DOI: 10.17707/AgricultForest.65.4.05

Khrystyna SHEREVERA, Tetiana SHEVCHENKO,
Halina SNIHUR, Irena BUDZANIVSKA, Oleksiy SHEVCHENKO [1]

FIRST REPORT OF LEEK YELLOW STRIPE VIRUS ON *ALLIUM SATIVUM* L. IN UKRAINE

SUMMARY

Garlic (*Allium sativum* L.) is a valuable crop, cultivated all over the world. Viral diseases of garlic are one of the factors that reduce quality of garlic yield. *Leek yellow stripe virus* belongs to genus *Potyvirus*, family *Potyviridae*, and garlic infected with LYSV is found in many places of garlic production. In 2018, we screened garlic-growing areas in different regions of Ukraine and collected plants with symptoms characteristic of viral infection: yellow striping and streaking, mosaic, spotting and plant growth retardation. DAS-ELISA with commercial diagnostic kit for LYSV (Adgia, USA) showed that 28% of collected plants were infected with LYSV. LYSV was detected in Vinnytsia region (Bershad district), Kyiv region (Boryspil district), and Poltava region (Semenivka district) in Ukraine, suggesting wide spread of the virus. According to available literature data, LYSV is often found in coinfection with OYDV. However, we found only 7% of plants to be mixed infected (LYSV+OYDV). Subsequent transmission electron microscopy revealed viral particles of 720-800 nm in length and 16 nm in diameter as characteristic for *Potyvirus* representatives. Further, LYSV infection was also confirmed by RT-PCR with coat protein gene-specific primers generating LYSV cDNA of expected length (~409 bp). This is the first report of LYSV-infected garlic plants in Ukraine that proves LYSV is widely spread in Ukraine.

Keywords: leek yellow stripe virus, onion yellow dwarf virus, garlic, potyvirus, Ukraine.

INTRODUCTION

Garlic stocks worldwide are infected with a complex of viruses, including two potyviruses: *Onion yellow dwarf virus* (OYDV) and *Leek yellow stripe virus* (LYSV). LYSV is a member of *Potyvirus* genus belonging to the largest *Potyviridae* family of plant viruses. LYSV has flexible filamentous particles ~800 nm long containing a single-stranded positive sense genomic RNA of about 10,000 nt (King et al., 2012).

[1]Khrystyna Sherevera, Tetiana Shevchenko, Halina Snihur, Irena Budzanivska, Oleksiy Shevchenko*(corresponding author: alexshevchenko@ukr.net), Virology Department, ESC "Institute of Biology and Medicine", Taras Shevchenko National University of Kyiv, 64/13 Volodymyrska Street, City of Kyiv, 01601, UKRAINE

Paper presented at the 10th International Scientific Agricultural Symposium "AGROSYM 2019".
Notes: The authors declare that they have no conflicts of interest. Authorship Form signed online.

Contrary to many other potyviruses, LYSV has narrow natural host range mostly limited to *Allium* sp. plants and induces persistent symptoms (yellow leaf striping and mosaic are most common). LYSV is mainly transmitted by aphids non-persistently, mechanically from plant to plant, as well as with planting material. The last route of transmission is especially important for commercial garlic production.

LYSV probably occurs worldwide and has been found in both temperate and subtropical regions of Asia (including China and India) (Chen et al., 2002; Gupta et al., 2013), North Africa, Europe, Oceania, and North and South America (Pappu et al., 2005; Oleas, Arahana, 2015). In Europe, LYSV was reported from Sweden, Finland, Denmark, France, Belgium, the Netherlands, Germany, Italy, Greece, Slovenia, Croatia, Serbia and Poland (Lot et al., 1994; Dovas et al., 2001; Chodorska et al., 2014; Vončina et al., 2016; Vučurović et al., 2016; EPPO, 2019).

Ukraine is one of the largest European countries enjoying strategic position between the eastern EU states and Black Sea/Middle East region, where LYSV was also detected in Turkey (Fidan, Baloglu, 2009) and Iran (Shahraeen, Lesemann, Ghotbi, 2008). Recently, authors have reported on the occurrence and possible wide spread of OYDV in Ukraine (Snihur et al., 2019). In this study, authors included the results of LYSV screening in various regions.

MATERIAL AND METHODS

Sampling was restricted to private gardens in 4 distant parts of Ukraine: Kyiv, Cherkassy, Vinnytsia, and Poltava regions. Garlic, onion and leek plants were visually examined; samples were collected from plants with LYSV-like symptoms typically including striping, mosaics, leaf discoloration, and/or stunting.

