Original scientific paper
Оригиналан научни рад
UDC 634.73:632.3(497.6)
DOI 10.7251/AGREN2202049D

University of Banjaluka, Faculty of Agriculture
Agro-knowledge Journal

Blueberry latent virus in highbush blueberry (*Vaccinium corymbosum* L.) in Bosnia and Herzegovina

Delić, D.[1], Mihaljica, M.[1], Vakić, M.[1], Sunulahpašić, A.[2], Tzanetakis E.I.[3]

[1]*University of Banjaluka, Faculty of Agriculture, Bulevar vojvode Petra Bojovića 1A, Banjaluka, Bosnia and Herzegovina, duska.delic@agro.unibl.org*
[2]*Ministry of Agriculture, Water Management and Forestry, Central Bosnia Canton, Travnik*
[3]*Department of Entomology and Plant Pathology, Division of Agriculture, University of Arkansas System, Fayetteville, USA*

Abstract

A small-scale survey for blueberry viruses in Bosnia and Herzegovina was performed in 2018. A total of 20 samples from three locations were collected and analyzed for the presence of 11 viruses. ELISA assays were performed for blueberry scorch virus, blueberry shock virus, blueberry shoestring virus, blueberry leaf mottle virus, tobacco ringspot virus, and tomato ring spot virus. The samples were tested for blueberry red ringspot virus by PCR, and blueberry fruit drop associated virus, blueberry latent virus, blueberry mosaic associated virus, blueberry necrotic ring blotch virus, blueberry virus A, and blueberry leaf mottle virus by RT-PCR. The analyses confirmed the presence of BlLV in eight samples with no other virus detected in any of the samples.

Key words: *Vaccinium corymbosum* L., BlLV, RT-PCR, sequencing, diversity

Introduction

Although highbush blueberry (*Vaccinium corymbosum* L.) was introduced in Bosnia and Herzegovina (B&H) in the 1980s, production has intensified in the last five years. Blueberry is cultivated on around 150 ha with orchards established with imported planting material, posing a risk of the introduction of exotic and

emerging pathogens. Blueberries are known to harbour several viruses belonging to diverse families (Martin et al. 2012). The most economically important viruses are: blueberry red ringspot virus (BRRV), blueberry leaf mottle virus (BLMoV), blueberry scorch virus (BlScV), blueberry shock virus (BlShV), blueberry shoestring virus (BSSV), peach rosette mosaic virus (PRMV), tobacco ringspot virus (TRSV), and tomato ringspot virus (ToRSV) (Prodorutti et al., 2007). Other reported viruses include blueberry latent virus (BBLV), blueberry latent spherical virus (BLSV), blueberry virus A (BVA), and blueberry mosaic-associated virus (BlMaV) whose economic impact is largely unknown (Isogai et al., 2011; Martin et al., 2011; Isogai et al., 2015; Thekke-Veetil et al., 2014). There are no significant data of the sanitary status of blueberry in Europe regarding viruses other than reports for BRRV in Czech Republic, Slovenia, Poland, and Serbia (Mavrič Pleško et al., 2010; Jevremović et al., 2016), BlScV in Italy, Poland, and the Netherlands (Ciuffo et al., 2005; Paduch-Cichal et al., 2011), BSSV, PRMV, and TRSV in Poland (Paduch-Cichal et al., 2011) and BlMaV in Slovenia, Turkey, and Serbia (Gauthier et al., 2015; Gazel et al., 2015; Jevremović et al., 2015).

In this report we present the results of the first small-scale survey for blueberry viruses in B&H.

Material and Methods

Plant material

Twenty samples of blueberries 'Bluecrop', 'Duke', 'Elliott', and 'Patriot' were randomly collected from production fields in Bugojno, Banjaluka, and Teslić during May/June 2018. Each sample consisted of 10 young but fully expanded leaves picked from one bush.

