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DETECTION AND MOLECULAR CHARACTERIZATION OF *Pepper mild mottle virus* IN SERBIA

Dragana MILOŠEVIĆ [1], Ivana STANKOVIĆ[2], Aleksandra BULAJIĆ[2], Maja IGNJATOV [1], Zorica NIKOLIĆ [1], Gordana PETROVIĆ [1], Branka KRSTIĆ [2]

[1]Institute of Field and Vegetable Crops, Novi Sad, Serbia
[2]Institute of Phytomedicine, Department of Phytopathology, University of Belgrade Faculty of Agriculture, Belgrade, Serbia

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During 2009 and 2010, a survey was conducted in pepper crops to detect the possible presence of *Pepper mild mottle virus* (PMMoV) in Serbia. A total of 239 pepper samples from 39 crops at 26 localities were collected and analyzed for the presence of PMMoV, *Cucumber mosaic virus* (CMV), *Potato virus Y* (PVY), and *Alfalfa mosaic virus* (AMV), using DAS-ELISA test. Although it was detected in a small percentage, PMMoV could pose a threat to pepper production in Serbia due to its rapid seed-borne spread. Presence of PMMoV was confirmed by serological and biological detection, followed by conventional reverse transcription RT-PCR, using primers specific for the RNA-dependent RNA polymerase (RdRp) and the coat protein (CP) genes. Molecular identification confirmed that the Serbian isolates belong to PMMoV pathotypes $P_{1,2}$ which do not break the resistance gene L^3. Reconstructed phylogenetic tree confirmed the allocation of the Serbian isolates together with the majority of PMMoV isolates which belong to pathotypes $P_{1,2}$. This study represents the first serological and molecular characterization of PMMoV infection of pepper in Serbia, and provides important data on the population structure. The obtained data could have great influence on pepper production in Serbia as well as future pepper resistance breeding in the country.

Key words: DAS-ELISA, phylogenic analysis, pepper, RT-PCR, sequencing, viruses

Corresponding author: Dragana Milošević, Institute of Field and Vegetable Crops, Makima Gorkog 30, 21000 Novi Sad, Serbia, phone:+381 21 4898155, fax:+381 21421249, E-mail: dragana.milosevic@nsseme.com

INTRODUCTION

Pepper (*Capsicum annuum* L.) is among the most important vegetable species both in Serbia and in other parts of the world. Pepper crops are produced in fields and greenhouses, but average yields are highly dependent on the presence of pests and diseases (JEE *et al.,* 2005, JEON *et al.,* 2006, IGNJATOV *et al.,* 2009, IGNJATOV *et al.* 2010, GAŠIĆ *et al.,* 2011, IGNJATOV *et al.,* 2014). One of the major factors limiting the yield and the quality of peppers are viral diseases (MILOŠEVIĆ *et al.,* 2014, PETROVIĆ *et al.,* 2010, ANANDAKUMAR *et al.,* 2008). Several viruses are known to infect peppers, and *Pepper mild mottle virus* (PMMoV) is one of the most important pathogens that affect commercial pepper production, causing significant economic losses worldwide (GENDA *et al.,* 2007). The virus is seed-borne in *Capsicum* species and the infected seed has probably been inadvertently distributed internationally. Thus PMMoV is considered to be more widespread than reported (LAMB *et al.,* 2001). In field pepper crops, infection with PMMoV may reach up to 100% which drastically reduces the yield of marketable fruits (GREEN, 2003). Recently, PMMoV has caused significant economic losses in Japanese (HAGIWARA *et al*., 2002) and Chinese (WANG *et al*., 2006) fields and greenhouse pepper production areas. The level of PMMoV seed-borne infection ranging between 0.23 and 100% has been reported, as well as retention of infectivity even after prolonged storage (GENDA *et al.,* 2005). The virus remains infective for prolonged periods in soils containing infected plant components (IKEGASHIRA *et al.,* 2010) and can act as sources of infection for subsequent susceptible crops (LAMB *et al.,* 2001). Symptoms caused by PMMoV include mild chlorosis and stunting especially if plants are infected when young, but symptoms may vary depending on cultivar (VELASCO *et al.,* 2002). The fruit may be small, malformed and mottled, with sunken or raised necrotic spots (PETROV, 2014). Most cultivars and species of pepper (genus *Capsicum*) are susceptible to PMMoV. However, this virus does not affect tomato, eggplant or tobacco, within the same family (Solanaceae). The virus is not known to be spread by insects, but is very easily spread mechanically by routine handling of the young plants, especially at transplanting (OZASLAN *et al.,* 2006). PMMoV belongs to the genus *Tobamovirus* and its genome consists of a positive-sense single-stranded RNA that encodes at least four proteins; namely, the 126-kDa and 183-kDa replicases, the movement protein (MP) and the coat protein (CP) (WANG *et al.,* 2006).

