UDC 578+633.8

DOI: https://doi.org/10.36495/2312-0614.2022.3.11-14

SCREENING OF CHERRY LEAF ROLL VIRUS IN *SAMBUCUS NIGRA* PLANTS IN UKRAINE

Goal. *Cherry leaf roll virus (CLRV) infects naturally a wide range of herbaceous and woody plants, different species of trees, shrubs, horticultural, ornamental, weed plants and causes significant economic losses in many hosts and countries. CLRV on elderberry species was reported in Europe, USA, Iran and North America. Recently this virus has been detected in sour and sweet cherry fruit orchards in Ukraine. Testing of other hosts than Prunus was performed in Ukraine only recently. The aim of the study was to test the black elderberry plants (Sambucus nigra L.). for the presence of CLRV in the Poltava region and Kyiv regions.* ***Methods***. *Sampling was carried out in the summer–autumn period in 2019—2021 in the territories of the Poltava and Kyiv regions and in Kyiv city. Samples of healthy elderberry plants were also analyzed. Visual diagnostics, enzyme-linked immunosorbent assay in DAS-ELISA modification, total RNA extraction, RT-PCR with primers for a 412 bp fragment of the 3' untranslated region of the CLRV genome, and statistical data analysis were used in this research. PCR products were separated on an 1.5% agarose gel. Commercial CLRV preparations were used for positive controls in DAS-ELISA.* ***Results***. *Thirty three elderberry samples with symptoms of leaf rolling and mosaics of varying degrees were selected in the territory of the Poltava and Kyiv regions and in Kyiv city in 2019 and 2021 and used in the study. ELISA and RT-PCR results showed that 82% of the tested black elderberry samples were infected by CLRV.* ***Conclusions.*** *The presence of CLRV, its harmfulness for elderberry plants, and Sambucus potential to serve as a reservoir for the virus indicate the necessity of testing a wider range of plant species for cherry leaf roll virus in Ukraine.*

[1]L. MISHCHENKO,
Dr. Sci., Prof.,
[1]A. DUNICH,
PhD.,
[2]A. DASHCHENKO,
PhD.,
[3]N. KOZUB,
Dr. Sci.,
[4]L. HLUSHCHENKO,
PhD
[1]Taras Shevchenko National University of Kyiv, ESC «Institute of Biology and Medicine», 64/13, Volodymyrska str., Kyiv, 01601, Ukraine
[2]National University of Life and Environmental Sciences of Ukraine, 15, Heroyiv Oborony str., Kyiv, 03041, Ukraine
[3]Plant Protection Institute of NAAS, 33, Vasylkivska str., Kyiv, 03022, Ukraine
[4]Research station of medicinal plants, Institute of agroecology and environmental management, NAAS, 16 A, Pokrovskaya str., vil. Berezotocha, Lubny distr., Poltava reg., 37535, Ukraine
e-mail: [1]lmishchenko@ukr.net

cherry leaf roll virus (CLRV); *Sambucus nigra* L.; elderberry; mosaics; leaf rolling; diagnostics methods; DAS-ELISA; RT-PCR

Introduction. Cherry leaf roll virus (CLRV, *Nepovirus, Comovirinae, Secoviridae*, segmented (+) ssRNA) naturally infects a wide range of herbaceous and woody plants, among which are *Betula* spp., *Fagus* spp., *Fraxinus* spp., *Juglans* spp., *Ulmus* spp., *Ramus* spp., *Sambucus* spp., *Prunus* spp., as well as *Ligustrum vulgare* L., *Ptelea trifoliata* L. and *Cornus florida* L. [1, 2].

CLRV is naturally transmitted through seeds of woody perennial hosts which include birch, cherry, elderberry, elm, and walnut [3, 4] or pollen [5]. It has also been experimentally graft-transmitted by bark patches of English walnut trees [6] and mechanically transmitted to a wide range of herbaceous species [7]. Unlike many other nepoviruses, CLRV appears not to be transmitted by soil-inhabiting nematodes [8, 9]. More recently, it has been suggested that CLRV particles released from roots of infected *Chenopodium quinoa* plants are able to migrate through nutrient solution and infect healthy *C. quinoa* plants [1].