Nucleic Acid Extractions

Total nucleic acids (TNA) were extracted from using the protocol described in Tzanetakis et al. (2007). Leaf tissue (50 mg) representative of all leaves in a sample was homogenized with liquid nitrogen in 1 ml of extraction buffer (200 mM Tris base, pH 8.5, 300 mM lithium chloride, 1.5% lithium dodecyl sulphate, 10 mM EDTA, 1% deoxycholic acid, 2% polyvinyl-pyrrolidone 40, and 1% [vol/vol] TCEP, added just before use). An equal volume of potassium acetate (2.8 M potassium and 5.8 M acetate, pH 6.5) was added to the extract and chilled at –20°C for at least 30 min. The mixture was centrifuged at 16,000 × g for 10 min in a microcentrifuge and 0.7 ml of the supernatant was transferred to a new tube. An equal amount of isopropanol was added and mixed via inversion and centrifuged for 20 min at 16,000 × g. The precipitated TNA was resuspended in 500 µl of wash buffer and 25 µl glass milk which was

pelleted by pulse centrifugation to 6000 rpm and washed with another 500 µl of wash buffer. After a brief centrifugation, glass milk was dried in a speed vac and the dried pellet was resuspended in 40 µl of RNase-free water.

ELISA

The grinding buffer of Martin and Bristow (1988) was used for tissue homogenization. Commercial antisera (AGDIA Inc., Elkhart, IN, USA) for BLMoV and TRSV were used in DAS-ELISA according to the manufacturer's recommendations with the exception of the grinding buffer. Antibodies for BlScV, BlShV, and ToRSV were provided by Dr. R.R. Martin and for BlSSV from Dr. A.C. Schilder (Michigan State University, East Lansing, MI, USA). The samples were considered positive after the measurements of absorbance values at 405nm on ELx808 plate reader (BioTek U.S., Winooski, VT, USA) were three times higher or more than those of the negative control.

PCR and RT-PCR

Reverse transcription was primed with random hexamers and performed using the Maxima H Minus Reverse Transcriptase (Thermo Fisher Scientific, USA) according to the manufacturer's guidelines. The cDNA quality was evaluated using the NADH dehydrogenase ND-2 subunit (ndhB gene) as an internal control (Tzanetakis et al., 2007). Taq DNA Polymerase (GenScript, USA) was used for PCR in separate reactions using the primers listed in Table 1. RT-PCR was employed for detection of BFDaV, BlLV, BlMaV, BNRBV, BVA, and BLMV, whereas BRRV was detected by PCR. Representative amplicons were sequenced at Eurofins Genomics, USA to confirm their identity.

Tab. 1. List of primers used for blueberry virus detection

Virus	Primers	Annealing Temp (°C)	Expected product size (bp)	Reference
BlLV	F: CTTATCAGAGCTTCTTCAGACTGG R: TCGTCACCCGCACATTTC	53	391	Martin et al., 2011
BFDaV	F: GACAACAGCATCTACATCTCTGC R: GGTCGTTCTACCACGTTTCTG	55	395	Diaz-Lara et al., 2016
BlMaV	F: CCWGTATCAAGCATAGTYACAAG R: AAGAAGGTRGTGATTGAGA	58	254	Thekke-Veetil et al., 2015
BNRBV	F: CCAGTTTGGAGGAATTGCAT R: GCGTTTCAGCACCACTAAC	55	432	Quito-Avila et al., 2013
BRRV	F:ATCAGTCCCAGAAGAAAAGAAGTA R: TCCGAAAAATAGATAGTGTCAGC	56	548	Polashock et al., 2009
BVA	F: AACTCATGGTTAAGCGTGAG R: AGTCCTGAGACTTATCGAAC	55	270	Thekke-Veetil et al., 2014
BLMV	F: GTTCCGTGAAATCGGTATAC R: CTCTGGTAGTTTCAAAACTACA	54	324	Tang, J (2014) unpublished