Population structure of tobamoviruses in pepper production area is of utmost importance. Five allelic *L* genes, L^1, L^{1a} L^2, L^3 and L^4, were categorized by their increasing effectiveness against four respective tobamoviruses (pathotypes: P_0, P_1, $P_{1,2}$ and $P_{1,2,3}$). Thus, viruses belonging to the P_0 pathotype are unable to infect plants carrying any *L* gene. Likewise, viruses belonging to the P_1, $P_{1,2,}$ and $P_{1,2,3}$ pathotypes can systemically infect plants with L^1 and L^{1a} genes; L^1 to L^2 genes; and L^1 to L^3 genes, respectively (SAWADA *et al.,* 2004). GENDA *et al.* (2007) and ANTIGNUS *et al.* (2008) reported a new tobamovirus, pathotype $P_{1,2,3,4}$, which is able to overcome the resistance conferred by the L^4 gene in *Capsicum* spp. previously held as the most effective genetic resource against tobamoviruses. The L^3 gene-mediated resistance is one of the most effective resistance genes against tobamoviruses.

This investigation provides the first information on the occurrence and distribution, as well as the variability of PMMoV population in Serbia. The presence of PMMoV was previously confirmed in our country (KRSTIĆ *et al.,* 1996) by biological characterization on test plants. Survey presented in this paper was carried out in 2009 and 2010, and was aimed to identify PMMoV on pepper plants in Vojvodina Province, and to estimate their relative incidence and distribution. In this study, the first molecular detection and identification, as well as the first data on molecular

characterization of PMMoV isolates originating from Serbia are reported. The main goal of phylogenetic analysis of PMMoV was to determine the genetic relationships of Serbian PMMoV pepper isolates with those responsible for breakage the resistance conferred by L^3 gene in *Capsicum* species and thus evaluate their capability to endanger pepper production in our country.

MATERIALS AND METHODS

Survey and sample collection

Survey and sample collection during 2009 and 2010 were carried out in order to determine the occurrence and distribution of PMMoV in pepper crops in three districts of Vojvodina Province (Bačka, Srem and Banat). A total of 239 (123 samples in 2009 and 116 samples in 2010) plants with symptoms resembling those of viral infection were collected after the visual inspection of 39 pepper fields at 26 localities. Samples were transported to testing laboratory, stored at 4°C until ELISA testing or stored at -18°C for molecular analyses.

Serological assay

Serological testing of 239 samples were performed utilizing double antibody sandwich (DAS)-ELISA kits with commercial antisera specific for detection PMMoV as well as additional three the most common pepper viruses: *Cucumber mosaic virus* (CMV), *Potato virus Y* (PVY) and *Alfalfa mosaic virus* (AMV) (Loewe Biochemica, Sauerlach, Germany), according to manufacturer's instructions. Plant tissue samples were ground in extraction buffer (1:10 wt/vol). After incubation with p-nitrophenyl phosphate (Sigma-Aldrich, St. Louis, MO), at room temperature (23°C) for 1 to 2 h in dark, absorbance at 405 nm (A405) was measured with an ELISA microplate reader (Multiscan Ascent, Thermo Scientific, Finland). Samples were considered to be positive if their average absorbance value were equal to or higher than twice that of the negative control. Commercial positive and negative controls (Loewe) for each virus were included in each ELISA.

