

Incidence and distribution of *Leek yellow stripe virus* in *Allium* crops in Serbia

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SUMMARY

Leek yellow stripe virus (LYSV) is one of the most frequent and important viruses in leek and garlic crops worldwide. In Serbia this virus is found both in leek and garlic, and often at high percentages. During two consecutive years, 2012 and 2013, a total 92 samples were collected from 11 inspected leek-, garlic- and onion-growing locations and they were analyzed for the presence of LYSV using DAS-ELISA. LYSV was detected in 31.5% of the tested samples. In 2012, the presence of LYSV was only detected in leek plants, and in 55.6% of the tested samples. During 2013, LYSV was detected in 85% of leek and 58.3% of garlic samples. In total, LYSV was detected in 56.4% of leek samples and 17.1% garlic samples. LYSV incidence was confirmed using RT-PCR with LYSV specific primers amplifying 1020 bp fragment representing coat protein and part of nuclear inclusion B genes. Molecular identification was confirmed by sequencing of two selected isolates, 181-13 (MG242625) from garlic and 298-13 (MG242624) from leek, and comparing them to the GenBank sequences of LYSV. Phylogenetic analysis of 55 sequences of LYSV from all over the world showed some correlation between host plant and geographical origin of the isolates, forming five separate clades. Two Serbian LYSV isolates fell into distant clades. The Serbian leek isolate 298-13 of LYSV belongs to clade B, while isolate 181-13 originating from garlic belongs in clade E.

Keywords: *Leek yellow stripe virus*; Leek; Garlic; RT-PCR; ELISA; Serbia

INTRODUCTION

Allium is one of the largest genera of monocotyledonous plants which comprises more than 800 species (Fritsch et al. 2010). Species of this genus are widespread in the

Holarctic region. The genus includes several economically very important species, such as onion (*Allium cepa* L.), shallot (*Allium cepa* var. *ascalonicum*), garlic (*Allium sativum* L. var. *sativum*), chives (*Allium schoenoprasum* L.), and leek (*Allium porrum*), which are cultivated

worldwide and broadly used as food and medicinal plants (Brewster, 1994; Fritsch & Friesen, 2002; Fritsch & Friesen, 2009; Block, 2010).

Onion and garlic are two most important food crops of the genus *Allium* worldwide, considering both the area harvested and quantities used for human consumption. China is the leading producer of these two species with 30% of worldwide onion production and 75% of garlic production. Onion is the third most widely produced vegetable, and is considered the principal species of the genus *Allium*. In Serbia, onion is grown on 18014 ha with an annual production of 139,000 t, while garlic is grown on 7744 ha with 21,000 t (FAO, 2011). Serbian production, especially of garlic, has been insufficient so far, and products need to be imported (RZS, 2013).

The most important diseases in allium production worldwide are caused by plant viruses, and those on onion and garlic are the most important. Other species are considered less prone to virus infections, and their diseases are of local importance (Katis et al., 2012).

A majority of virus diseases on different allium species is caused by viruses from three genera: *Potyvirus*, *Carlavirus* and *Allexivirus* (Katis et al., 2012). Yield losses caused by virus infections could be very important and even more significant if they are the consequence of mixed infection with *Potyvirus* species and species from another genus (Lot et al., 1998; Conci et al., 2003; Filho et al., 2006).