Collected samples were tested for LYSV by double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA), as described previously by Clark and Adams (1977), using specific polyclonal antibodies purchased from Adgia (USA) and following the manufacturer's recommendations. Briefly, 0,5 g leaf tissue was ground to a powder with a mortar and pestle in 10 mL phosphate-buffered saline, pH 7,4, containing 0,05% Tween 20, 2,0% polyvinylpyrrolidone (MW 40 000) and 0,2% bovine serum albumin. In the meantime, microtitre plates (Greiner Bio-One, Germany) were coated with LYSV-specific broad-spectrum polyclonal antibodies (1:100) in carbonate buffer according to the manufacturer's instructions. Leaf extracts were then added to the plates in duplicate wells and incubated overnight at 4°C. The presence of LYSV in the samples was detected in 200 µL homogenate by LYSV-specific antibodies conjugated to alkaline phosphatase using *p*-nitrophenyl phosphate substrate (Sigma, USA). Absorbance values at 405 nm were measured using a Thermo Labsystems Opsis MR microtitre plate reader (USA). Absorbance values, measured 60 min after adding the substrate, greater than three times those of the negative controls were considered positive.

For transmission electron microscopy (TEM), copper grids (Sigma, USA) were coated with chloroform-dissolved 0.2% polyvinyl formaldehyde (Serva, Germany) and dried overnight on filter paper at room temperature. The samples deposited onto grids were stained with 2.5% uranyl acetate and 0.02 N lead citrate (Serva, Germany), and examined using JEM 1400 (JEOL, Japan) transmission electron microscope. The samples were photographed at a magnification of 5,000-60,000x (Mendgen, 1991).

Total RNA was extracted from naturally infected LYSV-positive plant samples using RNeasy Plant Mini kit (Qiagen, UK). The two-step reverse transcription reaction (RT-PCR) was accomplished using pairs of specific primers (1-LYSVF: 5'-ACA AGT AAG AAA CAG AAG GAC AGC-3', 2-LYSVR: 5'-GAG GTT CCA TTT TCA ATG CAC CAC-3') complementary to the part of the *CP* gene of LYSV producing the amplicon with expected size of 409 bp (Parrano et al., 2012). Total RNA extraction and PCR amplification were assessed by electrophoresis in a 1.5% agarose gel in TBE buffer (89 mM TRIS borate and 2 mM EDTA, pH 8.3) stained with ethidium bromide using HyperLadder 100 bp markers (Bioline, Germany).

RESULTS AND DISCUSSION

During the last several years, large-scale production of garlic led to wide occurrence of virus-like symptoms on *A. sativum* plants in Ukraine. In 2018, we collected garlic plants exhibiting yellowing, leaf mosaic, stunting and wilting. Transmission electron microscopy of plant sap confirmed the presence of filamentous virions (720-820 x 16 nm) typical of *Potyvirus* genus.

Using ELISA, OYDV was identified for the first time in Ukraine (Snihur et al., 2019). However, some symptomatic and TEM-positive samples were confirmed as OYDV-negative suggesting possible plant (co)infection with a different potyvirus - *Leek yellow stripe virus* (LYSV).

In 2018/2019 yy, plant samples (including garlic, onion and leek) with LYSV-like mosaic and striping, leaf discoloration, and/or stunting symptoms were collected in different regions of Ukraine (Kyiv, Cherkassy, Vinnytsia, and Poltava regions). Sampling areas mostly included private gardens with some samples collected from commercial garlic fields.

On garlic plants, LYSV typically induced yellow striping, discoloration, spotting and plant growth reduction (Fig.1A). Plants co-infected with LYSV and OYDV typically demonstrated more severe visual symptoms of the disease (Fig.1B).

Using DAS-ELISA with virus-specific antibodies, LYSV was found in garlic plants but not in onion or leek. On average, 28% of collected plants were infected with LYSV. LYSV was detected in 3/4 screened regions of the country: Vinnytsia (Bershad district), Kyiv (Boryspil district), and Poltava (Semenivka district), suggesting wide spread of the virus in Ukraine (Table 1). According to available literature data, LYSV is often found in coinfection with OYDV. However, we found only 7% of plants to be mixed infected (LYSV+OYDV).

Mixed infection of garlic with LYSV and OYDV was detected in all three regions where LYSV was found.