Mechanical transmission of PMMoV

The infectious nature of the disease and the biological characterization of the three selected serologically positive samples (P-57-09, P-60-09, P-3-10) were performed by mechanical inoculation of 5 seedlings of each of six different test plants in the glasshouse conditions. Five healthy seedlings of *C. annuum*, *Licopersicon esulentum, Chenopoidium quinoa*, *Nicotiana glutinosa*, *N. tabacum* 'Samsun' and *N. benthamiana*, were used for virus isolation and biological characterization. All plants were well developed with 3-4 true leaves. The inoculum was prepared by homogenization of infected pepper leaves in 0.01 M phosphate buffer pH 7.0, using carborundum powder of 400 mesh as abrasive. The inoculated plants were grown in glasshouse conditions and symptoms on inoculated plants were recorded during the 3 weeks after inoculation. The presence or absence of the virus was tested by ELISA.

RT-PCR detection and sequence analysis based on the RdRp gene

In order to confirm serological detection of PMMoV, total RNA extracts were obtained from ELISA-positive symptomatic pepper leaves, using the RNeasy Plant Mini Kit (Qiagen, Hilden, Germany) following the manufacturer´s instructions. Tissue sample from healthy pepper

leaves and PCR water were used as negative controls. Detection of PMMoV was performed using primer pair P12/3/P12/3A (VELASCO *et al.,* 2002), previously described to amplify RNA-dependent RNA polymerase (RdRp) gene. RT-PCR was carried out with the One-Step RT-PCR kit (Qiagen) according to the manufacturer´s instructions. The reaction components included 400 µM each of the four dNTPs, 1 µl of RT-PCR enzyme mix, 0.6 µM of each primer P12/3/P12/3A, and 1 µl extracted RNA in a final volume of 25 µl. Amplifications were performed in a thermal cycler (Biometra, T-1 Thermocycler) with the following cycling parameters: reverse transcription at 50°C for 30 min and an initial PCR denaturation step at 95°C for 15 min, followed by 30 cycles consisting of a denaturation step of 30 s at 92°C, primer annealing for 30 s at 60°C, and extension for 45 s at 72°C. The final extension was performed at 72°C for 10 min. Amplified products were analyzed by 1% agarose gel electrophoresis, stained with ethidium bromide (0.5 µg/ml), and visualized under a UV transilluminator. The size of fragments was determined by comparison with MassRuler^{TM}DNA ladder, Mix (Fermentas Life Sciences GmbH, Lithuania).

The amplified products derived from the three isolates, P-3-10, P-57-09, P-60-09 were sequenced in both directions using the same primers directly after purification with a QIAquick PCR Purification Kit (Qiagen). Sequencing was performed on an automated sequencer (BMR Genomics, Padova, Italy). The nucleotide sequences of the amplification products were deposited in GenBank database and they were assigned accession numbers. Sequences of Serbian virus isolates were compared with the previously reported PMMoV isolates available in the GenBank (http://www.ncbi.nlm.nih.gov/BLAST/) using the ClustalW program (THOMPSON *et al.,* 1994) and MEGA5 software (TAMURA *et al.*, 2011). A p-distance model was applied for nucleotide (nt) and deduced amino acid (aa) sequence analyses and the divergence of selected sequences was calculated using sequences trimmed to the length of the shortest sequence.

RT-PCR detection, sequence analysis characterization based on CP gene

Further characterization of isolates was performed utilizing RT-PCR with specific primers PMF1/PMR1 (HAMADA *et al.,* 2002) previously described to amplify coat protein (CP) gene. The components of reaction mix were identical to those previously described. The cycling parameters were the following: reverse transcription at 50°C for 30 min and an initial PCR denaturation step at 95°C for 15 min, 35 cycles consisting of a denaturation step of 30 s at 94°C, primer annealing for 1 min at 50°C, and extension for 1 min at 72°C. Sequencing and sequence analysis was performed as previously described.