Leek yellow stripe virus (LYSV) is a member of the genus *Potyvirus*, family *Potyviridae*. The disease caused by the virus was first noticed in 1937 (Bremer, 1937), and then described by Kupke (1957). Much later it was shown to be caused by *Potyvirus Leek Yellow Stripe Virus* (Bos et al., 1978). LYSV is one of the most important and widespread viruses of garlic and leek (Bos, 1981; Diekmann, 1997). As a very widespread virus, LYSV has been detected in Brazil (Fajardo et al., 2001), Argentina (Conci et al., 2002), the USA (Pappu, 2005; Testen et al., 2014), China (Chen et al., 2002), Vietnam (Ha et al., 2008), India (Gupta et al., 2013), Ecuador (Oleas & Arahana, 2016), Morocco and Egypt (van Dijk, 1993), as well as in many European countries: France, Slovenia, Serbia, Croatia and Poland (Delecolle & Lot, 1981; Lot et al., 1998; Mavrič & Ravnikar, 2005; Chodorska et al., 2014; Vončina et al., 2016; Vučurović et al., 2016). In some countries, such as Israel (Salomon et al., 1996; Shibolet et al., 2001), Greece (Dovas et al., 2001), Italy (Dovas & Vovlas, 2003), Syria (Mohammad et al., 2007) and Turkey (Fidan & Baloglu, 2009), LYSV is considered the most frequent virus. LYSV is transmitted

by more than 10 aphid species in non-persistent manner (Verhoyen & Horvat, 1973; Bos et al., 1978; Lunello et al., 2002; Abd El-Wahab, 2009).

Although viruses infecting allium crops are widespread across the country and cause considerable yield losses, there have been only a few reports mainly focusing on first detection of a certain virus species in Serbia (Bagi et al., 2010; Stanković et al., 2012; Milošević et al., 2015; Vučurović et al., 2015; Vučurović et al., 2016).

Given that viruses cause diseases in Serbia every year, and they occur at high levels of infection in different allium crops in some seasons, significantly reducing yield and quality of products, the main goal of this research was to assess the presence and distribution of LYSV by monitoring different allium crops in Serbia. The purpose of this study was also to characterize Serbian LYSV isolates by phylogenetic analysis and to determine the genetic relationship of these isolates with those of different geographical origin.

MATERIALS AND METHODS

Field survey: collection of plant samples

During two consecutive years, 2012 and 2013, several major leek, onion and garlic producing areas in Serbia were inspected for the presence of symptoms indicating virus diseases, aiming to determine the presence and distribution of *Leek yellow stripe virus* in the most important allium crops in Serbia. Before sampling, disease incidence was estimated in each production field by counting plants with virus-like symptoms in a random batch of 100 plants in four replicates. Plants showing virus-like symptoms, which varied from chlorosis, different types of mosaic and yellow stripping to total deformation of plants and stunting, were observed and sampled during the surveys. Samples were transported and stored at 4°C until ELISA or RT-PCR testing.

In total, during both years of investigation, 11 allium growing locations were inspected and 92 samples of leek, onion and garlic were collected. In 2012, a total of 28 samples were collected from 3 locations: Mihailovo, Padinska Skela and Vladičin Han. The samples were collected from leek (9), onion (10) and garlic (9) plants. In 2013, a total of 64 samples were collected from 8 locations: Mladenovac, Medveđa, Arilje, Donji Tavankut, Padinska Skela, Porodin, Popinci and Pećinci. The samples were collected from leek (20), onion (32) and garlic (12) plants.

Serological detection

The collected samples were tested for the presence of LYSV, using enzyme-linked immunosorbent assay (ELISA) according to a standard DAS-ELISA protocol (Clark & Adams, 1977). Serological testing was performed utilizing double antibody sandwich (DAS)-ELISA kits (Bioreba AG, Reinach, Switzerland) with commercial antisera specific for detection of LYSV following the manufacturers' instructions. Plant tissue samples were ground in extraction buffer (1:10 w/v). After incubation with p-nitrophenyl phosphate (Sigma-Aldrich, St. Louis, MO), absorbance at 405 nm (A₄₀₅) was measured with an ELISA microplate reader (DAS srl, Italy). Both commercial positive and negative controls (Bioreba AG) were included in each ELISA test. Samples were considered positive if the average optical density (OD) was equal to or higher than two times the average OD of the negative control.

