Acta Agriculturae Serbica, 27 (53), 31–37, 2022

UDC 633.853:632.38(497.11)"2019"
doi: 10.5937/AASer2253031S

Publisher: Faculty of Agronomy Čačak

www.afc.kg.ac.rs

Molecular characterization of turnip yellows virus isolates from canola in Serbia

Ivana Stanković[1*], Katarina Zečević[1], Goran Delibašić[1], Lidija Ivanović[1], Dragana Milošević[2], Milena Marčić[3], Branka Krstić[1]

[1]*University of Belgrade, Faculty of Agriculture, Institute of Phytomedicine, Department of Phytopathology, Nemanjina 6, 11080 Belgrade, Serbia*
[2]*Institute of Field and Vegetable Crops, Maksima Gorkog 30, 21000 Novi Sad, Serbia*
[3]*Plant Protection Forecasting and Warning Service, Temerinska 131, 21000 Novi Sad, Serbia*

**Corresponding author: ivana.stankovic@agrif.bg.ac.rs*

Received 7 December 2021; Accepted 18 March 2022

A B S T R A C T

In November 2019, virus-like symptoms resembling those caused by turnip yellows virus (TuYV) were observed in many canola crops across Serbia. In order to identify their causal agent, a total of 206 samples were collected and analyzed for the presence of TuYV, cauliflower mosaic virus (CaMV) and turnip mosaic virus (TuMV), using commercial double-antibody sandwich (DAS)-ELISA kits. TuYV was detected serologically in 91.75% of tested samples collected at 24 locations in all of seven inspected districts. None of the samples tested positive for TuMV and CaMV. Further molecular characterization based on the partial P0 gene sequences of seven selected ELISA-positive samples showed that Serbian TuYV isolates collected in 2019 shared low nucleotide diversity, and that they were closely related to previously identified Serbian cabbage and mustard isolates of TuYV. Phylogenetic analysis showed that TuYV isolates from Serbia were clustered within the TuYV/BrYV group. Moreover, nucleotide and amino acid sequence comparison of all TuYV isolates originating from Serbia, supported with a phylogenetic tree, indicated the existence of two virus subpopulations in Serbia. Further research should focus on determining the variability of TuYV population in Serbia, based on a whole-genome analysis that will contribute to a better understanding of the epidemiology of this pathogen, aiming at developing and implementing appropriate control measures.

Keywords: Turnip yellows virus, canola, DAS-ELISA, molecular detection, phylogenetic analysis.

И З В О Д

Током новембра 2019. године, симптоми који упућују на присуство вируса жутице пострне репе (turnip yellows virus, TuYV) примећени су у многим усевима уљане репице гајених широм Србије. У циљу идентификације проузроковача обољења, сакупљено је укупно 206 узорака, који су серолошки тестирани на присуство TuYV, вируса мозаика карфиола (cauliflower mosaic virus, CaMV) и вируса мозаика пострне репе (turnip mosaic virus, TuMV) коришћењем комерцијалних DAS-ELISA китова. Присуство TuYV серолошки је доказано у 91,75% тестираних узорака сакупљених на 24 локалитета гајења у свих седам прегледаних округа, док присуство TuMV и CaMV у тестираним изорцима уљане репице није потврђено. Даља молекуларна карактеризација седам одабраних ELISA позитивних узорака на основу делимичне секвенце P0 гена указала је на висок степен нуклеотидне идентичности изолата TuYV прикупљених током 2019. године, као и на њихову велику сличност са претходно идентификованим изолатима овог вируса пореклом из купуса и слачице из Србије. Филогенетска анализа је показала груписање изолата TuYV из Србије у TuYV/BrYV групу. Међутим, нуклеотидна и аминокиселинска сличност секвенци свих изолата из Србије, као и филогенетска анализа, указују на постојање две популације овог вируса у нашој земљи. Даља истраживања треба усмерити ка утврђивању варијабилности популације TuYV у Србији анализом секвенци целог генома што ће допринети бољем разумевању епидемиологије овог патогена, а све у циљу развоја и примене адекватних мера контроле.

