ACTA SCIENTIARUM POLONORUM

Acta Sci. Pol. Hortorum Cultus, 19(5) 2020, 91–99

https://czasopisma.up.lublin.pl/index.php/asphc ISSN 1644-0692 e-ISSN 2545-1405 DOI: 10.24326/asphc.2020.5.10

ORIGINAL PAPER

Accepted: 18.10.2019

INCIDENCE OF VIRUSES IN CLOVES AND BULBILS OF GARLIC ECOTYPES IN CROATIA

Sara Godena[1], Dean Ban[1,2], Gvozden Dumičić[3], Smiljana G. Ban[1,2]

[1] Institute of Agriculture and Tourism, Carlo Hugues 8, 52440 Poreč, Croatia

[2] Centre of Excellence for Biodiversity and Molecular Plant Breeding, Svetošimunska 25, 10000 Zagreb, Croatia

[3] Institue for Adriatic Crops and Karst Reclamation, Put Duilova 11, 21000 Split, Croatia

ABSTRACT

In a survey on the sanitary status of garlic collection performed in 2016 at the Institute of Agriculture and Tourism in Poreč (Croatia), 24 ecotypes and cultivars were tested by DAS-ELISA test for the presence of four main garlic viruses: *Garlic common latent virus* (GCLV), *Shallot latent virus* (SLV), *Leek yellow stripe virus* (LYSV) and *Onion yellow dwarf virus* (OYDV). The least frequent virus was SLV, whereas the most frequent was LYSV. In most ecotypes the infection of cloves was lower than infection of bulbils, while for SLV we found higher infection in cloves. Total virus infection of Istrian ecotypes was lower than infection of Dalmatian ones. Total incidence of viruses in Croatian garlic material ranged from 40.6% to 100.0%, depending on ecotype. In imported cultivar 'Rose de Lautrec' we found no infection with SLV. A high percentage of mixed infections in garlic cloves was also found and for double infections it ranged from 69% to 88%. Infection with all tested viruses was observed in 78% cloves. The most infected combination was double infections with potyviruses LYSV+OYDV. To the best of our knowledge, this is the first report of SLV infection in garlic in Croatia.

Key words: *Allium sativum* var. *sativum*, GCLV, LYSV, OYDV, SLV

INTRODUCTION

Garlic (*Allium sativum* var. *sativum*) is one of the oldest vegetables cultivated in the world for over 6000 years [Fiume 2005, Winiarczyk et al. 2014]. The world's largest producer of garlic is China [FAOSTAT 2016], although the countries of the Mediterranean region are also important Allium producers [Katis et al. 2012].

Garlic is an indigenous crop in Croatia and it is widely cultivated mostly at small family farms and households. In 2016, Croatia had 245 ha under garlic production with 1443 t of total production [FAOSTAT 2016, Croatian Bureau of Statistics 2017]. Each region or even village has its own garlic ecotype and there is a substantial genetic variability present in a small region. Viruses persisted in garlic population and by vegetative propagation by cloves or bulbils from year to year, garlic crops are constantly infected [Katis et al. 2012].

Garlic viruses induce different symptoms like chlorotic streaking, mottling, twisted and curled leaves, diminution in vigor and formation of little bulbs and cloves which may lead to very important reduction in crop quantity (up to 60%) and quality [Dewan et al. 1995, Lot et al. 1998, Takaichi et al. 2001, Conci et al. 2003, Ramírez-Malagón et al. 2006, Lunello et al. 2007, Conci et al. 2010, Katis et al. 2012]. The main viruses in *Allium* crops are two economically important and the most common potyviruses, *Onion yellow dwarf virus* (OYDV) and *Leek yellow stripe virus* (LYSV), two latent carlaviruses, *Shallot latent virus*

✉ sara@iptpo.hr

(SLV) and *Garlic common latent virus* (GCLV) and eight species of allexiviruses, which have a worldwide distribution [Katis et al. 2012]. Natural and artificial infections also play an important role in yield, because plants originating from cloves of infected plants suffered greater yield losses than plants originating from healthy cloves that were mechanically inoculated [Lot et al. 1998].

Local ecotypes of garlic are maintained in gene banks as a part of national program for preserving agricultural biodiversity (Croatian Plant Genetic Resources). Garlic is vegetatively propagated each year and there are concerns about presence of virus infection, but also constraints for future program due to possibility for spreading infections among ecotypes.