CLRV on elderberry species was reported in Poland [10], Germany [11, 12], USA [12], North America [13], Canada [14], Iran (NCBI GenBank), and Hungary [12].

CLRV can lead to economic losses in walnut production by inducing walnut black line disease, which causes necrosis at grafting unions with some English walnut and rootstock combinations [6]. This may lead to subsequent dieback, a common disease symptom especially of woody plants, characterized by progressive death of twigs, branches, shoots, or roots, starting at the tips. Significant economic losses due to walnut blackline disease have been reported from California [6]. Kegler et al. found that CLRV can cause decline and dieback in sweet cherry (*Prunus avium*) [15], and CLRV was detected recently in several downy birch trees (*Betula pubescens* Ehrh.) in Finland [16] and has to be regarded as of economic importance.

CLRV has been recently detected in sour and sweet cherry fruit orchards in Ukraine [17]. Despite the diverse host range of the virus, which includes different species of trees, shrubs, horticultural, orna-

mental, weed plants, and significant economic losses in many hosts and countries due to CLRV infection, testing of other hosts than *Prunus* was performed in Ukraine only recently [18]. So, the aim of the study was to test the black elderberry plants for the presence of CLRV in the Poltava and Kyiv regions an in Kyiv city.

Materials and methods. *Sample collection and visual diagnostics.* Sampling was carried out in the summer–autumn period in 2019—2022 in the territories of the Poltava and Kyiv regions and in Kyiv city. Visual diagnostics revealed symptoms of viral infection on leaves of wild-grown black elderberry plants (*Sambucus nigra* L.). Samples of elderberry with symptoms and visually healthy plants were selected.

Enzyme-linked immunosorbent assay. To determine the presence of viral antigens, the double-antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) was used. The analysis was performed using commercial antibodies against CLRV manufactured by Loewe (Germany) in three replicates. Samples of healthy elderberry plants were used as negative controls. Commercial CLRV preparations (Loewe, Germany) were used for positive controls. The reaction results were recorded on a Thermo Labsystems Opsis MR (USA) reader with a Dynex Revelation Quicklink software at wavelength of 405 nm. Samples with absorbance values that exceeded the negative control at least three times were considered positive [19].

RNA extraction, RT-PCR. Total RNA was extracted from symptomatic leaves using GeneJet Plant RNA Puntification Kit; cDNA was synthesized using RevertAid Reverse Transcriptase (Thermo Fisher Scientific, USA) following the manufacturer's protocol. DreamTaq™ Green PCR Master Mix (Thermo Fisher Scientific, USA) and specific oligonucleotide primers for amplifying a 412 bp fragment of the 3' untranslated region (3'UTR) of the CLRV genome were used: forward CLRV-5 5'-TGGCGACCGTGTAACGGCA-3' and reverse CLRV-3 5'-GTCGGAAAGATTACGTAAAAGG-3' [20]. Amplification steps using Dream Taq Green PCR Master Mix (Thermo Scientific, USA) were performed using a Genetic Research Instrumentation LTD thermocycler (UK). The amplification reactions were set up as follows: initial denaturation for 3 min at 95°C, followed by 30 cycles of 95°C for 30 s, 55°C for 30 s and 72°C for 45 s. The final extension was at 72°C for 5 min. The primers are expected to amplify a 412-bp DNA fragment of CLRV 3'UTR. PCR products were separated on an 1.5% agarose gel with DNA markers CSL-MDNA-100bp (Cleaver Scientific, UK), and visualized under UV light.