A phylogenetic tree was reconstructed using 33 CP sequences of PMMoV isolates from different host plants and geographic origins which were retrieved from GenBank (Table 1) and three PMMoV CP sequences generated in this study. One sequence of each *Tobacco mosaic virus* (TMV; Acc. No. X70883) and *Tomato mosaic virus* (ToMV; Acc. No. AF411922) were used as the outgroup sequences. Phylogenetic tree was constructed using Maximum Parsimony method and bootstrap analysis with 1000 replicates implemented in MEGA5. All branches with bootstrap support value <50% were omitted. Intra- and inter-group diversity values were calculated as the average genetic distance using p-distance model.

Table. 1. Coat protein gene sequences of PMMoV isolates used in the phylogenetic analysis

Isolate name[a]	Country	Host plant	GenBank accession number
P-60-09	Serbia	pepper	KC288151
P-57-09	Serbia	pepper	KC288150
P-3-10	Serbia	pepper	KC288149
Iw	Japan	pepper	AB254821
L4BV	Japan	pepper	AB276030
P	Korea	pepper	AB084456
KR	Korea	unknown	AB126003
PO	Korea	pepper	AF103776
Ge1	Japan	pepper	AB062049
Ge5	Japan	pepper	AB062051
Ge4	Japan	pepper	AB062050
DF01	Brasil	pepper	AB550911
CN	Kina	pepper	AY859497
Taiwan	Taiwan	unknown	M87827
OH	Japan	pepper	AB062052
J	Japan	pepper	AB000709
S	Spain	pepper	M81413
Na	Japan	pepper	AB062054
Ia	Spain	pepper	AJ308228
I	Italy	pepper	X72587
Tosa	Japan	pepper	AB062053
LinsBR08	Brasil	pepper	AM411433
unknown	China	unknown	AY632863
PMMV1.2	Italy	unknown	AJ429088
Pe1	Japan	pepper	AB119482
P98/15	Spain	pepper	FR671392
P99/23	Spain	pepper	FR671393
P96/44	Spain	pepper	FR671388
P83/4	Spain	pepper	FN594853
P85/29	Spain	pepper	FN594869
P89/4.2	Spain	pepper	FN594881
P02/2	Spain	pepper	FN594870
P86/10	Spain	pepper	FN594889
PMMoV-BD	China	pepper	HQ699079
PMMoV-TRf1	Turkey	pepper	HE963026
PMMoV-TRf2	Turkey	pepper	HE963027
TMV[b]	Germany	unknown	X70883
ToMV	Brasil	tomato	AF411922

[a]All data are from GenBank, [b]*Tobacco mosaic virus* and *Tomato mosaic virus* sequences were used as outgroups

RESULTS

Virus detection and occurrence in pepper

PMMoV was detected by DAS-ELISA in five out of 239 serologically tested samples. In 2009 it was detected in three samples (2.44%) out of 123 tested. Single infection of PMMoV was detected in one sample while the mixed infections were detected in two samples, with PVY or PVY and CMV from the locality Čonoplja (West Bačka District). Due to mixed infections, symptoms varied greatly and preliminary diagnostics was not possible. During 2010, in 116 symptomatic pepper samples the presence of PMMoV was serologically confirmed in two samples (1.72%), Đurđevo (South Bačka District) in single infections and Čonoplja (West Bačka District) in mixed infections with PVY and CMV. Plants with single PMMoV infection exhibited similar mild to severe mosaic on the leaves, while plants with mixed infections exhibited more intensive symptoms of mosaic, followed by leaf malformations in mixed infection with CMV, and mild necrosis in infections with PVY. Regardless of the type of the infection, single or mixed, symptoms mostly developed on leaves, while only rarely, color-breaking on pepper fruits was visible.

Mechanical transmission of PMMoV

Biological characterization of isolates from single PMMoV infections (P-57-09, P-60-09, P-3-10) showed no differences in a type or time of symptoms expresion. Local as well as systemic symptoms were visible two to three weeks after inoculation depending on test plants. After inoculations with each of three isolates, all five inoculated *C. annuum* responded with typical advent mosaic symptoms on the inoculated and systemic leaves 21 days post inoculation (dpi), while all five *N. benthamiana* expressed systemic chlorosis, leaf deformation and growth reduction 14 dpi. On the other hand *N. glutinosa* and *C. quinoa* reacted with small necrotic local lesions only on inoculated leaves, 14 dpi. *N. tabacum* 'Samsun' and *L. esculentum* were not infected by any isolates. Virus presence/absence was confirmed by DAS-ELISA and RT-PCR.