Therefore, the aim of this work was to analyze the sanitary status of cloves and bulbils of garlic collected from various production regions in Croatia for the presence of four main garlic viruses using serological diagnostic method double-antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA).

MATERIAL AND METHODS

Plant material

Garlic accessions tested in this study originate from the area across Adriatic cost of Croatia and one

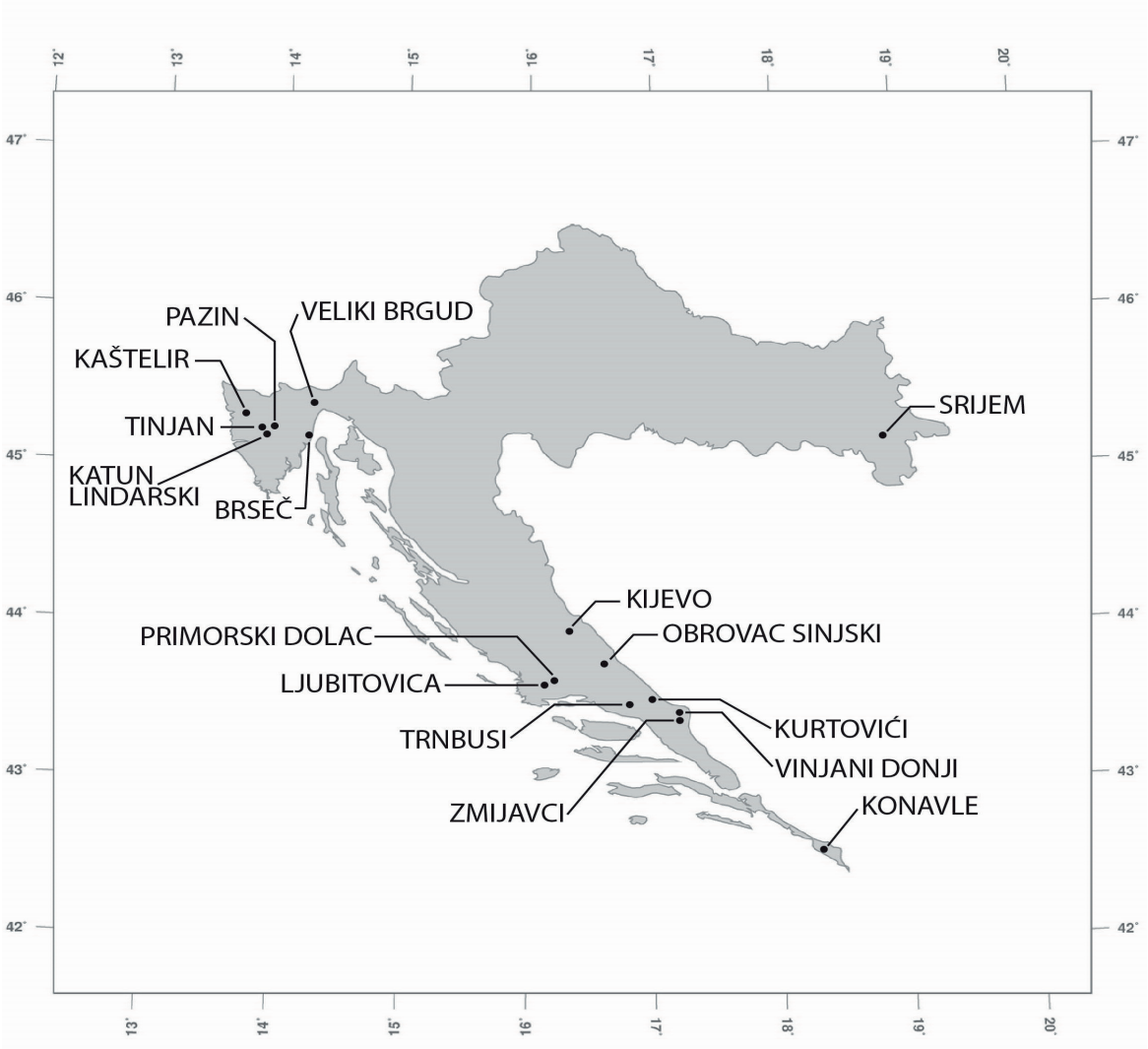

Fig. 1 The geographical origin of tested Croatian garlic ecotypes

accession (IPT205, Srijemski) is from inland (Fig. 1). They are maintained as a part of *Allium* program in Croatian Gene Bank (CGB) at Institute of Agriculture and Tourism in Poreč (IPT) from 2014 and at Institute for Adriatic Crops and Karst Reclamation in Split (IJK) from 2012. The introduced garlic cultivars are maintained in CGB in Poreč from 2014 (Chinese; accession No. IPT207) and 2015 (French; 'Rose de Lautrec'; IPT206).