Molecular detection of LYSV

Total RNAs from 100 mg of freeze-dried leaves of all 29 ELISA-positive samples were extracted with the RNeasy Plant Mini Kit (Qiagen, Hilden, Germany). PCR was conducted using the specific primer pair 1LYSV (5'-TCACTGCATATGCGCACCAT-3') and 2LYSV (5'-GCACCATACAGTGAATTGAG-3'), amplifying 1020 bp of the whole coat protein (CP) gene and part of the nuclear inclusion b (NIB) sequence (Fajardo et al., 2001). RT-PCR was carried out using the One-Step RT-PCR kit (Qiagen) according to the manufacturer's instructions. A Serbian isolate of LYSV from leek (GenBank Accession Number KR075504; Vučurović et al., 2016) was used as a positive control in RNA extraction and RT-PCR assays. The RNA extracted from healthy leek and garlic plants and PCR mix with RNase free water served as negative controls in each RT-PCR reaction. The RT-PCR reaction mixture included 400 µM each of the four dNTPs, 1 µl of RT-PCR enzyme mix, 0.6 µM each primer, and 1 µl extracted RNA in a final volume of 25 µl. Amplifications were performed in a thermal cycler (Applied biosystems 2720). Reverse transcription was performed at 50°C for 30 min, followed by an initial PCR denaturation step at 95°C for 15 min, and 35 cycles of denaturation at 95°C for 30 s, annealing at 50°C for 2 min, extension at 72°C for 2 min; and a final extension at 72°C for 10 min. Amplified products were analyzed by 1% agarose gel electrophoresis, stained with ethidium bromide, and visualized under a UV transilluminator.

Sequence analyses

Two previously serologically characterized samples were selected for these analyses. The origin, host plant and year of isolation of the two selected isolates are given in Table 1. Sequencing was performed with primers used for detection in both directions on an automated sequencer (ABI 3730XL Automatic Sequencer Macrogen, Korea). The sequences generated in this study were deposited in the National Center of Biotechnology Information (NCBI) GenBank database. Sequences of the Serbian LYSV isolates were compared with the respective sequences available in the GenBank database (<http://www.ncbi.nlm.nih.gov/BLAST/>) using the ClustalW program (Thompson et al., 1994) and MEGA5.2 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 the selected LYSV isolate sequences was calculated using sequences trimmed to the length of the shortest fragment.

A phylogenetic tree was constructed using two isolates of LYSV obtained during this study and 53 LYSV representative isolates from Serbia and other parts of the world which were retrieved from GenBank (Table 1). The best-fitting model of nt substitution was investigated using the MODELTEST implemented in MEGA5.2, and the Kimura 2-parameter model Gamma distributed (T92+G) was chosen. The reliability of the obtained tree was evaluated using the bootstrap method based on 1000 replicates, and bootstrap values <50% were omitted. Intra- and inter-group diversity values were calculated as the average genetic distance using T92+G.

RESULTS

Symptoms observed in the field and LYSV incidence

Symptoms suggesting virus infection were observed in leek, onion and garlic crops in Serbia during 2012 and 2013. The symptoms were observed mainly on leaves, exhibiting different chromatic changes, such as mosaic, yellow mosaic or stripe mosaic, chlorotic patterns, plant stunting or leaf deformations. Disease incidence in 2011 was estimated at 70% in leek, 25% in onion, and 60% in garlic, while in 2012 it was estimated at 20% in onion, and 65% in garlic.