Dot blot hybridization

The probe for detection of BlLV (fusion protein gen; Martin et al., 2011) was based on the PCR product of isolate B-1. The PCR amplicon (~35 ng) was purified using the GenJET PCR purification kit (Fermentas, Lithuania) and used for probe preparation and labelling with the DIG-High Prime Kit (Roche Applied Science, Germany) according to the manufacturer's recommendations. Integrity was verified by agarose electrophoresis (1% agarose gel in SB buffer). Each RNA sample was incubated at 95°C for 5 min and cooled on ice. Denatured RNAs were dotted and fixed on a Hybond N+ membrane (GE Healthcare, USA) by UV irradiation (UV cross-linking 1 min at both sides). Membranes were blocked with prehybridization buffer according to the instructions (GE Healthcare, USA). Hybridization was performed at 65°C overnight, followed by washing in 2x SSC/0.1% sodium dodecyl sulphate (SDS) at room temperature and in high stringency washes of 0.5× SSC/0.1 % SDS at 65°C. Immunodetection was done with anti-dioxigenin-AP (DIG-High Prime Kit, Roche Applied Science). Chemiluminescence substrate CSPD (DIG-High Prime Kit, Roche Applied Science) was used for visualization. Enzymatic dephosphorylation of CSPD was recorded on transilluminator ImageQuant LAS 500 (GE Healthcare, USA).

Results and Discussion

During inspection, majority of plants in the surveyed plantations were asymptomatic. None of the samples were positive for the BLMoV, BlScV, BlShV, TRSV, and ToRSV presence. A positive reaction was only observed in the positive control for each tested virus, with OD values at least 3 times higher than the values for negative control.

In eight samples RT-PCR produced the expected 391 bp PCR fragment for BlLV, with the virus present in all surveyed locations. Sequence analysis of the partial fusion protein gene (Acc. Nos MK511321-27; Table 2) showed 99.5-100% nt identity with BlLV isolates from Serbia, USA, Japan, and South Korea, whereas the amino acid identities ranged from 98.8-100%.

The presence of the virus was also confirmed with northern-blot hybridization.

Tab. 2: List of BlLV isolates sequenced during this study

Isolate name	Municipality	NCBI accession number
B1-B&H	Bugojno	MK511321
B4-B&H	Bugojno	MK511322
B6-B&H	Bugojno	MK511323
B9-B&H	Banjaluka	MK511324
B10-B&H	Banjaluka	MK511325
B11-B&H	Banjaluka	MK511326
B15-B&H	Banjaluka	MK511327
B18-B&H	Teslić	MK511328

BlLV is a member of genus *Amalgavirus*, family *Amalgaviridae*, with a genome composed of double-stranded RNA (dsRNA). BlLV is transmitted by seed almost by 100% but it is considered of minor importance as it does not cause symptoms in single infections and probably does not enhance symptoms in combination with other viruses (Martin et al., 2011). During our survey 8/20 samples were found to harbour the virus. High incidence of BlLV has also been reported in the USA, Japan, and Serbia (Martin et al. 2011; Isogai et al. 2011; Jevremović and Paunović, 2021). However, in the survey done in 2018 in USA that covered all blueberry regions, BlLV was detected only in 3.6% of the tested samples (Martin and Tzanetakis, 2018). In our survey the virus was found in all surveyed regions and tested cultivars.

Moreover, low sequence variability was found in the study performed by Jevremović and Paunović (2021) where high nucleotide sequence identities of BlLV isolates from geographically distant countries were also identified in our samples.

Conclusion

This study presents the first survey for viruses in highbush blueberry plantations in Bosnia and Herzegovina. The blueberry latent virus (BlLV) was confirmed in high prevalence and geographical distribution. BlLV is defined as a latent virus. However, imports of plant material from countries with reported viruses infecting blueberry involve a risk of introducing these pathogens to B&H. With a steady increase in area under highbush blueberry cultivation in Bosnia and Herzegovina, we may also expect to detect other viruses. The application of proper detection methods is of great importance for early detection and limited dispersal to virus-free areas.

Acknowledgement

This work was supported through a Fulbright Visiting Scholar Grant at the University of Arkansas, Division of Agriculture, Department of Plant Pathology and by the Ministry of Civil Affairs of Bosnia and Herzegovina (contract number 10/33-14-632-1/17).