Garlic cloves supplied from these collections were planted in the experimental fields in Poreč on December 17th, 2015 and in Split on October 28th, 2015 and were grown using standard growing practice for garlic production [Lešić et al. 2002, Dumičić et al. 2013]. The number of planted cloves was at least 82 to more than 500 (IPT accessions) and from 24 to 60 (IJK accessions). The plants were harvested on July 14th (210 days after planting) in Poreč and on July 7th in Split (254 days after planting).

Sampling

Symptomatic and asymptomatic garlic plants have been randomly collected in the fields of both locations. In total, 24 accessions have been tested (Tab. 1). For each ecotype five to eight plants were sampled for virus detection. For IPT accessions, cloves and bulbils were collected and for IJK accessions only cloves were sampled.

DAS-ELISA test

In total, 227 randomly chosen samples of garlic (154 cloves and 73 bulbils – i.e. vegetative topset of the inflorescences) were tested for the presence of viruses. Plant tissue derived from cloves (one clove removed from each bulb) and bulbils was tested on the presence of four main garlic viruses *Onion yellow dwarf virus* (OYDV), *Leek yellow stripe virus* (LYSV), *Shallot latent virus* (SLV) and *Garlic common latent virus* (GCLV).

For virus detection, DAS-ELISA was performed according to Clark and Adams [1977] using commercial kits with commercial positive and negative controls (Catalogue no. 1202-24, 1204-24, 1199-24 and 1222-24 Neogen, Scotland) and following manufacturer's instructions. Two replicates were used for each sample. Volume used was 100 µL/well. Reactions were read after one and after two hours of incubation with absorbance microplate reader (Sunrise™ Tecan, Switzerland) at wavelenght λ = 405 nm and absorbance was analyzed with Magellan™ software. Samples were consider positive if the average OD value from each of the duplicate sample wells is $\geq 2 \times$ OD of that in the negative control of healthy plant extract according to OEPP/EPPO [2015].

RESULTS

At harvest in collection fields, 40 representative garlic cloves of each ecotype were sampled and height, weight and diameter of each clove were measured. Weight of garlic bulbs ranged from 17.7 g (IJK12) to 90.8 g (IJK22) – Table 1. Of the tested accessions, 15 had weight lower than 40 g/bulb and 9 weighed more than 40 g/bulb. The height of the bulbs ranged from 26.3 mm (IPT015) to 56.6 mm (IJK22), with majority of bulbs higher than 30 mm (16 accessions).

Symptoms of yellow stripes on garlic leaves were observed in the field in Poreč from the end of May. The presence of viruses in cloves of Croatian material was generally higher in IJK (Dalmatian) accession compared to IPT (Istrian) accessions (Tab. 2). In cloves and bulbils of IPT collection viruses ranged from 0.0% (0/8) to 100.0% (8/8), while in cloves of IJK collection viruses ranged from 20.0% (1/5) to 100.0% (5/5). Croatian garlic material was totally infected only with LYSV. In both plant samples (cloves and bulbils), in imported material viruses ranged from 0.0% (0/4) to 100.0% (4/4). Tested imported garlic materials were totally infected (e.g. 5/5 and 8/8, respectively) with both potyviruses LYSV and OYDV.

Overall, the least frequent virus in all tested garlic samples was carlavirus SLV, which was not detected neither in bulbils nor in cloves of ecotype IPT206 (from France), whereas the most frequent virus was LYSV. In both cloves and bulbils, the virus incidence for GCLV, SLV and OYDV ranged between 12.5% (1/8 tested samples) to 100.0%, (8/8), while incidence of LYSV was 100.0% (8/8 tested samples). The carlavirus GCLV was detected in all ecotypes except for IPT016 and IPT020; SLV was detected in all accessions except for IPT019 and IPT206; while LYSV and OYDV were detected in all accessions. For accessions were cloves and bulbils were tested we found lower cloves infection for GCLV and OYDV, but SLV showed lower infection rate of bulbils compared to cloves infection (Tab. 2).