Results and discussion. Thirty three elderberry samples of with symptoms of leaf rolling and mosaics were selected in the territory of the Poltava and Kyiv regions and in Kyiv city in 2019 and 2021 and used in the study (Table 1, Fig. 1-3 a).

In 2022, the especially high fruit yield was observed on healthy elderberry plants in comparison with virus-infected plants (Fig. 3, b, c).

ELISA results showed that 27 elderberry samples contained CLRV antigens (Fig. 4).

The results of RT-PCR are consistent with the data obtained by DAS-ELISA and demonstrate the presence of CLRV in the studied samples of elderberry (Fig. 5).

Elderberry in Ukraine and not only in our country is a valuable medicinal plant and a widely used source of dietary supplements. Our previous studies with viral diseases of purple coneflower, valerian and ginseng have shown that viruses can significantly deteriorate the

1. Black elderberry samples used in the study

	Sample number	Collection date	Place of collection
1	1,2	06.08.2019	Poltava region
2	3,4,5,6,7	05. 30.2021	Poltava region
3	8,9,10,11,12,13, 14,15,16,17,18	06.13.2021	Poltava region, Myrhorod district
4	19	06.19.2021	Poltava region, Lubny city
5	20,21	06.21.2021	Kyiv region, Fastiv district
6	22,23,24,25,26,27	06.18.2021	Poltava region
7	28	06.18.2021	Poltava region
8	29,30,31,32,33	06.17.2021	Kyiv city

a *b* *c* *d* *e*

Fig. 1. Elderberry with CLRV symptoms, Poltava region 8th June 2019: a, d — healthy; b — leaf rolling; c — mosaics and leaf rolling; e — mosaics

a b c d e

Fig. 2. Elderberry with CLRV symptoms, Poltava region, 30th May 2021 (a—c) and 13th June 2021 (d, e): a — a branch with mosaic leaves and a reduced inflorescence; b — an inflorescence from a healthy plant; c — leaf mosaics; d — leaf mosaics and mild leaf rolling; e — healthy branch

a b c

Fig. 3. Elderberry with CLRV symptoms: a — CLRV mild mosaic and leaf rolling symptoms on elderberry at the flowering stage, Kyiv region, 21st June, 2021; b — severe symptoms of leaf mosaics during ripening of elderberry clusters, c — healthy plants, 29th August, 2022, Poltava region

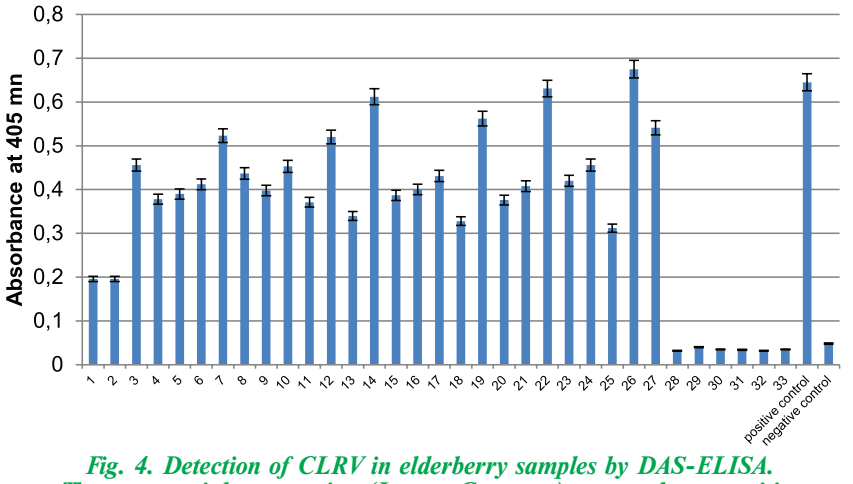

Fig. 4. Detection of CLRV in elderberry samples by DAS-ELISA. The commercial preparation (Loewe, Germany) was used as a positive control and a healthy-looking plant was used as a negative control. Three technical replicates (individual plants) were performed. The figure shows the average values of optical density for each sample

