided in two main subgroups named subgroups I and II based on biological, serological and nucleotide sequence properties. Subgroup I is further divided into subgroups IA and IB. Isolates of subgroups IA and II are distributed throughout the world, whereas isolates of subgroup IB mainly originate from East Asia. However, several IB isolates were also found in the Mediterranean region, California, Brazil, and Australia

(Palukaitis et al., 1992; Palukaitis and García-Arenal, 2003; Jacquemond, 2012; Giakountis et al., 2018).

In Serbia, CMV has had devastating effects on the production of several economically important crops, including tomato (Nikolić et al., 2018; Stanković et al., 2021), pepper (Milošević et al., 2017), tobacco (Stanković et al., 2011), cucurbits (Vučurović et al., 2011, 2012; Milojević et al., 2013b) and safflower (Milošević et al., 2020). In addition, CMV has been detected on various ornamental plants such as *Peperomia tuisana* (Milojević et al., 2013a), *Tulipa* sp. (Milojević et al., 2014), *Calendula officinalis* (Milošević et al., 2015), *Wisteria sinensis* (Milojević et al., 2016), but there is no data on the occurrence of CMV on impatiens.

In May 2020, New Guinea impatiens plants with chlorotic and necrotic concentric rings, mosaic, leaf malformation, and filiformism were noticed in a greenhouse in the Rača Kragujevačka locality. Given that impatiens is one of the most popular ornamental plants with intensive and growing production in Serbia, the objective of this research was to identify the pathogen responsible for these severe symptoms. Here we report the biological, serological, and molecular identification of CMV infecting impatiens as a new virus-host for Serbia. Furthermore, RT-PCR-RFLP analysis of five CMV genomic regions of a Serbian impatiens isolate reveals its belonging to the IA subgroup.

MATERIAL AND METHODS

Plant material collection and serological testing

In May 2020, impatiens plants with virus-like symptoms were observed in a greenhouse in Rača Kragujevačka locality (Šumadija District, Serbia). Twenty-two samples of symptomatic leaves were collected and transported to the laboratory for testing using a double-antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA).

The DAS-ELISA test was performed using commercial antisera (Bioreba, AG, Reinach, Switzerland) against the most common impatiens viruses including INSV, TSWV, CMV, TuMV, and TMV. According to the manufacturer's instructions, plant tissue samples were ground with extraction buffer using a mortar and pestle in a ratio of 1:10 (wt/vol). After incubation with *p*-nitrophenyl phosphate (Sigma-Aldrich, St. Louis, MO) at 23 °C for 1-2 hours in the dark, absorbance at 405 nm was determined with an ELISA microplate reader (DAS srl, Italy). Samples were considered positive if their average optical density (OD) was two times higher than the average OD of the negative control. Commercial positive controls for the above-mentioned viruses, as well as negative controls (Bioreba) were included in each ELISA test.

RNA extraction and RT-PCR amplification

Total RNAs of a selected Serbian CMV isolate 106-20 from symptomatic impatiens were extracted from 100 mg of freeze-dried leaves with the RNeasy Plant Mini Kit (Qiagen GmbH, Germany). In order to obtain amplicons of all five CMV genes, five sets of primers specific for different CMV genomic regions were used (Table 1) (Finetti Sialer et al., 1999; Milojević et al., 2012; Zečević et al., 2023). RT-PCR was performed with the One-Step RT-PCR kit (Qiagen) using Total RNAs of Serbian CMV isolate from tomato (GenBank Accession Number KT270490) and PCR mix with RNase-free water as a positive and negative control, respectively.

One-step RT-PCR was performed in a thermal cycler (Applied Biosystem 2720 Thermal Cycler), and the reaction mixture contained 5 µl 5x Qiagen OneStep RT-PCR Buffer, 1 µl 400 µM dNTPs, and 1 µl extracted RNAs. The final primer concentrations were as follows: 1.5 µM RNA1a-fwd, RV11 and CMVCPrev, 3 µM 2brev and CMV3a-rev, 4.5 µM RNA1a-rev and CMVCPfwd, 6 µM RW8, 2bfwd and CMVMP3. The volume of the mixture was adjusted to 25 µl with RNase-free water. Reverse transcription was performed at 50 °C for 30 min, followed by an initial PCR denaturation step at 95 °C for 15 min, a three-step cycle (denaturation, annealing, and extension) using conditions and number of cycles depending on the used primers (Table 1), and a final extension at 72 °C for 10 min.