Table 1. Accession number, ecotype name and main bulb characteristics of tested garlic

Accession No.	Ecotype/cultivar	Bulb		
		weight (g bulb^{-1} ±SD)	height (mm bulb^{-1} ±SD)	diameter (mm bulb^{-1} ±SD)
Croatian material				
IPT010[a]	Pazin – crveni	26.5 ±0.5[c]	28.0 ±0.5	39.0 ±1.7
IPT011	Kaštelir 1	27.4 ±1.9	27.3 ±0.8	39.9 ±1.3
IPT012	Kaštelir 2	31.3 ±2.7	28.0 ±0.7	41.9 ±1.7
IPT013	Pazin red	26.0 ±0.8	26.4 ±0.2	39.0 ±0.5
IPT014	Tinjan	31.9 ±2.2	31.5 ±1.8	40.6 ±0.9
IPT015	Pazin – bijeli	21.0 ±2.3	26.3 ±1.3	35.3 ±1.6
IPT016	Katun lindarski 1	23.0 ±0.9	26.4 ±0.7	38.3 ±0.6
IPT017	Katun lindarski 2	26.4 ±1.7	28.1 ±0.8	38.6 ±0.9
IPT019	Veliki brgud	25.2 ±2.4	27.1 ±0.5	40.6 ±1.2
IPT020	Brseč	30.4 ±3.1	28.1 ±0.7	41.5 ±1.6
IPT205	Srijemski	44.6 ±3.5	33.2 ±0.6	48.7 ±1.3
IJK3[b]	Zmijavci	31.7 ±3.6	29.0 ±1.3	40.3 ±2.0
IJK7	Tinjan	38.0 ±3.7	40.6 ±1.5	47.4 ±1.7
IJK8	Kurtovići	22.4 ±2.0	31.2 ±1.2	33.0 ±1.4
IJK9	Obrovac Sinjski	40.5 ±1.3	32.8 ±1.8	43.8 ±1.2
IJK12	Konavle	17.7 ±1.4	31.4 ±1.5	34.4 ±1.1
IJK13	Primorski dolac	56.3 ±2.6	43.7 ±0.7	59.1 ±0.9
IJK14	Trnbusi 1	36.9 ±1.7	39.4 ±1.2	45.2 ±0.9
IJK20	Trnbusi 2	44.2 ±2.4	34.8 ±0.9	48.1 ±0.9
IJK22	Vinjani donji	90.8 ±0.7	56.6 ±1.1	74.0 ±1.5
IJK23	Kijevo	47.3 ±2.6	38.3 ±2.1	51.1 ±0.9
IJK26	Ljubitovica	58.0 ±4.6	39.0 ±0.9	53.8 ±1.0
Introduced material				
IPT206	‘Rose de Lautrec’	42.6 ±5.7	33.6 ±0.6	48.9 ±1.5
IPT207	Chinese	45.3 ±4.4	30.4 ±0.5	51.7 ±1.2

[a] accessions maintained at Institute of Agriculture and Tourism in Poreč in Istria region (IPT);
[b] accessions maintained at Institute for Adriatic Crops and Karst Reclamation in Split in Dalmatia region (IJK);
[c] mean value ±SD, n = 40

Table 2. Infection – number of infected samples/ number of tested samples of Croatian and imported garlic material in cloves (C) and bulbils (B) and total infection of both (cloves and bulbils) as revealed by DAS-ELISA test for carlaviruses (GCLV and SLV) and potyviruses (LYSV and OYDV)