References

Ciuffo, M., Pettiti, D., Gallo, S., Masenga, V., and Turina, M. (2005). First report of Blueberry scorch virus in Europe. *Plant Pathology*, *54*(4), 565. doi: 10.1111/j.1365-3059.2005.01198.x

Diaz-Lara, A., and Martin, R.R. (2016). Blueberry fruit drop-associated virus: A new member of the family Caulimoviridae isolated from blueberry exhibiting fruit-drop symptoms. *Plant Disease*, *100*(11), 2211–2214. doi: 10.1094/PDIS-06-16-0792-RE

Gauthier, N. W., Polashock, J., Veetil, T.T., Martin, R.R., and Beale, J. (2015). First report of blueberry mosaic disease caused by Blueberry mosaic associated virus in Kentucky. *Plant Disease*, *99*(3), 421. doi: 10.1094/PDIS-09-14-0946-PDN

Gazel, M., Elçi, E., Çelik, H., Gündüz, K., Mavric Plesko, I., Virscek Marn, M., and Çağlayan, K. (2015). *The presence of Blueberry mosaic associated virus in* Vaccinium spp. *in Turkey*. Paper presented at the 23rd International Conference on Virus and Other Graft Transmissible Diseases of Fruit Crops, ICVF2015, Morioka, Japan.

Isogai, M., Nakamura, T., Ishii, K., Watanabe, M., Yamagishi, N., and Yoshikawa, N. (2011). Histochemical detection of Blueberry latent virus in highbush blueberry plant. *Journal of General Plant Pathology*, *77*(5), 304-306. doi: 10.1007/s10327-011-0323-0

Isogai, M., Suzuki, K., Yashima, S., Watanabe, M., and Yoshikawa, N. (2015). *Blueberry mosaic associated virus detected in a highbush blueberry tree with mosaic symptoms in Japan*. Paper presented at the 23rd International Conference on Virus and Other Graft Transmissible Diseases of Fruit Crops, ICVF2015, Morioka, Japan.

Jevremović, D., Leposavić, A., and Paunović, S. (2015). First report of Blueberry mosaic-associated virus in highbush blueberry in Serbia. *Journal of Plant Pathology*, *97*(3), 541. doi: 10.4454/JPP.V97I3.004

Jevremović, D., Leposavić, A., and Paunović, S. (2016). Incidence of viruses in highbush blueberry (*Vaccinium corymbosum* L.) in Serbia. *Pesticidi i fitomedicina*, *31*(1-2), 45-50. doi: 10.2298/PIF1602045J

Jevremović, D., and Paunović, S.A. (2021). Molecular detection, geographical distribution and genetic diversity of blueberry latent virus in highbush blueberries in Serbia. *J Plant Dis Prot*, *128*(2), 601–605. doi: 10.1007/s41348-020-00418-7

Martin, R.R., and Bristow, P.R. (1988). A Carlavirus associated with blueberry scorch disease. *Phytopathology*, *78*(12), 1636-1640.

Martin, R.R., Zhou, J., and Tzanetakis, I.E. (2011). Blueberry latent virus: an amalgam of the Partitiviridae and Totiviridae. *Virus Research*, *155*(1), 175-180. doi: 10.1016/j.virusres.2010.09.020

Martin, R.R., Polashock, J.J., and Tzanetakis, I.E. (2012). New and emerging viruses of blueberry and cranberry. *Viruses*, *4*(11), 2831-2852. doi: 10.3390/v4112831

Martin, R., and Tzanetakis, I. (2018). High Risk Blueberry Viruses by Region in North America; Implications for Certification, Nurseries, and Fruit Production. *Viruses*, *10*(7), 342. doi:10.3390/v10070342

Mavrič Pleško, I., Viršček Marn, M., and Koron, D. (2010). Detection of Blueberry red ringspot virus in highbush blueberry cv. 'Coville' in Slovenia. *Julius-Kühn-Archiv*, *427*, 204-205.