Table 1. *Leek yellow stripe virus* isolates with CP gene sequences available in GenBank and used for phylogenetic analyses

Isolate*	Country of origin	Host plant	Accession number
181-13	Serbia	<i>Allium sativum</i>	MG242625
298-13		<i>Allium porrum</i>	MG242624
277-12			KR075504
Netherlands1	Netherlands		AB194627
Netherlands2			AB194628
Okayama1	Japan		AB194625
Okayama2			AB194624
Lind3	Indonesia		AB005612
L	Argentina		AY007693
L	Germany		X89711
382-LYSV2	Italy	<i>Allium sativum</i>	HQ873737
399-LYSV2			HQ873738
381-LYSV2			HQ873739
402-LYSV2			HQ873740
400-LYSV2			HQ873741
378-LYSV2			HQ873742
379-LYSV2			HQ873743
401-LYSV2			HQ873744
225-LYSV1			HQ873755
380-LYSV2			HQ873756
228-LYSV1			HQ873757
227-LYSV1			HQ873758
397-LYSV2			HQ873759
224-LYSV1			HQ873760
393-LYSV7	United States of America		HQ873735
392-LYSV7			HQ873736
398-LYSV2			HQ873745
250-LYSV7			HQ873746
249-LYSV7			HQ873747
251-LYSV7			HQ873748
283-LYSV6			HQ873749
284-LYSV6			HQ873750
281-LYSV6			HQ873751
280-LYSV6			HQ873752
285-LYSV6			HQ873753
282-LYSV6			HQ873754
Hokkaido.043	Japan		AB194656
Hokkaido.042			AB194626
Aomori.021			AB194636
3mEl7			AB194621
1A3l			AB194623
/	Japan	<i>Allium sativum</i>	D11118
/**		/	D28590
Aomori.044		/	AB194637
W-Ku		/	AB194622
Saga.Kikai#2		/	AB194639
pLYSVg	New Zealand	/	AY842136
Aomori.041	Japan	/	AB194629
LYSV-5CZ	Czech Republic	/	DQ299380
FLC-CP	Israel	/	AF071525
LYSV-22CZ	Czech Republic	/	DQ299381
LYSV-MG	Brazil	<i>Allium sativum</i>	KP258216
Saga.Kikai#1	Japan	/	AB194638
yn1	China	/	AJ409307
Yuhang GYH	China	/	NC_004011

* - GenBank data; ** - Data unavailable

Serological detection

LYSV was found in 31.5% of the 92 leek, onion and garlic samples collected during the surveys and tested by DAS-ELISA. LYSV was identified in 17.9% and 37.5% of the tested samples in 2012 and 2013, respectively (Table 2).

Considering allium species, the presence of LYSV was detected in leek and garlic plants in 56.4% and 17.1% of the tested samples, respectively. In this investigation, the presence of LYSV was not detected in onion plants.

In 2012, LYSV was only detected in leek plants (55.6%) collected from two fields of one location, while in 2013 its presence was confirmed in all collected plants from one leek location, 40% of plants from another, and in 58.3% of the tested garlic samples from two out of three inspected locations.

Molecular detection and sequence analyses

The LYSV primers specifically amplified target cDNA fragments of a predicted size of 1020 bp and detected the presence of LYSV in all 29 ELISA-positive samples. No reaction was recorded in healthy controls. The identities of the obtained amplicons from two selected isolates were confirmed by sequencing. The sequences of isolates selected in this study were submitted to GenBank and were assigned with accession numbers for leek isolate 298-12 (MG242624) and garlic isolate 181-13 (MG242625).

The partial NIB-CP sequence of the Serbian LYSV isolate 298-12 showed the highest nt identity of 97.9% (98.3% aa identity) with a Japanese isolate (AB194622) from garlic, while partial NIB-CP sequence of the Serbian LYSV isolate 181-13 showed the highest nt identity of 90.1% (94.9% aa identity) with a Brazilian isolate (KP258216) from garlic.

The two Serbian LYSV isolates were 21.1% different from each other on the nucleotide level (17.4% aa). Compared to the previously described Serbian leek isolate KR075504, the isolates 298-13 and 181-13 showed 84.1% (86.8% aa) and 79.5% (80.9% aa) nucleotide identities, respectively.