A B

Table 1. Double-antibody enzyme-linked immunosorbent assay for the detection of *Onion yellow dwarf virus* and *Leek yellow stripe virus* by region in Ukraine (2018/2019 yy)

Region	**Virus-positive samples (from total number of samples)**	
	OYDV	**LYSV**
Vinnytsia	13 (18)	7 (18)
Kyiv	11 (18)	4 (18)
Poltava	5 (9)	3 (9)
Cherkassy	3 (5)	0 (5)
TOTAL	**32 (50)**	**14 (50)**
Incidence of infection (%)	**64%**	**28%**

TEM of OYDV-negative and LYSV-positive plant samples showed flexuous viral particles of ~800 nm in length and ~16 nm in diameter typical for potyviruses (Fig.2) which was in line with ELISA data.

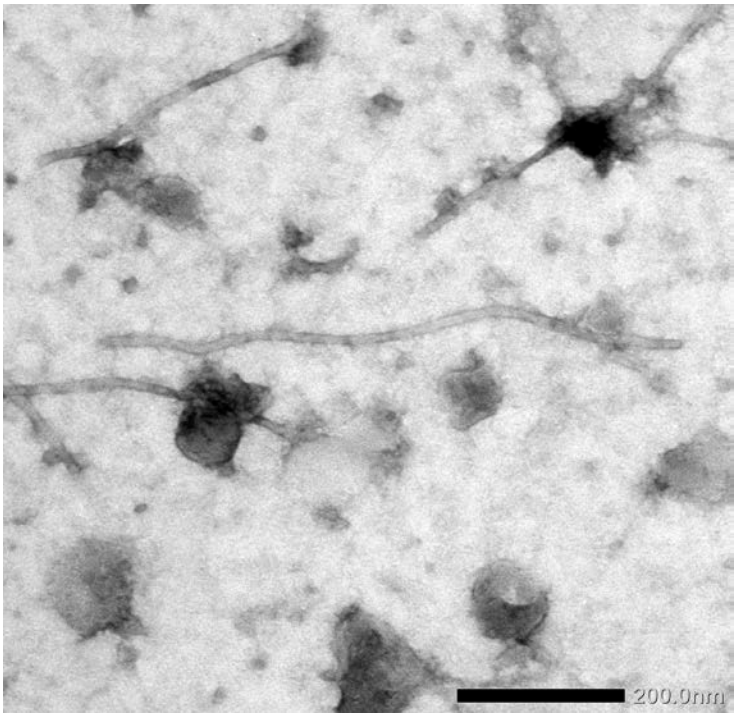

Figure 2. Transmission electron microscopy showing numerous potyvirus particles in partly purified sap of LYSV-positive garlic plant sample. Scale bar corresponds to 200 nm

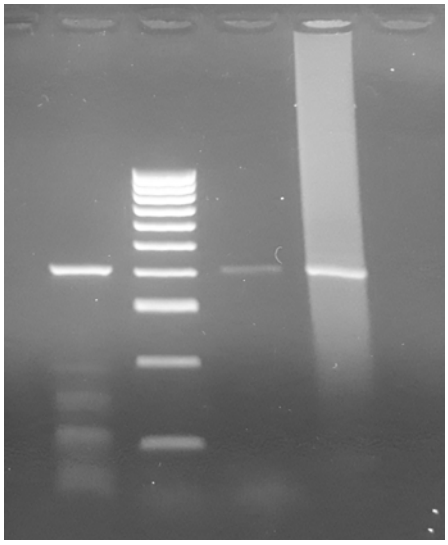

Figure 3. Amplicons of LYSV isolates corresponding to the part of *CP* gene (409 bp expected size): 1, 3, 4 – isolates from Vinnytsia, Kyiv and Poltava regions, respectively; 2 – MW markers (HyperLadder 100 bp)

Using LYSV coat protein-specific primers (Parrano et al., 2012), partial nucleotide sequence of three LYSV isolates was amplified and visualized by electrophoresis in a 1.5% agarose gel (Fig.3).

From Fig.3 it follows that all three amplified cDNAs of LYSV isolates collected from different parts of Ukraine have identical and expected size. These will be further used for phylogenetic studies of LYSV population in Ukraine.

Rather high rate of LYSV infection in private gardens (28% of symptomatic plants) may be explained by using contaminated planting material aided by flying vectors and mechanical transmission. In the view of official 'absence' of LYSV in neighboring countries (except Poland), we hypothesize that LYSV-infected seed garlic might have been the initial source of this virus in Ukraine. As we have shown before, one of the Ukrainian OYDV isolates (Accession number MK177281) was most phylogenetically related to Chinese OYDV isolates Yuhang (AJ510223) and YH1 (AJ292231) (Snihur et al., 2019), suggesting China as a potential origin of (at least) one of the OYDV isolates found in Ukraine. LYSV occurrence may follow the same pattern, as a significant part of (seed) garlic was (and is) imported to Ukraine from China.