Table 1. Primers used for RT-PCR amplification of different genes of CMV

Gene	Primer name	Primer sequence (5′ to 3′)	Cycling (temperature/time)			No. of cycles	Amplicon size (bp)
			Denaturation	Annealing	Extension		
1a	RNA1a-fwd	TGGTAGCCTCCCACGGCGATA	94°C/60 s	51°C/60 s	72°C/60 s	35	1198
	RNA1a-rev	GAYTGCATRGACATACCATT					
2a	RV11	GTTTATTTACAAGAGCGTACGG	94°C/30 s	53°C/60 s	72°C/60 s	35	650
	RW8	GGTTCGAARRWATAACCGGG					
2b	2bfwd	TTTGTTGAYMGRYTGAAGTTT	94°C/60 s	46°C/60 s	72°C/60 s	5	804
	2brev	CCTTCCGAAGAAAYCYAGGA		50°C/60 s		30	
MP	CMVMP3	GAGTGYGACCTAGGYCGRCATCA	94°C/60 s	60°C/60 s	72°C/60 s	35	728
	CMV3a-rev	CTAARGACCGTTAACCACCTGC					
CP	CMVCPfwd	TGCTTCTCCRCGARWTTGCGT	94°C/60 s	52°C/60 s	72°C/60 s	35	871
	CMVCPrev	CGTAGCTGGATGGACAACCCG					

The size of the amplified products was determined after separation on a 1% agarose gel in TBE buffer and incubation in ethidium bromide (EB) solutions with UV transilluminator.

Restriction enzyme digestion of the amplified amplicons

The amplified fragments of all five genes were digested using restriction fragment length polymorphism (RFLP) with the following restriction enzymes: *Hind*III, *Sac*II (1a gene), *Mlu*I (2a gene), *Stu*I, *Sal*I (2b gene), *Bae*I (MP gene), *Sfc*I and *Hae*III (CP gene), which were able to distinguish subgroups of CMV based on the obtained characteristic restriction patterns (Zečević et al., 2023). Ten µl of each of the five amplified PCR products was diluted in RNase-free water in ratios from 1:9 to 3:7 (depending on the yield of the PCR product) and digested with 0.1 µl of restriction enzyme (10U/µl), according to the manufacturer's instructions. Digested RT-PCR products were run in 1.2% agarose gel in TBE buffer, stained with ethidium bromide, and visualized on a UV transilluminator.

Mechanical transmission

Crude sap extracted from a symptomatic impatiens sample (106-20) was mechanically inoculated onto five plants each of *Chenopodium quinoa*, *Nicotiana debneyii*, and *N. glutinosa*. Mechanical inoculation was performed with 0.01 M phosphate buffer (pH 7) and silicon carbide abrasive when the test plants were at the 2 to 3 true leaf stage. The inoculated test plants were kept in a greenhouse at a temperature between 22 and 25 °C and regularly examined for the development of symptoms during the period of up to four weeks after inoculation. The test plants were assayed by DAS-ELISA to confirm the presence of CMV.

RESULTS AND DISCUSSION

Symptomatology and virus detection

In May 2020, impatiens New Guinea plants with virus-like symptoms including chlorotic and necrotic concentric rings (Figure 1a), mosaic, leaf malformation and filiformism (Figure 1b) were observed in a greenhouse in the Rača Kragujevačka locality (Šumadija District). Disease incidence was estimated at 20% of the total number of 3000 plants grown. Symptomatic impatiens plants were collected and tested for the presence of the five most common impatiens viruses (INSV, TSWV, CMV, TuMV and TMV) utilizing DAS-ELISA kits.