A neighbour-joining tree (Figure 1) was constructed using a fragment of 394 nt from sequences generated in this study, one generated in a study of Vučurović et al. (2016) and 52 LYSV isolates from all over the world. A phylogenetic analysis revealed five major groups of LYSV isolates (Clades A-E) with high bootstrap values (91, 96, 100, 99, and 99, respectively) together with two unclassified isolates originating from the Czech Republic (DQ299380 and DQ299381). Overall genetic diversity of LYSV sequences in the phylogenetic tree was 0.203 ± 0.021 . Clade A comprised garlic isolates from Italy and Israel with 0.013 ± 0.004 genetic diversity among the sequences of this clade. Clade B included garlic isolates from Serbia, USA, Japan and New Zealand (0.02 ± 0.004). Clade C contained garlic isolates from the USA and Italy (0.002 ± 0.001).

Table 2. Incidence of *Leek yellow stripe virus* in allium crops in Serbia during 2012 and 2013

Year	Allium species	Location	No of fields	No of samples collected	LYSV
2012	<i>Allium cepa</i>	Mihailovo	1	5	0*
		Padinska Skela	1	5	0
	<i>Allium sativum</i> var. <i>sativum</i>	Vladičin Han	2	9	0
	<i>Allium porrum</i>	Padinska Skela	2	9	5 (55.6)
	Subtotal		7	28	5 (17.9)
2013	<i>Allium cepa</i>	Mladenovac	1	6	0
		Medveda	1	8	0
		Arilje	2	9	0
		Donji Tavankut	1	5	0
		Pećinci	1	1	0
		Popinci	1	3	0
	<i>Allium sativum</i> var. <i>sativum</i>	Mladenovac	1	4	2 (50)
		Arilje	1	7	5 (71.4)
		Beograd	1	1	0
	<i>Allium porrum</i>	Padinska Skela	1	15	15 (100)
		Porodin	1	5	2 (40)
	Subtotal		11	64	24 (37.5)
	Total		18	92	29 (31.5)

* - Number of infected samples (percentage of infection in parentheses calculated over total number of samples)