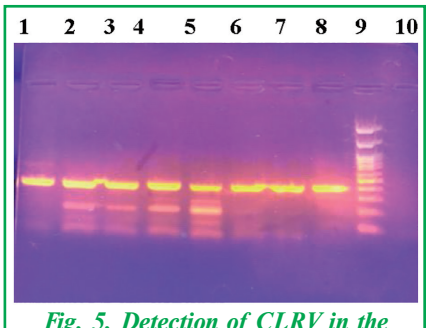

Fig. 5. Detection of CLRV in the infected leaves using RT-PCR. Gel electrophoresis shows 412 bp RT-PCR fragments amplified using RNA from infected elderberry leaves with primers for the viral 3'UTR (lanes 1-8). Lane 9 contains DNA markers CSL-MDNA-100bp (Cleaver Scientific, UK). RNA isolated from a healthy-looking plant was used as a negative control (lane 10)

quality of medicinal raw materials by reducing the amount of biologically active substances in plants [21, 2]. On the other hand, there is a danger that elderberry plants can be reservoirs of the virus. It was revealed that the isolate derived from *S. nigra* could be transmitted by sap inoculation to peach and cherry [9]. Langer et al. conducted investigation with three CLRV isolates (elderberry isolate E603, walnut isolate E326, rhubarb isolate E395) from different phylogenetic groups, which were selected and mechanically inoculated on five natural woody host plants by stem slashing. They proved that one year after inoculation the CLRV isolate from black elderberry was able to infect four out of five plant species: (*Sambucus nigra, Sorbus aucuparia, Juglans regia, Betula pendula,* but not *Prunus avium*) [14]. This suggests

that for this isolate host adaption is not stringent and/ or transmission barriers between host species are developed differentially.

This work was supported by the National Academy of Agrarian Sciences of Ukraine in the framework of the project 17.01.01.18.F. «Development of methodological principles for the formation of collections of genetic resources of medicinal and essential oil plants» of research program 17 «Genetic resources» (0121U108614).

CONCLUSIONS

Thus, studies conducted in the period of 2019—2021 showed that 27 samples of elderberry plants in the Poltava and Kyiv regions were affected by CLRV. The other studied samples with symptoms of mosaics of different degree and leaf rolling could also be infected by viruses but not by CLRV. These cases require further virusologic research. The results indicate the importance of further research in this field, as the presence of CLRV, its harmfulness for elderberry plants, and *Sambucus* potential to serve as a reservoir for the virus, indicate the necessity of testing a wider range of plant species for this virus.

REFERENCES

1. *Bandte M., Buttner C.* (2000). A review of an important virus of deciduous trees-cherry leafroll virus: occurrence, transmission and diagnosis. *Pflanzenschutzberichte*. Vol. 59. P. 1–19.

2. *Obermeier C., Rebenstorf K., Bandte M., Buttner C.* (2003). Impact and distribution of cherry leaf roll virus (CLRV) in forest trees and horticultural plants. In: Dujesiefken, D., Kockerbeck, P. (Eds.), Yearbook of Arboriculture. Thalacker Medien, Braunschweig, 2003. P. 219–225.

3. *Cooper J.I., Massalski P.R., Edwards M.L.* (1984). Cherry leaf roll virus in the female gametophyte and seed of birch and its relevance to vertical transmission. *Annals of Applied Biology.* Vol. 105. P 55–64. doi: 10.1111/j.1744-7348.1984.tb02802.x

4. *Massalski P.R., Cooper J.I.* (1984) The location of virus-like particles in the male gametophyte of birch, walnut, cherry naturally infected with CLRV and its relevance to vertical transmission of the virus. *Plant Pathology.* Vol. 33. P. 255–262. doi: 10.1111/j.1365-3059.1984.tb02647.x

5. *Card S.D., Pearson M.N., Clover G.R.G.* (2007). Plant pathogens transmitted by pollen. *Australas Plant Pathology*. Vol. 36. P. 455–461. doi: 10.1071/AP07050

6. *Mircetich S.M., Sanborn R.R., Ramos D.E.* (1980). Natural spread, graft transmission, and possible etiology of walnut black line disease. *Phytopathology*. Vol. 70. P. 962–968. doi: 10.1094/Phyto-70-962.