Ecotype/ region	GCLV		SLV		LYSV		OYDV		Infection of C n = 154	Infection of B n = 73
	C	B	C	B	C	B	C	B		
Croatian material										
IPT010[a]	8/8	6/8	7/8	6/8	8/8	8/8	4/8	6/8	27/32	26/32
IPT011	7/8	7/8	7/8	8/8	8/8	8/8	5/8	8/8	27/32	31/32
IPT012	8/8	8/8	7/8	8/8	8/8	8/8	7/8	8/8	30/32	32/32
IPT013	6/8	8/8	8/8	8/8	8/8	8/8	5/8	8/8	27/32	32/32
IPT014	8/8	n.t.[c]	7/8	n.t.	8/8	n.t.	5/8	n.t.	28/32	n.t.
IPT015	5/8	n.t.	7/8	n.t.	8/8	n.t.	2/8	n.t.	21/32	n.t.
IPT016	0/8	7/8	3/8	8/8	8/8	8/8	2/8	8/8	13/32	31/32
IPT017	1/8	6/6	3/8	5/6	8/8	6/6	2/8	5/6	14/32	22/24
IPT019	1/8	7/8	4/8	0/8	8/8	8/8	1/8	8/8	14/32	23/32
IPT020	0/8	8/8	5/8	1/8	8/8	8/8	4/8	8/8	17/32	25/32
IPT205	1/8	n.t.	6/8	n.t.	8/8	n.t.	2/8	n.t.	17/32	n.t.
IJK3[b]	5/5	n.t.	1/5	n.t.	5/5	n.t.	5/5	n.t.	16/20	n.t.
IJK7	5/5	n.t.	5/5	n.t.	5/5	n.t.	5/5	n.t.	20/20	n.t.
IJK8	5/5	n.t.	5/5	n.t.	5/5	n.t.	5/5	n.t.	20/20	n.t.
IJK9	5/5	n.t.	5/5	n.t.	5/5	n.t.	5/5	n.t.	20/20	n.t.
IJK12	4/5	n.t.	5/5	n.t.	5/5	n.t.	5/5	n.t.	19/20	n.t.
IJK13	5/5	n.t.	5/5	n.t.	5/5	n.t.	5/5	n.t.	20/20	n.t.
IJK14	5/5	n.t.	5/5	n.t.	5/5	n.t.	5/5	n.t.	20/20	n.t.
IJK20	3/5	n.t.	5/5	n.t.	5/5	n.t.	5/5	n.t.	18/20	n.t.
IJK22	5/5	n.t.	5/5	n.t.	5/5	n.t.	4/5	n.t.	19/20	n.t.
IJK23	5/5	n.t.	5/5	n.t.	5/5	n.t.	5/5	n.t.	20/20	n.t.
IJK26	4/4	n.t.	4/4	n.t.	4/4	n.t.	4/4	n.t.	16/16	n.t.
Imported material										
IPT206[b]	4/4	2/4	0/4	0/4	4/4	4/4	4/4	4/4	12/16	10/16
IPT207[a]	7/8	4/6	4/8	1/6	8/8	6/6	8/8	6/6	27/32	17/24

[a] n = 8 (IPT ecotypes), [b] n = 5 (IJK ecotypes), [c] n.t. – not tested

Table 3. Percentage of multiple infections in cloves and bulbils as revealed by DAS-ELISA test

Various multiple infection of cloves and bulbils	Infection of cloves (percentage)	Infection of bulbils (percentage)
GCLV + SLV	73	75
GCLV + LYSV	85	94
GCLV + OYDV	69	92
SLV + LYSV	88	82
SLV + OYDV	72	79
LYSV + OYDV	84	98
GCLV + SLV + LYSV	82	84
GCLV + SLV + OYDV	71	82
LYSV + OYDV + GCLV	79	95
SLV + LYSV + OYDV	81	86
GCLV + SLV + LYSV + OYDV	78	87

In this survey multiple infections of garlic cloves ranged from 69% (GCLV + OYDV) to 88% (SLV + LYSV) – Table 3. The presence of three viruses was found in 71% to 82% tested cloves, while multiple infections with all four tested viruses was observed in 78% of the tested cloves. Similar situation was observed for tested bulbils and at least 75% bulbils had double infection, at least 82% were infected with three viruses and 87% was infected with four viruses. The combination with the highest frequency of bulbils infection was combination of potyviruses LYSV + OYDV (Tab. 3).

DISCUSSION

The presence of viruses is a serious problem in garlic production since they could substantially c yield and quality. In this study, we have tested cloves of local ecotypes of garlic primarily from the Croatian coastal area, and bulbils when they were avilable. Generally, in both collections we found a high incidence of infected cloves and the most widespread virus was LYSV. Only in ecotype IPT206 ('Rose de Lautrec') the incidence of SLV was 0.0% for both cloves and bulbils. Compared to other Croatian ecotypes it seems that IPT019 (Veliki brgud) has lower rate of infection for majority of tested viruses (Tab. 2).

In addition to a single virus presence in garlic, we have observed a high rate of mixed infections in cloves and bulbils. More than 69% of the tested cloves were infected with more than one virus, and 75% to 98% of the bulbils had mixed infections. The phenomenon of mixed infections is very common in garlic [Keller and Senula 2001, Katis et al. 2012] and mixed infection at 86% for OYDV, 57% for LYSV, and 29% for GCLV were reported previously [Parrano et al. 2012]. In our study, mixed infection by potyviruses (LYSV and OYDV) was observed at high rate, which, in other study resulted in severe symptoms in plants, with a yield loss from 33% [Takaichi et al. 2001] to 91% [Lot et al. 1998].