Detection and sequence analysis based on RdRp gene

The presence of PMMoV in all ELISA positive samples was confirmed by target cDNA amplification of fragments of predicted size of 836 bp using specific pair of primers P12/3/P12/3A (VELASCO *et al.,* 2002).The identities of RT-PCR products derived from the isolates P-57-09, P-60-09, P-3-10, representing the partial nucleotide sequences of the RdRp gene, were confirmed. Sequence analysis of RdRp gene revealed high nt identity of 99.4 to 100% among Serbian PMMoV isolates from pepper, and 97.2 to 99.7% of nt identity (93.3-99.2% aa identity) with other PMMoV isolates deposited in GenBank. Serbian isolates showed the highest nt identity of 99.7% with PMMoV pepper isolates AB276030 and AB254821 from Japan and AB126003 from Korea. The highest aa identity of the isolate P-60-09, as well as for isolates P-3-10 and P-57-09 was found with PMMoV pepper isolates AB254821 from Japan (99.6% and 98.7%, respectively).

Characterization based on CP gene

The nucleotide sequences of the fragment of the CP gene derived from the isolates P-57-09, P-60-09, P-3-10, shared nucleotide identity from 99.5 to 100%, while identity with isolates retrieved from GenBank was 97.6 and 100% (98.5-100% aa identity). Isolates P-60-09, P-57-09 and P-3-10 exhibited the highest nucleotide identity (100%, 99.5% and 99.5%, respectively) with three PMMoV isolates from Hungary (AM491594-6, AM491598 and AM491601), one isolate

from Spain (FR671383), and two isolates from France (GQ427044 and GQ427043). Accession numbers of the sequences obtained in this study for all three pepper virus isolates from Serbia are presented in Table 2.

Table. 2. List of PMMoV isolates analyzed in this study

Isolate	Year of isolation	Target DNA sequences	Acc. No.
P-3-10	2010	CP gene	KC288149
		RdRp gene	KC288154
P-57-09	2009	CP gene	KC288150
		RdRp gene	KC288152
P-60-09	2009	CP gene	KC288151
		RdRp gene	KC288153

All three PMMoV isolates from Serbia were highly identical (98.1-100%) at the CP aa level with PMMoV pathotype $P_{1,2}$ isolates. The aa sequence alignment for CP revealed that Serbian PMMoV isolates share molecular prints defined by three amino acids Ser, Met and Ala at positions 6, 139 and 148, respectively (Figure. 1).