7. *Jones A.T.* (1985). Cherry leaf roll virus. CMI/AAB Descriptions of plant viruses. 306, 9 p

8. *Jones A.T., McElroy F.D., Brown D.J.F.* (1981). Tests for transmission of cherry leaf roll virus using *Longidorus, Paralongidorus* and *Xiphinema* nematodes. *Annals of Applied Biology.* Vol. 99. P. 143–150. doi: 10.1111/j.1744-7348.1981.tb05141.x

9. *Wang S., Gergerich R.C., Wickizer S.L., Kim K.S.* (2002). Localization of transmissible and nontransmissible viruses in the vector nematode *Xiphinema americanum*. *Phytopathology*. Vol. 92. P. 646–653. doi: 10.1094/PHYTO.2002.92.6.646

10. *Berniak H., Kaminska M.* (2010). Viral diseases of *Sambucus* plants. *Zeszyty Problemowe Postępów Nauk Rolniczych.* Vol. 554. P 15-20.

11. *Buchhop J., von Bargen S., Buttner C.* (2009). Differentiation of cherry leaf roll virus isolates from various host plants by immunocapture-reverse transcription-polymerase chain reaction-restriction fragment length polymorphism according to phylogenetic relations. *Journal of Virological Methods*. Vol. 157(2). P. 147-154. doi: 10.1016/j.jviromet.2008.12.010

12. *Rebenstorf K., Candresse T., Dulucq M.J., Büttner C., Obermeier C.* (2006). Host species-dependent population structure of a pollen-borne plant virus, cherry leaf roll virus. *Journal of Virology*. Vol. 80(5). P. 2453–2462. doi: 10.1128/JVI.80.5.2453-2462.2006

13. *Ellis P.J., Converse R.H., Stace-Smith R.* (1992). Viruses of *Sambucus canadensis* in North America. *Acta Horticulturae*. Vol. 308. P. 69-80. doi: 10.17660/ActaHortic.1992.308.8

14. *Hansen AJ, Stace-Smith R.* (1971). Properties of a virus isolated from golden elderberry, *Sambucus nigra aurea*. *Phytopathology*. 61:1222–1229.

15. *Kegler H., Richter J., Schmidt H.B.* (1966). Untersuchungen zur Identifizierung und Differenzierung des Blattrollvirus der Kirsche (cherry leaf roll virus). *Phytopathologische Zeitschrift.* Vol. 56. P. 313–330.

16. *Jalkanen R., Buttner C., von Bargen S.* (2007). Cherry leaf roll virus, CLRV, abundant on *Betula pubescens* in Finland. *Silva Fennica.* Vol. 41. P. 755–762. doi: 10.14214/sf.927

17. *Pavliuk L., Udovychenko K., Riaba I., Bublyk M.* (2021). Detection of sour and sweet cherry viruses in Ukraine. *Agronomy Research.* 19(X).

18. *Mishchenko L. Dunich A., Molodchenkova O., Hlushchenko L.* (2021). First report of *Cherry leaf roll virus* from *Sambucus nigra* in Ukraine. *Journal of Plant Pathology*. Vol. 103, P. 1077. doi 10.1007/s42161-021-00884-4

19. *Crowther J.R.* (1995). ELISA. Theory and practice. New York: Humana Press. 223 p.