Latent carlaviruses were widely more prevalent in Germany, Czech Republic and Poland [Keller and Senula 2001, Klukáčková et al. 2007, Smékalová et al. 2010, Winiarczyk et al. 2014], but in our work economically important potyviruses were more frequent, as reported in Italy [Dovas and Volvas 2003]; Israel [Koch et al. 1995]; Argentina [Conci et al. 2002, Conci

et al. 2003, Lunello et al. 2007, Perotto et al. 2014]; Japan [Takaichi et al. 2001] and Mexico [Velásquez-Valle et al. 2012].

In this study, incidence of viruses in cloves of 11 ecotypes from southern parts of Dalmatia (IJK3-IJK26) was very high, whereas Vončina et al. [2016] in northern Dalmatia found much lower incidence of GCLV in leaves (94.5% vs. 11.6%) and LYSV (100% vs. 7.4%). The incidence of virus infection in ten ecotypes from Istria (IPT010-IPT020, without IPT205) was lower for GCLV, SLV, and OYDV compared to the south of the country (Tab. 2). A high incidence of viruses has been found in imported bulbs from China (IPT207) and incidence was extremely high for potyviruses. Similar was also observed in the Czech Republic in the analyzed samples from China [Klukáčková et al. 2007].

The time of sampling is also very important for subsequent results in diagnosis. Our samples were 210 to 254 days old when tested, which is the optimum time to detect virus according to previous studies to ensure virus detection [Conci et al. 2010]. The concentration of LYSV differs according to the sampling time, cultivar and region, which is probably due to the physiological state of the crop, virus-cultivar relation and the ambient temperature [Conci et al. 2002]. Also, potyviruses and carlaviruses are transmitted by various migratory species of aphids (*Myzus ascallonicus*, *Aphis fabae* complex and the genus *Macrosiphum*) in the non-persistent manner [OEPP/EPPO 1995, Brunt et al. 1996, Katis et al. 2012, Perotto et al. 2014, Winiarczyk et al. 2014], therefore, virus infection can be spread very fast in new crop particularly if they are present in propagation material. Total aphid catch is also very important and it is a better predictor of virus spread than catches of any single species or a combination of a few key species [Perotto et al. 2014].

On some garlic ecotypes in this work, yellow stripes on leaves were observed by the end of May till July when garlic was harvested. A single infections with either OYDV or OYDV in combination with other viruses like LYSV [Lot et al. 1998, Klukáčková et al. 2007] always cause bright yellow-green stripes on garlic leaves; however, symptoms may be different depending on whether garlic was propagated by cloves or bulbils [Parrano et al. 2012]. Although, it was proposed that potyviruses reduces bulb and clove sizes [Lot et al. 1998, Takaichi et al. 2001, Lunello et al. 2007] we couldn't directly relate bulb characteristics to frequency of infection or specific virus. Takaichi et al. [2001] stated that the disease symptoms were observed in proportion to the infection rate of LYSV, but the apparent reduction in bulb weight was not directly related to the infection of LYSV.

In our survey, for accessions where bulbils and cloves were tested, the incidence of viruses in bulbils was higher than in cloves for OYDV, similarly to Koch et al. [1995] who reported that aerial bulblets were generally more infected than cloves. However, the frequency of SLV was higher in cloves than in bulbils in four ecotypes. According to Winiarczyk et al. [2014], viruses are most frequently identified in the underground bulbs than in the inflorescence and leaves, especially for potyviruses OYDV and LYSV. Other authors stated that virus status of garlic bulbils is not different from that of cloves [Parrano et al. 2012]. Accumulation of virus in different plant and in different part of the plant is probably influenced by developmental stage of plant [Conci et al. 2010]. Potyviruses are not uniformly distributed and an irregular distribution of positive and negative cloves within a single bulb was also observed [Ramírez-Malagón et al. 2006]. However, Koch et al. [1995] and Conci et al. [2010] proposed that testing of few bulbs per clove could be sufficient for determination of health status of plant. Regarding leaves, no one type or position of leaf is preferable for detecting virus presence [Conci et al. 2010].

According to our results, it seems that different garlic tissues, such as cloves and bulbils, are suitable for virus detection, as previously reported by some authors [Koch et al. 1995, Conci et al. 2002, 2010, Winiarczyk et al. 2014]. Aerial bulbils are suitable test material because they contain the highest concentration of OYDV and can be used instead of valuable planting material [Koch et al. 1995].