Кључне речи: Turnip yellows virus, уљана репица, DAS-ELISA, молекуларна детекција, филогенетска анализа.

1. Introduction

Brassica napus L. (family Brassicaceae), known as canola or oilseed rape, is an oilseed crop serving as an important source of dietary proteins, energy and anti-nutrients, mainly glucosinolates (Sakač et al., 2006). This oilseed plant is primarily cultivated for the production of vegetable oil for human consumption due to its low level of erucic acid and high level of unsaturated fatty acids, including Omega-3. Furthermore, it is also used to obtain biodiesel, biological lubricants or technical fatty acids for industry purposes or as a valuable source of protein for animal feed (Daun, 2011; Lin et al., 2013). Given these agronomical benefits, the production of canola is growing rapidly on a global scale and expanding to new countries. In Serbia, canola is also very popular and its cultivation has been increasing in recent years

(Marjanović Jeromela et al., 2016). However, the production of this profitable crop is significantly threatened by the emergence of various plant viruses, of which turnip yellows virus (TuYV) is one of the most economically important in Europe (Stevens et al., 2008; Newbert, 2016; Orfanidou et al., 2021).

TuYV was first reported in the United Kingdom as a European strain of beet western yellows virus (BWYV), which was not able to cause sugar beet infection (Duffus and Russell, 1970). The International Committee for the Taxonomy of Viruses (ICTV) later re-classified TuYV and BWYV as separate species in the genus *Polerovirus*, family *Luteoviridae*, based on differences in their host range, serological specificity and amino acid sequence identity of any gene product greater than 10%, and due to the failure of cross-protection (Mayo, 2002; King et al., 2011).

TuYV, as other members of the *Polerovirus* genus, has non-enveloped icosahedral particles. The genome is a single-stranded positive sense RNA with a viral genome-linked protein (VPg) on the 5' terminus (D'Arcy and Domier, 2005). The ORFs are arranged in a 5' (ORF 0, 1 and 2) and 3' (ORF 3, 3a, 4, and 5) block, separated by an intergenic non-coding region (iNCR). ORFs 0-2 encode the proteins P0, P1 and the P1-P2 fusion protein, associated with pathogenicity, host range, silencing suppression and replication on the viral sense. P3 is the major coat protein (CP), and P3a is a complement to ORF4 and aids cellular movement, while P4 is the putative movement protein (MP), and P3-P5 fusion protein is a minor coat protein which participates in virus accumulation and persistence within the vector. Genes of the 3' block encode proteins either on the viral or viral complementary sense (Mayo et al., 1989; Mayo and Miller, 1999; Pfeffer et al., 2002; Brault et al., 2005; D'Arcy and Domier, 2005; Smirnova et al., 2015).

The virus is particularly significant as a pathogen of oilseed crops but its host range is wide and includes many important crops, as well as a large number of weed species in the families Brassicaceae, Fabaceae, Amaranthaceae and Asteraceae (Jay et al., 1999; Farzadfar et al., 2007; Sharma et al., 2013; Wang et al., 2015). Canola plants infected with TuYV produce a broad range of symptoms such as mild reddening of leaf margins and interveinal yellowing or reddening, but sometimes the symptoms resemble those caused by stress or nutrient deficiency (Stevens et al., 2008). TuYV is transmitted by numerous aphid species in a circulative, non-propagative manner. The green peach aphid *Myzus persicae* is the most efficient vector, while other aphid species such as *Brevicoryne brassicae*, *Aphis gossypii*, and *Macrosiphum euphorbiae* have much lower transmission rates (Schliephake et al., 2000; Hauser et al., 2002).

In Serbia, TuYV was recorded for the first time on canola in 2014 (Milošević et al., 2015). A follow-up study conducted the following year showed that the virus was present in several canola growing areas at high incidence rates (Milošević et al., 2016). In addition, virus spreading to other brassica crops including mustards (Milošević et al., 2019) and cabbage (Milošević et al., 2020) was also reported.