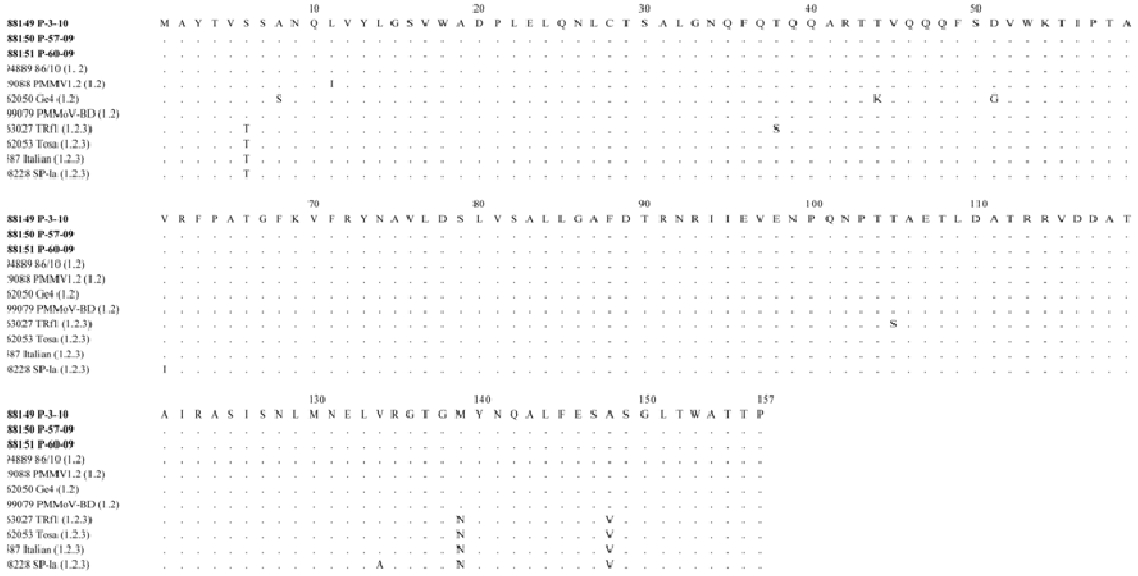

Figure. 1. Alignment of amino acid sequences of the CP of pepper infecting tobamoviruses. HE963027 TRf1, AB062053 Tosa, X72587 I, AJ308228 Ia are L^3-resistance overcoming isolates, whereas FN594889 86/10, AJ429088 PMMV1.2, AB062050 Ge4, HQ699079 PMMoV-BD and Serbian isolates (P-57-09, P-60-09, P-3-10) are not.

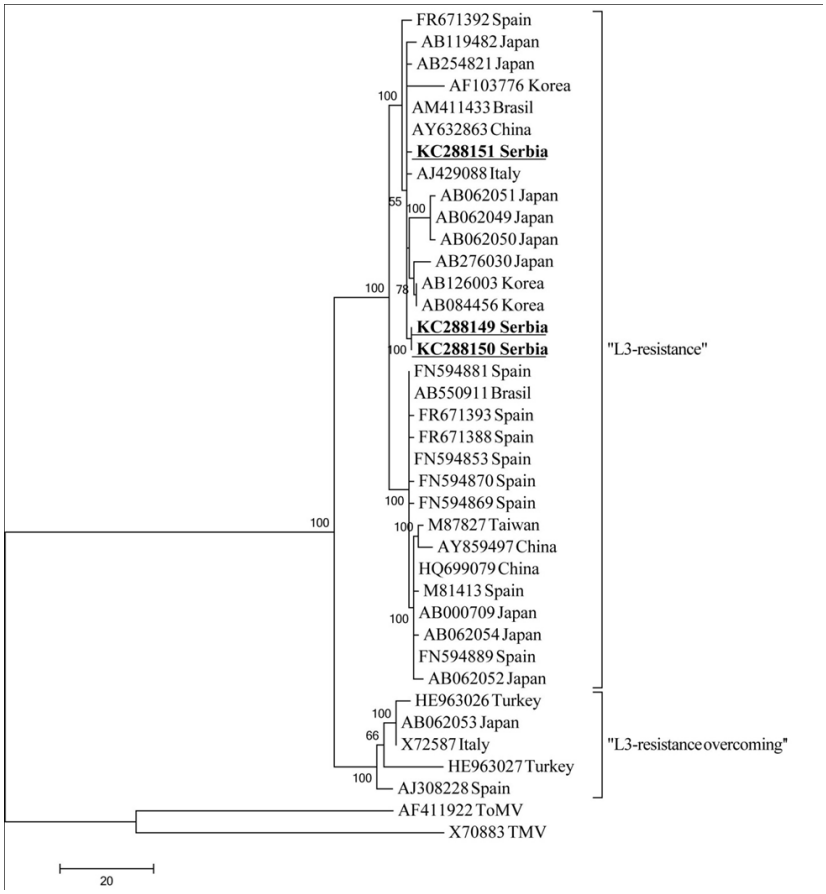

Figure. 2. Maximum parsimony tree based on nucleotide sequences of partial CP sequence of KC288149, KC288150, KC288151, isolates of *Pepper mild mottle virus* (PMMoV) using X70883 and AF411922 isolates as outgroup sequences. The three Serbian isolates are underlined.