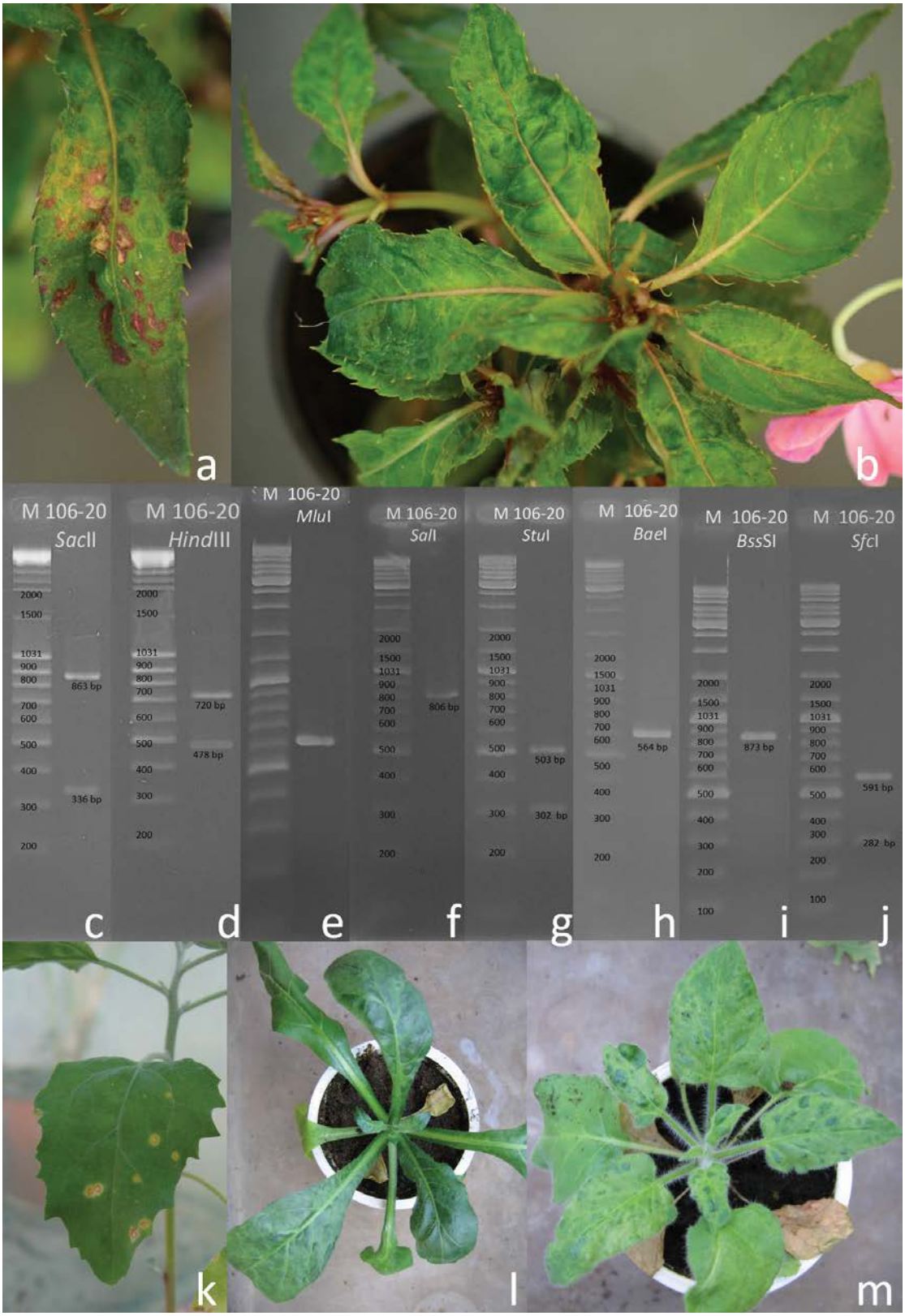

Figure 1. Symptoms of viral infections on impatiens plants (a and b): a – chlorotic and necrotic concentric rings (TSWV), b – mosaic, leaf malformation and filiformism (CMV); Restriction analysis of RT-PCR amplified products of Serbian CMV isolate 106-20 from impatiens using different restriction enzymes (c-j): c – *Sac*II, d – *Hind*III, e – *Sal*I, f – *Stu*I, g – *Mlu*I, h – *Bae*I, i – *Hae*III, j – *Sfc*I. Lane M – MassRuler™DNA ladder, Mix (Fermentas); Symptoms on inoculated plants with Serbian CMV isolate 106-20 from impatiens (k-m): k – local chlorotic lesions on the inoculated leaves of *C. quinoa*, l and m – severe mosaic and leaf malformation on the upper leaves of *N. debneyii* and *N. glutinosa*

Out of 22 samples collected, the majority of impatiens plants with symptoms of chlorotic and necrotic concentric rings (81.81%) were positive for TSWV. However, CMV was detected in four samples with mosaic, leaf malformation and filiformism. None of the tested samples reacted with INSV, TuMV or TMV antisera.