20. *Werner R., Mühlbach H.P., Büttner C.* (1997). Detection of Cherry leaf roll nepovirus (CLRV) in birch, beech and petunia by immunocapture RT-PCR using a conserved primer pair. *European Journal of Forest Pathology.* Vol. 27(5). P. 309-318. doi:10.1111/j.1439-0329.1997.tb01085.x

21. *Dunich A.A., Mishchenko L.* (2015). Purple coneflower viruses: species diversity and harmfulness. *Biopolymers and Cell*. Vol. 31(1). P. 15–28. doi:10.7124/bc.0008C8

22. *Mishchenko L.T., Dunich A.A., Daschenko A.V., Polischuk V.P.* (2015). Viral diseases of medicinal plants. Kyiv: Phytosociocentr. 2015. 320 p. [In Ukrainian]

23. *Langer J., von Bargen S., Büttner C.* (2008). Epidemiological investigations on cherry leaf roll virus. First Symposium on Horticulture in Europe. P. 255-256.

[1]Міщенко Л.Т., [1]Дуніч А.А., [2]Дащенко А.В., [3]Козуб Н.О., [4]Глущенко Л.А.
[1]ННЦ «Інститут біології та медицини», Київський національний університет імені Тараса Шевченка, вул. Володимирська, 64/13, м. Київ, 01601, Україна,
[2]Національний університет біоресурсів і природокористування України, вул. Героїв Оборони, 15, м. Київ, 03041, Україна,
[3]Інститут захисту рослин НААН, вул. Васильківська, 33, м. Київ, 03022, Україна,
[4]Дослідна станція лікарських рослин Інституту агроекології та природокористування НААН, вул. Покровська, 16А, с. Березоточа, Лубенський р-н, Полтавська обл., 37535, Україна
e-mail: [1]lmishchenko@ukr.net, [1]korenevochka1983@ukr.net, [2]dannaval@ukr.net, [3]natalkozub@gmail.com, [4]l256@ukr.net

Скринінг вірусу скручування листків черешні у рослинах бузини в Україні

Мета. *Вірус скручування листків черешні (cherry leaf roll virus (CLRV)) у природі уражує широкий спектр трав'янистих і деревних рослин, різні види дерев, кущів, садових, декоративних, дикорослих рослин і спричинює значні економічні втрати у багатьох країнах світу. CLRV на різних видах бузини повідомлявся в Європі, США, Ірані та Північній Америці. Останнім часом цей вірус було виявлено в садах черешні та вишні в Україні. Тестування інших рослин-хазяїв, окрім Prunus, розпочато в Україні лише нещодавно. Метою роботи було дослідження рослин бузини чорної (Sambucus nigra L.). на наявність CLRV у Полтавській, Київській областях та в м. Київ.* **Методи**. *Відбір проб проводили в літньо-осінній період 2019—2022 рр. на території Полтавської, Київської областей та в м. Київ. Також аналізували зразки здорових рослин бузини. У дослідженні були використані методи: візуальна діагностика, імуноферментний аналіз у модифікації DAS-ELISA, екстракція сумарної РНК, ЗТ-ПЛР з праймерами до фрагмента завдовжки 412 п.н. 3'-нетрансльованої ділянки геному CLRV, статистичні методи обробки даних. Продукти ПЛР розділяли в 1,5% агарозному гелі. Як позитивний контроль в DAS-ELISA використовували комерційні препарати CLRV.* **Результати**. *На території Полтавської, Київської областей та в м. Київ у 2019—2021 рр. відібрано та досліджено 33 зразки бузини з ознаками скручування листків та мозаїки листків різного ступеня. Результати ELISA та ЗТ-ПЛР показали, що 82% досліджених зразків чорної бузини були інфіковані CLRV.* **Висновки**. *Наявність CLRV, його шкідливість для рослин бузини та здатність Sambucus бути резерватором вірусу свідчать про необхідність дослідження ширшого спектра видів рослин на вірус скручування листків черешні в Україні.*

cherry leaf roll virus (CLRV); бузина; мозаїка, скручування листків; методи діагностики; DAS-ELISA; RT-PCR

Received on 05.23.2022