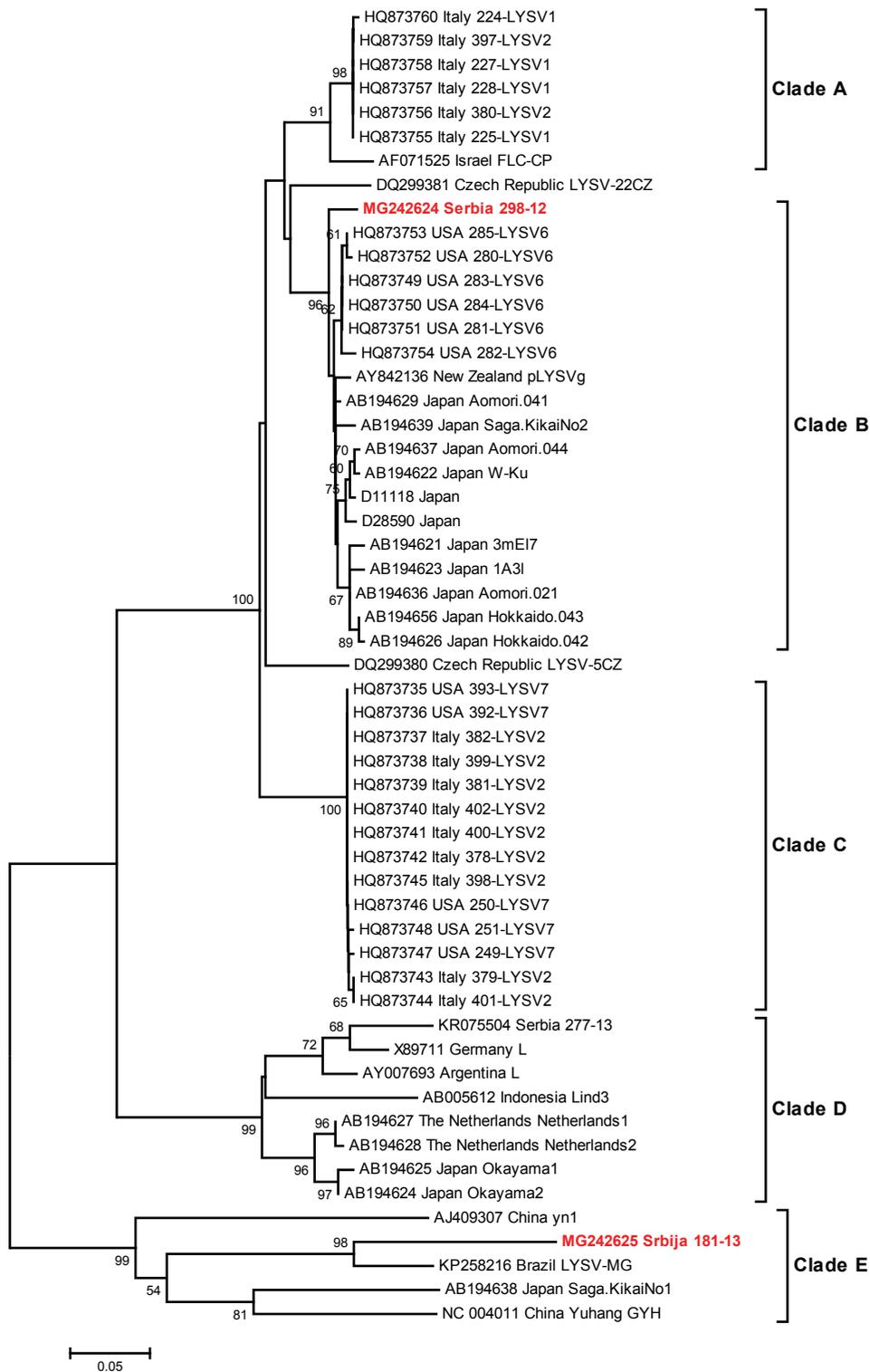


Figure 1. Neighbour-joining tree based on nucleotide sequences of 55 LYSV isolates. Phylogram was generated with MEGA5.2 software using Kimura 2-parameter model Gamma distributed. Bootstrap analysis was performed with 1000 replicates, and bootstrap values (>50%) are shown next to relevant branches. Scale bars: substitutions per site. The Serbian LYSV isolates generated in this study are printed in red.

Clade D contained diverse leek isolates from Serbia, Japan, The Netherlands, Germany and Argentina (0.104 ± 0.014). The fifth clade E comprised garlic isolates from China, Japan and Brazil (0.343 ± 0.037). Genetic diversity among the five clades was ranging from 0.088 ± 0.018 to 0.514 ± 0.064 . Such clustering of 55 LYSV isolates in five distinct groups was supported with high bootstrap values and with high nucleotide homology among the sequences belonging to the same group. Phylogenetic analysis showed the clustering of Serbian LYSV isolates into two distant clades. The Serbian LYSV isolate 181-13 from garlic clustered with several other garlic isolates into the most diverse Clade E, while the isolate 298-13 originating from leek fell into Clade B in which only garlic isolates were grouped.

DISCUSSION

Allium species, especially onion, garlic and leek as the most important crops economically, are prone to virus infections (Katis et al., 2012). The most common viruses that have been associated with allium crops worldwide are members of the genera *Potyvirus*, *Carlavirus* and *Allexivirus*. Viruses belonging to these three genera cause the most important yield losses in allium production worldwide (Conci et al., 2003; Filho et al., 2006; Katis et al., 2012), and LYSV is one of the most important allium viruses around the world (Takaichi et al., 1998; Dovas et al., 2001). Serbian production of allium species which mostly includes onion, garlic and leek, is frequently endangered by viruses which cause prominent virus-like symptoms. There are few reports on the occurrence of LYSV in garlic (Bagi et al., 2010) and leek (Vučurović et al., 2016) in Serbia, but our results have shown that LYSV is an important virus of garlic and leek due to its wide distribution and high incidence.