Impatiens plants infected with CMV most often show symptoms in the form of mosaic, leaf distortion and dwarfing (Hu and Chang, 2006; Choi et al., 2015; Diningsih et al., 2020), some of which were also noted in these studies.

RT-PCR and restriction enzyme analysis

The partial or complete fragment of all five CMV genes of a Serbian impatiens isolate 106-20 was successfully amplified using RT-PCR with specific primers. The primer pairs used in this study for the 1a, 2a, 2b, MP and CP genes amplified specific target cDNA fragments of 1198, 637, 805, 729 and 873 bp, respectively. The positive control also yielded fragments of the expected size, while no amplification products were recorded in the negative controls.

An accurate, simple and fast RFLP method was used to determine CMV subgroup and to classify the haplotype of the selected CMV isolate 106-20. PCR amplified fragments of all five genes were digested using eight restriction enzymes (Figure 1c to 1j), as suggested by Zečević et al. (2023) because this enzyme combination can be used for the appropriate subgrouping of CMV isolates. Based on restriction patterns the isolate 106-20 from impatiens belongs to subgroup IA.

*Sac*II digestion of the amplified 1a gene fragment revealed that 106-20 isolate shared a pattern that is typical of CMV subgroup I, whereas digestion with the enzyme *Hind*III suggested that the isolate belonged to subgroup IA. The restriction enzyme *Mlu*I, did not cut the RT-PCR product of the 2a gene of Serbian isolate, indicating that it belongs to subgroup IA. After enzyme digestion of the amplified 2b gene fragment with restriction enzyme *Sal*I, impatiens isolate 106-20 showed restriction patterns that is typical of subgroup I isolate, and digestion with the enzyme *Stu*I produced a pattern typical of subgroup IA. The RT-PCR product of MP gene of impatiens isolate was successfully digested by the enzyme *Bae*I and according to digestion result it was grouped into subgroup IA. Digestion of amplified CP gene fragment with *Hae*III revealed the restriction pattern typical of subgroup I, and after digestion with the enzyme, *Sfc*I showed the restriction pattern of subgroup IA. The genetic characterization of the Serbian CMV impatiens isolates based on specific restriction patterns of the amplified PCR products of all five genes revealed that the selected isolate has the haplotype IA; IA, IA; IA, IA.

According to the previous analysis of the genetic structure of CMV population in Serbia, most of the isolates from Serbia belong to subgroup IA (Petrović et al., 2010; Vučurović et al., 2011, 2012; Milojević et al., 2013a; Stanković et al., 2021; Zečević et al., 2023), which is the dominant and largest subgroup in the CMV population in the world (Roossinck, 2002; Bonnet et al., 2005; García-Arenal and Palukaitis, 2008; Jacquemond, 2012). In Serbia, isolates belonging to CMV subgroup II were also detected, although their percentage in the natural population of CMV in Serbia is low (Milošević et al., 2015, 2020; Stanković et al., 2021; Zečević et al., 2023). As well as isolates from subgroup IA, the isolates from subgroup II are distributed worldwide, but probably due to their less pronounced symptoms are found less frequently than those from subgroup IA (Xu et al., 1999; Tian et al., 2009).

The majority of CMV research has focused on characterization based on the CP gene, as it is the most conservative and informative region of the CMV virus (Roossinck, 2002; Moury et al, 2004; Jacquemond, 2012), but recent studies have shown the importance of genetic characterization of CMV isolates based on each of the three RNAs because they have evolved separately (Roossinck, 2002) and because recombinant and reassortant isolates represent over 11% of the population of this virus (García-Arenal and Palukaitis, 2008). Since genetic exchange and recombination may

also be important factors affecting the evolution of the virus, analysis of all five genes of CMV is necessary for understanding the genetic structure of virus populations and their evolutionary mechanisms, which could be an important aspect of managing viral diseases by developing appropriate and efficient control strategies.