A Maximum Parsimony tree (Figure 2) of 33 PMMoV isolates, reconstructed using the complete nucleotide sequences of the coat protein gene (550 nt), showed the presence of two major groups ("L^3 resistance" and "L^3 overcoming resistance"), with the high bootstrap value (100%). Genetic diversity between these two groups was 0.060 ± 0.010, and within the group it was 0.016±0.003 for "L^3-resistance" group and 0.010±0.004 for the second group. Three Serbian PMMoV isolates originating from pepper belongs to the "L3-resistance" group – of the P1,2 pathotypes which are not able to break resistance conferred to gene L3 together with isolates from Asia (Japan, China, Korea, Taiwan), Europe (Spain, Italy) and America (Brazil). Group "L3 overcoming resistance" - P1,2,3 pathotypes includes isolates from Italia (X72587), Spain

(AJ308228), Japan (AB062053) and Turkey (HE963026 and HE963027) which promote breakage resistance gene L3 (Figure 2).

DISSCUSSION

Pepper has great economic importance worldwide and belongs to the group of the most important vegetable crops in Serbia, where it is grown on about 20,000 ha with average yield of 8.3 t/ha, both in greenhouses and in open field. Spice pepper production is mainly concentrated in the Province of Vojvodina where almost 80% of total production in Serbia is allocated (GVOZDENOVIĆ, 2010). Regardless of the low level of PMMoV infection detected during this survey, considering its efficient mechanical and seed-borne spreading, as well as the importance of pepper growing in Serbia, there is a strong possibility for devastating consequences. ÇAĞLAR *et al.* (2013) also reported unexpectedly low level of infection, considering that Tobamoviruses generally spread quickly in nature. Symptoms observed on pepper plants infected with PMMoV included mild to severe leaf mosaic and were in accordance with previously described (MARTÍNEZ-OCHOA *et al*., 2003; GÜLDÜR and ÇAĞLAR, 2006). Mixed infections with two or more viruses were dominant. Similar data was given by APPIAH *et al.* (2014) where all the plants infected of PMMoV included a mixed infection with CMV. The virus isolates from pepper plants were able to induce symptoms on commonly used PMMoV test plants. All inoculated *N. benthamiana* plants produced symptoms typical of PMMoV, chlorotic spots on inoculated leaves accompanied with deformation (MNARI-HATTAB and EZZAIER, 2006). All five *N. glutinosa* and *C. quinola* produced small necrotic local lesions on inoculate leaves within 14 dpi which is consistent with symptoms caused by PMMoV (TOYODA *et al.,* 2004). *N. tabacum* 'Samsun' and *L. esculentum* were not infected by any of Serbian isolates which is in accordance with the literature data (MNARI-HATTABAND and EZZAIER, 2006.). The virus was transmitted mechanically to *C. annuum* and induced symptoms resembling those observed on the source plants. Mechanical transmission to the test plants confirmed the infectious nature of pepper disease and the symptoms developing on selected test plants confirmed the biological identification of PMMoV.

Identity of RdRp genes between the PMMoV isolates obtained in this work ranged from 99.4 - 100%, implying low genetic variability, while their identity with isolates from different geographic regions also showed a high degree of nucleotide and amino acid homology (97.2 to 99.7% nt and 93.3 to 99.2% aa). Based on studies conducted by Kálmán (2001), Hungarian isolates showed a high degree of nucleotide sequence identity with the previously described isolates (94 - 99%), while the degree of amino acid sequence identity varied from 95 to 100%.