In November 2019, virus-like symptoms, including red discoloration at leaf margins and interveinal yellowing, were observed in all major canola-producing areas throughout Serbia. The aim of this study was to identify the etiological agent responsible for the observed symptoms by serological and molecular testing of diseased canola plants. In addition, the study focused on determining the genetic relationship of new Serbian TuYV isolates with those from the GenBank database originating from different parts of the world, and with those previously identified in Serbia.

2. Material and methods

2.1. Plant material collection

In the autumn of 2019, plants with typical symptoms of virus infection were observed in many canola crops throughout Serbia. Five to ten symptomatic samples were randomly collected from each of 27 different locations across seven administrative districts of Serbia by using the "W model" of moving in the crop.

Samples of symptomatic leaves were packed in plastic bags, transported to the laboratory and then stored in a refrigerator or freezer until serological or molecular testing.

2.2. Serological testing

To indentify the causal agent, the collected samples were initially analized by double-antibody sandwich (DAS)-ELISA test using commercial polyclonal antisera (Loewe Biochemica, Sauerlach, Germany) for three most widespread brassica viruses: TuYV, turnip mosaic virus (TuMV), and cauliflower mosaic virus (CaMV) (Nooh, 2012). Samples for the DAS-ELISA test were prepared by grounding symptomatic plant tissue using a mortar and pestle in the presence of extraction buffer (1:10 wt/vol), according the manufacturer's instruction. Absorbance at 405 nm was determined spectrophotochemically using an ELISA microplate reader (DAS srl, Palombara Sabina, Italy) and samples that showed an absorbance value at least two times higher than absorbance of the negative control were considered positive. Both commercial positive and negative controls (Loewe), as well as extracts from healthy canola leaves were included in each ELISA test.

2.3. RT-PCR assay and sequence analysis

The identities of seven selected TuYV positive samples, one sample representing each district, were further confirmed by the amplification and sequencing of the partial P0 gene. The extraction of total RNAs was done with the RNeasy Plant Mini Kit (Qiagen, Hilden, Germany) and subjected to RT-PCR. RT-PCR was performed using One-Step RT-PCR kit (Qiagen) and TuYV-specific primers TuYVorf0F and TuYVorf0R (Schubert et al., 1998). Ampifications were performed using a T-1 thermal cycler (Biometra, Göttingen, Germany) with a reaction volume of 25 µl containing 5 µl 5x Qiagen OneStep RT-PCR buffer (containing 12.5 mM $MgCl_2$), 1 µl 400 µM dNTPs, 1.5 µl 0.6 µM of each primers, 1 µl enzyme mix, 1 µl extracted RNA and 14 µl nuclease-free water. RT-PCR cycling conditions were as folows: 30 min at 50°C for reverse transcription, 15 min at 95°C for initial denaturation, followed by 35 cycles with denaturation at 94°C for 30 s, annealing at 55°C for 30 s and extension at 72°C for 1 min and a final extension step at 72°C for 10 min. The size of the amplified products was determined using UV

transilluminator after 1% agarose gel electrophoresis in TBE buffer and stained with ethidium bromide.

RNAs extracted from a healthy canola plant and a Serbian TuYV isolate from cabbage (GenBank Accession No. MN602973) were included in each RT-PCR reaction as negative and positive controls, respectively.

Amplified products of seven selected isolates were purified (QIAquick PCR Purification Kit, Qiagen), Sanger sequenced in Macrogen-Europe Laboratory (Amsterdam, the Netherlands). and deposited in GenBank (Table 1).

The sequences obtained in this study were compared with each other and with previously identified Serbian TuYV sequences, as well as with TuYV sequences from other parts of world deposited in GenBank using the BLAST algorithm and the ClustalW program (Thompson et al., 1994) and MEGA7 software (Kumar et al., 2016).

2.4. Phylogenetic analysis

A phylogenetic tree was constructed by the maximum parsimony algorithm, using 43 P0 gene sequences of different poleroviruses. These included sequences generated in this study, six previously sequenced TuYV isolates from Serbia, as well as sequences of TuYV and other poleroviruses retrieved from GenBank (Table 1).