Bioassay

In order to biologically characterize the selected CMV isolate 106-20, the crude sap extract of symptomatic New Guinea impatiens was successfully mechanically transmitted onto *Chenopodium quinoa*, *Nicotiana debneyii* and *N. glutinosa*. All inoculated plants developed characteristic CMV symptoms as previously reported by Milojević et al. (2013b, 2014), Choi et al. (2015) and Diningsih et al. (2020). Local chlorotic lesions on the inoculated leaves of *C. quinoa* and severe mosaic and leaf malformation on the upper leaves of *N. debneyii* and *N. glutinosa* were observed 5 and 13 days post-inoculation, respectively (Figure 1k to 1m). CMV infection was confirmed in all mechanically inoculated plants using the DAS-ELISA assay.

CONCLUSIONS

To our knowledge, this is the first report of CMV infecting impatiens in Serbia. This study reveals that Serbian CMV isolate showing mosaic, leaf malformation and filiformism on impatiens belongs to subgroup IA with the haplotype IA; IA, IA; IA, IA. As a new CMV host in Serbia, impatiens represent a potential virus reservoir and an additional source of inoculum. The occurrence of CMV on impatiens, in addition to other viruses, may have a destructive impact on its production since it is widely grown in our country. Since impatiens are frequently grown together with other ornamental hosts of CMV, and the virus has a wide host range including a variety of ornamentals, this is a very important discovery that poses a serious threat to the ornamental industry in Serbia.

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REFERENCES

Ashfaq, M., Saleem, A., Waqas, M., Mukhtar, T. (2017) Natural occurrence and host range studies of *Cucumber Mosaic Virus* (CMV) infecting ornamental species in Rawalpindi-Islamabad area of Pakistan. Philippine Agricultural Scientist, 100 (1), 55-61.

Bonnet, J., Fraile, A., Sacristán, S., Malpica, J. M., García-Arenal, F. (2005) Role of recombination in the evolution of natural populations of *Cucumber mosaic virus*, a tripartite RNA plant virus. Virology, 332 (1), 359-368. DOI: https://doi.org/10.1016/j.virol.2004.11.017

Daughtrey, M. L. (1996) Detection and identification of tospoviruses in greenhouses. Acta Horticulture, 431, 90-98. DOI: https://doi.org/10.17660/ActaHortic.1996.431.8

de Ávila, A. C, de Haan, P., Kitajima, E. W., Kormelink, R., de Resende, R. O., Goldbach, R. W., Peters, D. (1992) Characterisation of a distinct isolate of *Tomato spotted wilt virus* (TSWV) from *Impatiens* sp. in the Netherlands. Journal of Phytopathology, 134, 133-151. DOI: https://doi.org/10.1111/j.1439-0434.1992.tb01222.x

Diningsih, E., Yanda, R. F., Rahardjo, I. B., Wegadara, M., Thamrin, M. (2022) Identifying *Cucumber mosaic virus* (*Cucumovirus*, *Bromoviridae*) isolates on *Impatiens* (*Balsaminaceae*): a preliminary study using molecular characterization. Acta Horticulture, 1334, 193-198. DOI: https://doi.org/10.17660/ActaHortic.2022.1334.23

Diningsih, E., Yanda, R. P., Marwoto, B. (2020) Identification of the main virus infecting impatiens (*Impatiens balsamina* L.). IOP Conf. Series: Earth and Environmental Science 468, 012035. DOI: https://doi.org/10.1088/1755-1315/468/1/012035

Duarte, L. M. L., Rivas, E. B., Harakava, R., Veauvy, M. C. D., Alexandre, M. A. V. (2013) Genealogy of *Cucumber mosaic virus* Isolated from Ornamental Species. American Journal of Plant Sciences, 4 (5), 1081-1087. DOI: https://doi.org/10.4236/ajps.2013.45134

Elliott, D. R., Lebas, B. S., Ochoa-Corona, F. M., Tang, J., Alexander, B. J. (2009) Investigation of *Impatiens necrotic spot virus* outbreaks in New Zealand. Australasian Plant Pathology, 38, 490-495. DOI: https://doi.org/10.1071/AP09031

Finetti Sialer, M. M., Cillo, F., Barbarossa, L., Gallitelli, D. (1999) Differentiation of *Cucumber mosaic virus* subgroups by RT-PCR-RFLP. Journal of Plant Pathology, 81 (2), 145-148.