Phylogenetic analysis of the CP sequences of the PMMoV documented the presence of two distinct groups of isolates, previously designated as L^3 resistance" – $P_{1,2}$ pathotypes, isolates which are unable to break resistance gene L^3 (including the Serbian isolate), and "L^3 overcoming resistance" – $P_{1,2,3}$ pathotypes, isolates which promote breakage resistance gene L^3 (TOMITA *et al*., 2011). All around the world a lot of concern has been related to the isolates L^3 – pathotypes $P_{1,2,3}$ because their occurrence so far was associated with major losses in pepper crops in Europe (LETSCHERT *et al.,* 2002; VELASCO *et al.,* 2002). In our study we showed that all detected PMMoV belong to L^2 gene resistance-breaking tobamovirus and that potentially more dangerous L^3 resistance" – $P_{1,2}$ pathotypes are not present yet in major field of pepper in Serbia. It is necessary to organize the preventive measures to avoid the introduction by controlling international trade with plants and seeds of *Capsicum* spp. especially with countries that announced the presence of disease breaking isolates such as Spain (VELASCO *et al.,* 2002), Italy (GARCIA-LUQUE *et al.,* 1993), Japan (TSUDA *et al*., 1998) and Turkey (ÇAĞLER *et al.,* 2013). Due to seed-borne nature of the disease

and mechanical spreading, PMMoV control is based exclusively on the use of healthy seeds, careful handling the pepper plants prior to setting in the field, and sanitation of diseased plants as soon as symptoms are noticed. Thus, the Serbian pepper seed production and even more the import of pepper seed must be firmly controlled for the presence of tobamoviruses, and especially PMMoV in the future.

By first molecular detection and isolation and characterization of PMMoV in Serbia, this study provides the first necessary step for maintaining good practice in managing PMMoV in our country. As there is a great risk that disease resistance breaking isolates of PMMoV might be introduced and further spread via contaminated seed, all future efforts in the phytosanitary system will be focused on early detection and diagnostics which will provide necessary conditions for successful pepper production in Serbia.

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DETEKCIJA I MOLEKULARNA KARAKTERIZACIJA VIRUSA BLAGOG ŠARENILA PAPRIKE (*Pepper mild mottle virus*) U SRBIJI

Dragana MILOŠEVIĆ[1], Ivana STANKOVIĆ[2], Aleksandra BULAJIĆ[2], Maja IGNJATOV[1], Zorica NIKOLIĆ[1], Gordana PETROVIĆ[1], Branka KRSTIĆ[2]

[1]Institut za ratarstvo i povrtarstvo, Novi Sad, Srbija
[2]Univerzitet u Beogradu, Poljoprivredni fakultet, Beograd, Srbija

Izvod

Tokom 2009 i 2010, sprovedeno je ispitivanje prisustva virusa blagog šarenila paprike (*Pepper mild mottle virus*, PMMoV) u usevima paprike u Srbiji. Ukupno 239 uzoraka paprike iz 39 useva sa 26 lokaliteta je prikupljeno i serološki testirano na prisustvo PMMoV, virusa mozaika krastavca (*Cucumber mosaic virus,* CMV), virusa crtičastog mozaika krompira (*Potato virus Y*, PVY) i virusa mozaika lucerke (*Alfalfa mosaic virus,* AMV), korišćenjem komercijalno dostupnih kitova za DAS-ELISA test. Iako je otkriven u malom procentu, PMMoV bi mogao predstavljati ozbiljnu pretnju proizvodnji paprike u Srbiji, s obzirom na to da se lako prenosi putem semena. Prisustvo PMMoV je potvrđeno serološkom i biološkom detekcijom, kao i primenom RT-PCR metode korišćenjem specifičnih prajmera za RNA-zavisnu RNA polimerazu i gen za protein omotača (CP gen). Molekularnom identifikacijom potvrđeno je da srpski izolati pripadaju PMMoV, patotipu $P_{1,2}$, koji nije u mogućnosti da prevaziđe otpornost gena L^3. Filogenetsko stablo potvrdilo je grupisanje srpskih izolata zajedno sa većinom izolata koji pripadaju patotipu $P_{1,2}$. Obavljena istraživanja pružaju prve informacije o serološkoj i molekularnoj karakterizaciji virusa blagog šarenila paprike poreklom iz paprike u Srbiji, pružajući važne podatke o strukturi populacije. Dobijeni podaci mogu imati velik uticaj na proizvodnju paprike u Srbiji, a takođe i velik značaj u budućem oplemenjivačkom radu na selekciji otpornih genotipova paprike.

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