During this two-year survey, the incidence of virus-like symptoms in inspected crops ranged from 15 to 75%, indicating the importance of virus diseases. Of a total of 92 symptomatic plants, 29 (31.5%) were positive for the presence of LYSV, but the percentage of infected plants was significantly higher (from 40 to 100%) in fields where the virus was found. Similarly, it has been observed in Poland, the Czech Republic and Brazil (Winiarczyk et al., 2014; Klukáčková et al., 2007; Fayad-André et al., 2011). During the present survey, LYSV was detected in leek and garlic crops. The incidence of LYSV was much higher in leek (56.4%) than in garlic crops (17.1%). Viral incidence has been reported from several European countries. In some countries, LYSV was found to be one of the most common viruses in

garlic crops, while in other countries it was common in leek crops. In Greece, the infection with LYSV was confirmed in 83.7% (Dovas et al., 2001) and in Italy in 83% (Dovas & Vovlas, 2003) of garlic samples analyzed. Infection of leek has been reported from major growing areas in Italy (Grancini, 1951), France (Cornuet, 1959), and Turkey (Korkmaz & Cevik, 2009). In Greece, LYSV was detected as the only virus infecting leek with a high incidence of up to 90% (Dovas et al., 2001). In Poland and the Czech Republic, infection of garlic with LYSV was less prevalent than the infection with other allium viruses (Winiarczyk et al., 2014). The presence of LYSV has not been detected in onion plants, which is not surprising because onions and shallots exhibit minimum susceptibility to that virus (Bos, 1981).

The primers designed on the most conservative part of the LYSV genome (Fajardo et al. 2001) were able to amplify sequences from the chosen Serbian LYSV isolates. Sequence analyses of two Serbian isolates revealed over 90% of nt identities (over 94% of aa) with LYSV sequences from GenBank, confirming affiliation of those isolates to the LYSV according to species demarcation criteria in the genus *Potyvirus* (Aleman-Verdaguer et al., 1997; Shukla & Ward, 1988).

The nucleotide and haplotype diversity of LYSV isolates has been reported (Parrano et al., 2012). The sequence analysis performed in this study showed a high genetic diversity among Serbian LYSV isolates originating from different locations and hosts. The phylogenetic analysis also showed high variation between LYSV isolates originating from leek and garlic in Serbia. The nature of the genetic diversity among LYSV isolates has not yet been clarified, but there are some factors that might affect the genetic composition of a virus population, such as geographical barriers that separate virus and host populations, susceptibility of different tissues and systemic vs. localized infections (Takaki et al., 2005; Li & Roossinck, 2004). However, the clustering of LYSV isolates has been reported to be partially correlated with geographic origin or host type (Chen et al., 2002; Parrano et al., 2012).

The structure of reconstructed phylogenetic tree was similar to those previously reported by Takaki et al. (2005) and Parrano et al. (2012), dividing the world LYSV population into three main clades, named A, B and C. The phylogenetic tree constructed with 55 sequences of partial CP gen, including two obtained from LYSV isolates (181-13 and 298-13) during this study and one from an isolate (277-13) previously reported by Vučurović et al. (2016) showed the clustering of LYSV isolates into five clades. Such grouping was supported by very high bootstrap values, but two sequences from

the Czech Republic could not be grouped in any clade, showing polytomy and indicating a lack of data which could point to the origin of those sequences.