To the best of our knowledge, this is the first report of SLV in garlic in Croatia. Viruses OYDV, LYSV and GCLV were previously detected from garlic leaves in Croatia [Vončina et al. 2016]. In this survey these three viruses were detected in cloves and bulbils.

ACKNOWLEDGEMENTS

The author would like to thank to Igor Pasković, Igor Palčić, Marko Černe (Institute of Agriculture and

Tourism Poreč, Croatia), Branimir Urlić, and Marija Mandušić (Institute for Adriatic Crops, Split, Croatia) for their valuable technical assistance and help during this research. Also, we would like to thank the Ministry of Agriculture Republic of Croatia for financial support in collecting and maintaining studied collections and the project KK.01.1.1.01.0005 Biodiversity and Molecular Plant Breeding for supporting author.

REFERENCES

Brunt, A.A., Crabtree, K., Dallwitz, M.J., Gibbs, A.J., Watson, L., Zurcher, E.J. (1996). Plant Viruses Online: Descriptions and List from the VIDE Database. Available: http://sdb.im.ac.cn/vide/sppindex.htm [date of access: 9.03.2018].

Clark, M.F., Adams, A.N. (1977). Characteristics of the microplate method of enzyme-linked immunosorbent assay for the detection of plant viruses. J. Gen. Virol., 34, 475–483. DOI: 10.1099/0022-1317-34-3-475

Conci, V.C., Canavelli, A., Lunello, P., Di Rienzo, J., Nome, S.F., Zumelzu, G., Italia, R. (2003). Yield losses associated with virus-infected garlic plants during five successive years. Plant Dis., 87(12), 1411–1415. Available: https://apsjournals.apsnet.org/doi/pdf/10.1094/PDIS.2003.87.12.1411 [date of access: 16.02.2017].

Conci, V.C., Canavelli, A.E., Balzarini, M.G. (2010). The distribution of garlic viruses in leaves and bulbs during the first year of infection. J. Phytopathol., 158, 186–193. DOI: 10.1111/j.1439-0434.2009.01601.x

Conci, V.C., Lunello, P., Buraschi, D., Italia, R.R., Nome, S.F. (2002). Variations of Leek yellow stripe virus concentration in garlic and its incidence in Argentina. Plant Dis., 86(10), 1085–1088.

Croatian Bureau of Statistics (2017). 16-7. Production of Vegetables. In: Statistical Yearbook of the Republic of Croatia 2017, Ostroški, Lj. (ed.). Zagreb, 262–263.

Dewan, C., Pearson, M.N., Scheffer, J.J.C. (1995). Natural field infection of garlic by garlic yellow streak virus in the Pukekohe area of New Zaeland and associated problems with the introduction of new garlic cultivars. N. Z. J. Crop Hortic. Sci., 23, 97–102.

Dovas, C.I., Volvas, C. (2003). Viruses infecting *Allium* spp. in southern Italy. J. Plant Pathol., 85(2), 135.

Dumičić, G., Čagalj, M., Urlić, B., Runjić, M., Goreta Ban, S. (2013). Komponente prinosa češnjaka (*Allium sativum* L.) [Yield components of garlic (*Allium sativum* L.)]. Zbornik radova 48. hrvatskog i 8. međunarodnog simpozija agronoma, 17th–22nd February 2013, Dubrovnik, Croatia, 349–352 [in Croatian].

FAOSTAT – Food and Agricultural Organization of the United Nations (2016). Statistics. Available: http://faostat.fao.org [date of access: 14.03.2018].

Fiume, F. (2005). Garlic. Available: http://francescofiume.altervista.org/Garlic.html [date of access: 11.09.2019.]

Katis, N.I., Maliogka, V.I., Dovas, C.I. (2012). Virus of the genus *Allium* in the Mediterranean region. In: Advances in virus research, Loebenstein, G., Lecoq, H. (eds). vol. 84, Elsevier Inc., Amsterdam, 163–208.