García-Arenal, F., Palukaitis, P. (2008) *Cucumber mosaic virus*. In: Mahy, B. W. J., van Regenmortel, M. H. V. eds. Desk Encyclopedia of Plant and Fungal Virology. Amsterdam: Elsevier, pp. 171-176. DOI: https://doi.org/10.1016/B978-012374410-4.00640-3

Giakountis, A., Tsarmpopoulos, I., Chatzivassiliou, E. K. (2018) *Cucumber mosaic virus* Isolates from Greek Legumes are Associated with Satellite RNAs that are Necrogenic for Tomato. Plant Disease, 102 (11), 2268-2276. DOI: https://doi.org/10.1094/PDIS-08-17-1259-RE

Hu, W. C., Chang, Y.C. (2006) *Cucumber mosaic virus* in New Guinea impatiens in Taiwan. Acta Horticulture, 722, 241-246. DOI: https://doi.org/10.17660/ActaHortic.2006.722.30

Choi, S. K., Choi, G. S., Kwon, S. J., Cho, I. S., Yoon, J. Y. (2015) First report of *Cucumber mosaic virus* in African Impatiens (*Impatiens walleriana*) in Korea. Research in Plant Disease, 21 (4), 341-345. DOI: https://doi.org/10.5423/RPD.2015.21.4.341

Jacquemond, M. (2012) *Cucumber mosaic virus*. Advances in Virus Research, 84, 439-504. DOI: https://doi.org/10.1016/B978-0-12-394314-9.00013-0

Milojević, K., Radović, N., Stanković, I., Vučurović, A., Nikolić, D., Bulajić, A., Krstić, B. (2016) First Report of *Cucumber mosaic virus* Infecting *Wisteria sinensis* in Serbia. Plant Disease, 100: 1799. DOI: https://doi.org/10.1094/PDIS-01-16-0096-PDN

Milojević, K., Stanković, I., Vučurović, A., Nikolić, D., Ristić, D., Bulajić, A., Krstić, B. (2014) First Report of *Cucumber mosaic virus* in *Tulipa* sp. in Serbia. Plant Disease, 98 (10), 1449-1449. DOI: https://doi.org/10.1094/PDIS-05-14-0527-PDN

Milojević, K., Stanković, I., Vučurović, A., Ristić, D., Milošević, D., Bulajić, A., Krstić, B. (2013a) First Report of *Cucumber mosaic virus* Infecting *Peperomia tuisana* in Serbia. Plant Disease, 97 (7), 1004-1004. DOI: https://doi.org/10.1094/PDIS-01-13-0089-PDN

Milojević, K., Stanković, I., Vučurović, A., Ristić, D., Nikolić, D., Bulajić, A., Krstić, B. (2013b) Biological and molecular characterization of *Cucumber mosaic virus* infecting watermelon in Serbia. Plant Protection, 64 (1), 14-25.

Milojević, K., Stanković, I., Vučurović, A., Ristić, D., Nikolić, D., Bulajić, A., Krstić, B. (2012) First Report of *Cucumber mosaic virus* Infecting Watermelon in Serbia. Plant Disease, 96 (11), 1706. DOI: https://doi.org/10.1094/PDIS-07-12-0631-PDN

Milošević, D., Ignjatov, M., Marjanović-Jeromela, A., Nikolić, Z., Tamindžić, G., Miljaković, D., Stanković, I. (2020) Presence and molecular characterization of *Cucumber mosaic virus* on safflower in Serbia. Field and Vegetables Crops Research, 57 (2), 49-54. DOI: https://doi.org/10.5937/ratpov57-25745

Milošević, D., Ignjatov, M., Nikolić, Z., Gvozdanović-Varga, J., Tamindžić, G., Stanković, I., Krstić, B. (2015) The presence of *Cucumber mosaic virus* in pot marigold (*Calendula officinalis*) in Serbia. Field and Vegetables Crops Research, 52 (2), 67-73. DOI: https://doi.org/10.5937/ratpov52-7814

Milošević, D., Stanković, I., Ignjatov, M., Nikolić, Z., Krstić, B. (2017) Presence and distribution of pepper viruses in Serbia. Plant Doctor, 45 (6), 647-656.