Genetic diversity of the sequences in clades A-C is relatively low, indicating high similarity among those sequences, while genetic differences are significantly greater for sequences in clade D and especially clade E, but lower than the values showing genetic diversities between groups, which confirms the grouping in the phylogram. Higher values of the genetic diversity among sequences of clades E and D can indicate older populations because generally it is expected of older populations to have greater genetic variability, compared to younger ones (García-Arenal et al., 2001). Some clades presented evidence that the clustering of LYSV is based on geographic origin with some exceptions. Phylogenetic structuring based on the geographic origin is shown in clade A, which comprised Italian LYSV isolates with an Israel isolate as an exception. Certain grouping of isolates according to geographical origin can indicate virus migration and influence of different evolutionary forces on the shaping of virus population, as it is recorded for the *Papaya ringspot virus* and LYSV (Bateson et al., 1994; Takaki et al., 2005). Some clades have shown clustering based on host plant. Clade C consists of LYSV isolates originating from garlic, while clade D comprises isolates originating from leek only. Interestingly, three isolates originating from Serbia were clustered into different and distant clades (B, D and E). The high sequence divergence among the Serbian LYSV isolates and their clustering into three distant clades indicate that the virus has been well established in Serbia.

Two isolates originating from leek (298-13 and 273-13) fell into distant clades. Leek LYSV 298-13 isolate, identified in this study, was clustered in clade B, which comprised only isolates originating from garlic, while another leek isolate 273-13 clustered in clade D together with other isolates from leek. The clustering of the LYSV 298-13 isolate from leek with isolates from garlic indicated a gene flow from one to another allium crop. This is quite possible because transmission of LYSV by aphids occurs readily. Lunello et al. (2007) showed that the LYSV-Arg isolate from leek was transmitted readily to garlic both mechanically and by aphids.

The results of this study demonstrated the important role of LYSV in leek and garlic production in Serbia and emphasized the need to control the virus. Knowing that LYSV produces great damage in terms of yield, especially in combination with other viruses, further investigation is needed, focusing especially on the incidence and distribution of other viruses infecting allium species.

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Prisustvo i rasprostranjenost virusa žute prugavosti praziluka u usevima različitih vrsta lukova u Srbiji

REZIME

Virus žute prugavosti praziluka (*Leek yellow stripe virus*, LYSV) je jedan od najčešćih i najznačajnijih virusa na praziluku i belom luku. U Srbiji je virus detektovan na obe kulture, i praziluku i belom luku, a često se javlja u visokim procentima. Tokom dve uzastopne godine (2013. i 2014.) pregledano je 11 lokaliteta gajenja praziluka, belog i crnog luka i sakupljena su 92 uzorka koja su DAS-ELISA metodom testirana na prisustvo LYSV. Prisustvo LYSV je dokazano u 31,5% testiranih uzoraka. Tokom 2012., prisustvo LYSV dokazano je samo u praziluku, u 55,6% testiranih uzoraka. Tokom 2013., LYSV je dokazan u 85% uzoraka praziluka i 58,3% uzoraka belog luka. Ukupno, prisustvo LYSV je dokazano u 56,4% uzoraka praziluka i 17,1% uzoraka belog luka. Prisustvo LYSV u testiranim uzorcima potvrđeno je primenom RT-PCR metode i specifičnih prajmera za LYSV koji umnožavaju fragment od 1020 bp koji obuhvata gen za proteinski omotač i deo gena za nuklearne inkluzije B. Molekularna identifikacija LYSV obavljena je sekvenciranjem dva odabrana izolata iz belog luka 181-13 (MG242625) i praziluka 298-13 (MG242624) i poređenjem dobijenih sekvenci sa sekvencama LYSV iz GenBank baze podataka. Filogenetske analize 55 sekvenci izolata LYSV iz različitih delova sveta ukazale su na delimičnu korelaciju između biljke domaćina i geografskog porekla izolata, formiranjem pet odvojenih grupa izolata u stablu. Dva izolata iz Srbije grupisala su se u udaljene grupe. Izolat iz Srbije iz praziluka 298-13 grupisao se u grupu B, dok se izolat iz belog luka 181-13 grupisao u grupu E.

Ključne reči: Virus žute prugavosti praziluka; Praziluk; Beli luk; RT-PCR; ELISA; Srbija