Paduch-Cichal, E., Kalinowska, E., Chodorska, M., Sala-Rejczak, K., and Novak, B. (2011). Detection and identification of viruses of highbush blueberry and cranberry using serological ELISA test and PCR technique. *Acta Scientiarum Polonorum-Hortorum Cultus*, *10*(4), 201-215.

Polashock, J.J., Ehlenfeldt, M.K., and Crouch, J.A. (2009). Molecular detection and discrimination of blueberry red ringspot virus strains causing disease in cultivated blueberry and cranberry. *Plant Disease*, *93*(7), 727–733. doi: 10.1094/PDIS-93-7-0727

Prodorutti, D., Pertot, I., Giongo, L., and Gessler, C. (2007). Highbush blueberry: Cultivation, protection, breeding and biotechnology. *The European Journal of Plant Science and Biotechnology, 1*(1), 44-56.

Quito-Avila, D.F., Brannen, P.M., Cline, W.O., Harmon, P.F., and Martin, R.R. (2013). Genetic characterization of Blueberry necrotic ring blotch virus, a novel RNA virus with unique genetic features. *Journal of General Virology*, *94*(6), 1426-1434. doi: 10.1099/vir.0.050393-0

Thekke-Veetil, T., Ho, T., Keller, K.E., Martin, R.R., and Tzanetakis, I.E. (2014). A new ophiovirus is associated with blueberry mosaic disease. *Virus Research*, *189*, 92-96. doi: 10.1016/j.virusres.2014.05.019

Thekke-Veetil, T., Polashock, J. J., Marn, M. V., Plesko, I. M., Schilder, A. C., Keller, K. E., Martin, R. R., and Tzanetakis, I. E. (2015). Population structure of Blueberry mosaic associated virus: Evidence of reassortment in geographically distinct isolates. *Virus Research*, *201*, 79-84. doi: 10.1016/j.virusres. 2015.02.022

Tzanetakis, I.E., Postman, J.D., and Martin, R.R. (2007). Identification, detection and transmission of a new Vitivirus from Mentha. *Archives of Virology*, *152*(11), 2027–2033. doi: 10.1007/s00705-007-1030-1

Blueberry latent вирус у боровници (*Vaccinium corymbosum* L.) у Босни и Херцеговини

Делић, Д.[1], Михаљица, М.[1], Вакић, М.[1], Сунулахпашић, А.[2], Тзанетакис, Е.И.[3]

[1]Универзитет у Бањој Луци, Пољопривредни факултет, Булевар војводе Петра Бојовића 1А, Бања Лука, Босна и Херцеговина, duska.delic@agro.unibl.org
[2]Министарство пољопривреде, водопривреде и шумарства, Средњобосански кантон, Травник
[3]Одјељење за ентомологију и фитопатологију, Одсјек за пољопривреду, Универзитет у Арканзасу, Фејетвил, САД

Сажетак

У 2018. години обављено је истраживање мањег обима присуства вируса боровнице у Босни и Херцеговини. Укупно је прикупљено 20 узорака са три локације и анализирано на присуство 11 вируса. ELISA тестови су коришћени за испитивање узорака на присуство blueberry scorch virus, blueberry shock virus, blueberry shoestring virus, blueberry leaf mottle virus, tobacco ringspot virus и tomato ring spot virus. Узорци су тестирани и на red ringspot virus помоћу PCR и blueberry fruit drop associated virus, blueberry latent virus, blueberry mosaic associated virus, blueberry necrotic ring blotch virus, blueberry virus A, blueberry leaf mottle virus користећи RT-PCR методе. Анализе су потврдиле присуство BlLV у осам узорака, при чему ни у једном од узорака није потврђено присуство других вируса.

Кључне ријечи: *Vaccinium corymbosum* L., BlLV, RT-PCR, секвенцирање, диверзитет

Corresponding author: Duška Delić
E–mail: duska.delic@agro.unibl.org

Received: March 04, 2022
Accepted: May 23, 2022