Moury, B. (2004) Differential selection of genes of *Cucumber mosaic virus* subgroups. Molecular Biology and Evolution, 21 (8), 1602-1611. DOI: https://doi.org/10.1093/molbev/msh164

Nikolić, D., Vučurović, A., Stanković, I., Radović, N., Zečević, K., Bulajić, A., Krstić, B. (2018) Viruses affecting tomato crops in Serbia. European Journal of Plant Pathology, 152, 225-235. DOI: https://doi.org/10.1007/s10658-018-1467-y

Palukaitis, P., García-Arenal, F. (2003) *Cucumoviruses*. Advances in Virus Research, 62, 241-323. DOI: https://doi.org/10.1016/s0065-3527(03)62005-1

Palukaitis, P., Roossinck, M. J., Dietzgen, R. G., Francki, R. I. B. (1992) *Cucumber mosaic virus*. Advances in Virus Research, 41, 281-348. DOI: https://doi.org/10.1016/s0065-3527(08)60039-1

Petrović, D., Bulajić, A., Stanković, I., Ignjatov, M., Vujaković, M., Krstić, B. (2010) Presence and distribution of pepper viruses in Serbia. Field and Vegetable Crops Research, 47 (2), 567-76.

Roossinck, M. J. (2002) Evolutionary History of *Cucumber mosaic virus* Deduced by Phylogenetic Analyses. Journal of Virology, 76 (7), 3382-3387. DOI: https://doi.org/10.1128/jvi.76.7.3382-3387.2002

Stanković, I., Bulajić, A., Vučurović, A., Ristić, D., Milojević, K., Berenji, J., Krstić, B. (2011) Status of tobacco viruses in Serbia and molecular characterization of tomato spotted wilt virus isolates. Acta virolologica, 55 (4), 337-47. DOI: https://doi.org/10.4149/av_2011_04_337

Stanković, I., Vučurović, A., Zečević, K., Petrović, B., Nikolić, D., Delibašić, G. (2021) Characterization of *Cucumber mosaic virus* and its satellite RNAs associated with tomato lethal necrosis in Serbia. European Journal of Plant Pathology, 160 (2), 301-13. DOI: https://doi.org/10.1007/s10658-021-02241-8

Tian, Z., Jiyan, Q., Jialin, Y., Chenggui, H., Weicheng, L. (2009) Competition Between *Cucumber mosaic virus* Subgroup I and II Isolates in Tobacco. Journal of Phytopathology, 157 (7-8), 457-464. DOI: https://doi.org/10.1111/j.1439-0434.2008.01531.x

Vučurović, A., Bulajić, A., Stanković, I., Ristić, D., Berenji, J., Jović, J., Krstić, B. (2012) Non-persistently aphid-borne viruses infecting pumpkin and squash in Serbia and partial characterization of *Zucchini yellow mosaic virus* isolates. European Journal of Plant Pathology, 133 (4), 935-47. DOI: https://doi.org/10.1007/s10658-012-9964-x

Vučurović, A., Bulajić, A., Stanković, I., Ristić, D., Berenji, J., Krstić, B. (2011): Characterization of *Cucumber mosaic virus* Originating from Cucurbits in Serbia. Pesticides and Phytomedicine, 26 (4), 325-36. DOI: https://doi.org/10.2298/PIF1104325V

Xu, P., Li, M., Lin, Q., Xie, L. (1999) Comparative Studies on Properties of Five *Cucumber mosaic virus* Isolates Infecting Passiflora in China. Virologica Sinica, 14 (1), 73-79.

Zečević, K., Stanković, I., Petrović, B., Krstić, B. (2023) Molecular characterization and differentiation of *Cucumber mosaic virus* subgroups in Serbia by RT-PCR-RFLP. Archives of Biological Sciences, 75 (4), 431-442. DOI: https://doi.org/10.2298/ABS230718035Z