Obtained results clearly demonstrate the importance of routine control of both imported planting/seed material and cultivated crops on a regular basis which remain highly efficient measures in preventing the spread of the mechanically and aphid-transmitted virus and reducing consequential damages.

CONCLUSIONS

Leek yellow stripe virus (LYSV) is one of the potyviruses infecting garlic in many countries including southern/eastern EU states (Vončina et al., 2016; Vučurović et al., 2017), but was never reported from Ukraine. Using double-antibody sandwich enzyme-linked immunosorbent assay with virus-specific antibodies, LYSV was detected in 28% of samples from 3/4 screened regions of Ukraine, similarly to previously described OYDV in Ukraine (Snihur et al., 2019). These findings were also supported by two other independent techniques (TEM and RT-PCR). Obtained data suggest significant spread of LYSV in Ukraine and raises questions of proper control of the quality of imported planting/seed material..

REFERENCES

Chen J., Chen J.P., Adams M.J. (2002). Characterisation of some carla- and potyviruses from bulb crops in China. (Brief report). Archives of Virology, 147(2), pp. 419-428.

Chodorska M., Paduch-Cichal E., Kalinowska E., Gaczkowska O., Lis M., Sierant B., Szyndel M.S. (2014). First report of Leek yellow stripe virus in foreign and polish garlic plants in central Poland. Journal of Plant Pathology, 96(4 supp.), pp. 4-120.

Clark M. F., & Adams A. M. (1977). Characteristics of the microplate method of enzyme-linked immunosorbent assay for detection of plant viruses. The Journal of General Virology, 34, pp. 475–483.

Dovas C., Hatziloukas E., Salomon R., Barg E., Shiboleth Y., Katis N.I. (2001). Incidence of viruses infecting Allium spp. in Greece. European Journal of Plant Pathology, 107, pp. 677–684.

EPPO Global Database, Leek yellow stripe virus: https://gd.eppo.int/taxon/LYSV00

Fidan H., Baloglu S. (2009). Detection of Onion Yellow Dwarf Virus and Leek Yellow Stripe Virus in Garlic in Turkey. Plant Disease, 93(6), pp. 672-672.

Gupta N., Prabha K., Islam S., Baranwal V.K. (2013). First report of *Leek yellow stripe virus* in garlic from India. Journal of Plant Pathology, 95(4 supp.), S4.69-S4.77.

King A.M.Q., Adams M.J., Carstens E.B., & Lefkowitz E.J. (eds.) (2012). Virus taxonomy: classification and nomenclature of viruses: Ninth Report of the International Committee on Taxonomy of Viruses. San Diego: Elsevier Academic Press.

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 Disease, 82(12), pp. 1381-1385.

Mendgen K. Electron Microscopy of Plant Pathogens. Edited by D.E. Lesemann. Springer, 1991. 336 p.

Oleas A., Arahana V. (2015). First Report of *Leek yellow stripe virus*, *Shallot latent virus*, and *Onion yellow dwarf virus* in Garlic From Ecuador. Plant Disease, 100(1), p. 232.

Pappu H.R., Hellier B.C., Dugan F.M. (2005). First report of *Onion yellow dwarf virus*, *Leek yellow stripe virus*, and *Garlic common latent virus* in garlic in Washington State. Plant Disease, 89(2), p. 205.

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. Phytopathologia Mediterranea, 51(3), pp. 549-565.

Shahraeen N., Lesemann D.E., Ghotbi T. (2008). Survey for viruses infecting onion, garlic and leek crops in Iran. EPPO Bulletin, 38, pp. 131–135.

Snihur H., Shevchenko T., Sherevera Kh., Budzanivska I., Shevchenko O. (2019). First report of *Onion yellow dwarf virus* in Ukraine. Journal of Plant Pathology, DOI: 10.1007/s42161-019-00350-2.

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 Disease, 100(3), pp. 656-656.

Vučurović I., Nikolić D., Radović N., Vučurović A., Ristić D., Krstić B., Stanković I. (2017). Incidence and distribution of *Leek yellow stripe virus* in *Allium* crops in Serbia. Pestic. Phytomed. (Belgrade), 32(3-4), pp. 145–155.