Keller, E.R.J., Senula, A. (2001). Progress in structuring and maintaining the garlic (*Allium sativum*) diversity for the European Genres Project. Acta Hortic., 555, 189–193. DOI: 10.17660/actahortic.2001.555.28

Klukáčková, J., Navrátil, M., Duchoslav, M. (2007). Natural infection of garlic (*Allium sativum* L.) by viruses in the Czech Republic. J. Plant Dis. Prot., 114(3), 97–100. DOI: 10.1007/BF03356714

Koch, M., Ta'anami, Z., Levi, S., Salomon, R. (1995). Testing garlic cloves and bublets for Onion yellow dwarf virus by ACP-ELISA. Phytoparasitica, 23(1), 27–29. DOI: 10.1007/BF02980393

Lešić, R., Borošić, J., Buturac, I., Herak Ćustić, M., Poljak, M., Romić, D. (2002). Povrćarstvo. Zrinski, Čakovec [in Croatian].

Lot, H., Chovelon, V., Souche, S., Delecolle, B. (1998). Effects of Onion yellow dwarf and Leek yellow stripe viruses on symptomatology and yield loss of three French garlic cultivars. Plant Dis., 8(12), 1381–1385. DOI: 10.1094/PDIS.1998.82.12.1381

Lunello, P., Di Renzo, J., Conci, V.C. (2007). Yield loss in garlic caused by *Leek yellow stripe virus* Argentinian isolate. Plant Dis., 91(2), 153–158. DOI: 10.1094/PDIS-91-2-0153

OEPP/EPPO (1995). EPPO Standards PP 2/4(2) Guidelines on good plant protection practice: *Allium* crops. Bulletin OEPP/EPPO Bulletin, 1–12.

OEPP/EPPO (2015). EPPO Standard PM 7/125 (1) ELISA tests for viruses. Bulletin OEPP/EPPO Bulletin, 45(3), 445–449. DOI: 10.1111/epp.12259

Parrano, L., Afunian, M., Pagliaccia, D., Douhan, G., Vidalakis, G. (2012). Characterization of viruses associated with garlic plants propagated from different reproductive tissues from Italy and other geographic regions. Phytopathol. Mediterr., 51(3), 549–565. DOI: 10.14601/Phytopathol_Mediterr-10479

Perotto, M.C., Di Rienzo, J.A., Lanati, S., Panonto, S., Macchiavelli, R., Cafrune, E.E., Conci, V.C. (2014). Temporal and spatial spread of potyvirus infection and its

relationship to aphid populations visiting garlic crops. Australas. Plant Pathol., 43, 623–630. DOI: 10.1007/s13313-014-0312-9

Ramírez-Malagón, R., Pérez-Moreno, L., Borodanenko, A., Salinas-González, G.J., Ochoa-Alejo, N. (2006). Differential organ infection studies, potyvirus elimination and field performance of virus-free garlic plants produced by tissue culture. Plant Cell Tissue Organ Cult., 86, 103–110. DOI: 10.1007/s11240-006-9102-6

Smékalová, K., Stavlíková, H., Dušek, K. (2010). Distribution of viruses in the garlic germplasm collection of the Czech Republic. J. Plant Pathol., 92(1), 273–274. DOI: 10.4454/jpp.v92i1.43

Takaichi, M., Yamamoto, M., Nagakubo, T., Oeda, K. (2001). Mixed virus infections of garlic determined by Multivalent Polyclonal Antiserum and virus effects on disease yymptoms. Plant Dis., 85(1), 71–75. Available: https://apsjournals.apsnet.org/doi/pdf/10.1094/PDIS.2001.85.1.71 [date of access: 16.02.2017].

Velásquez-Valle, R., Reveles-Hernandez, M., Amador-Ramirez, M.D. (2012). Viral distribution on asymptomatic onion (*Allium cepa* L.) plants. Rev. Mex. Cienc. Agric., 3(7), 1425–1434.

Vončina, D., Ćurić, K., Fabek, S., Toth, N. (2016). First report of *Onion yellow dwarf virus*, *Leek yellow stripe virus* and *Garlic common latent virus* on Garlic in Croatia. Plant Dis., 100(3), 656. DOI: 10.1094/PDIS-08-15-0952-PDN

Winiarczyk, K., Solarska, E., Sienkiewicz, W. (2014). Prevalence of infections with *Onion yellow dwarf virus*, *Leek yellow stripe virus* and *Garlic common latent virus* in plants from the genus *Allium.* Acta Sci. Pol. Hortorum Cultus., 13(3), 123–133.

Godena, S., Ban, D., Dumičić, G., Ban, S.G. (2020). Incidence of viruses in cloves and bulbils of garlic ecotypes in Croatia. Acta Sci. Pol. Hortorum Cultus, 19(5), 91–99. DOI: 10.24326/asphc.2020.5.10