Table 1.
P0 gene sequences of poleroviruses used in the phylogenetic analysis

Isolate name*/Virus species	Country	Host plant	GenBank Accession number
*348-19/TuYV***	*Serbia*	*Brassica napus*	*OK166814*
351-19/TuYV	*Serbia*	*B. napus*	*OK166815*
358-19/TuYV	*Serbia*	*B. napus*	*OK166816*
361-19/TuYV	*Serbia*	*B. napus*	*OK166817*
367-19/TuYV	*Serbia*	*B. napus*	*OK166818*
371-19/TuYV	*Serbia*	*B. napus*	*OK166819*
374-19/TuYV	*Serbia*	*B. napus*	*OK166820*
114-TuYV/TuYV	Serbia	*B. napus*	KR351306
119-TuYV/TuYV	Serbia	*B. napus*	KU351664
345Cb/TuYV	Serbia	*B. oleracea* var. *capitata*	MN602973
364Cb/TuYV	Serbia	*B. oleracea* var. *capitata*	MN165558
88Sal/TuYV	Serbia	*Sinapis alba*	MK144816
98Bni/TuYV	Serbia	*B. nigra*	MK144817
FL1/TuYV	France	*Lactuca sativa*	NC_003743
Geo15/TuYV	Greece	*B. napus*	MT955611
5509/TuYV	Australia	*B. napus*	MT586587
C2016b/TuYV	Australia	*B. napus*	MT586588
C20A/TuYV	Australia	*B. napus*	MT586597
Geo7/TuYV	Greece	*B. napus*	MT955610
1-2/TuYV	Greece	*B. napus*	MT955609
5512b/TuYV	Australia	*B. napus*	MT586580
P5-8/TuYV	Australia	*Pisum sativum*	MT586586
L31-4/TuYV	Australia	*Lens culinaris*	MT586574
Br12/TuYV	Australia	*Beta vulgaris*	MT586598
5248/TuYV	Australia	*S. arvensis*	MT586581
MK111/TuYV	Australia	*Cicer arietinum*	MT586573
5512a/TuYV	Australia	*B. napus*	MT586593
5514a/TuYV	Australia	*B. napus*	MT586594
Anhui/TuYV	China	*Nicotiana tabacum*	KR706247
AJS/BrYV	China	*B. rapa*	HQ388350
WN1/BrYV	Japan	*S. alba*	LC428359
BBJ/BrYV	China	*B. napobrassica*	HQ388349
R3b/BrYV	Japan	*Raphanus raphanistrum*	LC428363
CC1/BrYV	Japan	*B. rapa*	LC428358
BChV-CR/BChV	USA	*B. vulgaris*	AF352025
BChV-GW/BChV	USA	*B. vulgaris*	AF168609
Unknown/CABYV	China	*Cucurbita pepo*	EU000535
N /CABYV	France	unknown	X76931
IM/BWYV	China	*B. vulgaris*	EU636991
BJA/BWYV	China	*B. vulgaris*	HM804471
S19/BWYV	Japan	*Spinacia oleracea*	LC428356
LS/BWYV	Korea	*Leonurus sibiricus*	KM076647
Rouen 1/BWYV	France	*Nepenthes mirabilis*	KU521325

*All data are from GenBank

**Isolates in italics were amplified and sequenced in this study, other data are from GenBank

The philogenetic tree was constructed using MEGA7 and the bootstrap method based on 1000 replicates. The Kimura 2-parameter model with Gamma distribution (K2+G) was selected as the best-fitting model of nt substitution and was used to calculate intra- and inter-group diversity.

3. Results and discussions

3.1. Field symptoms and presence of TuYV

During visual inspection of canola crops from October to November 2019, virus-like symptoms were observed in all inspected locations throughout Serbia. Canola plants showed symptoms on leaves, including reddening of leaf margins (Figure 1a), accompanied by interveinal yellowing (Figure 1b) to reddening and leaf curling (Figure 1c). Disease incidence mostly ranged between 20 and 40%, but in some crops it was much higher (about 70%).

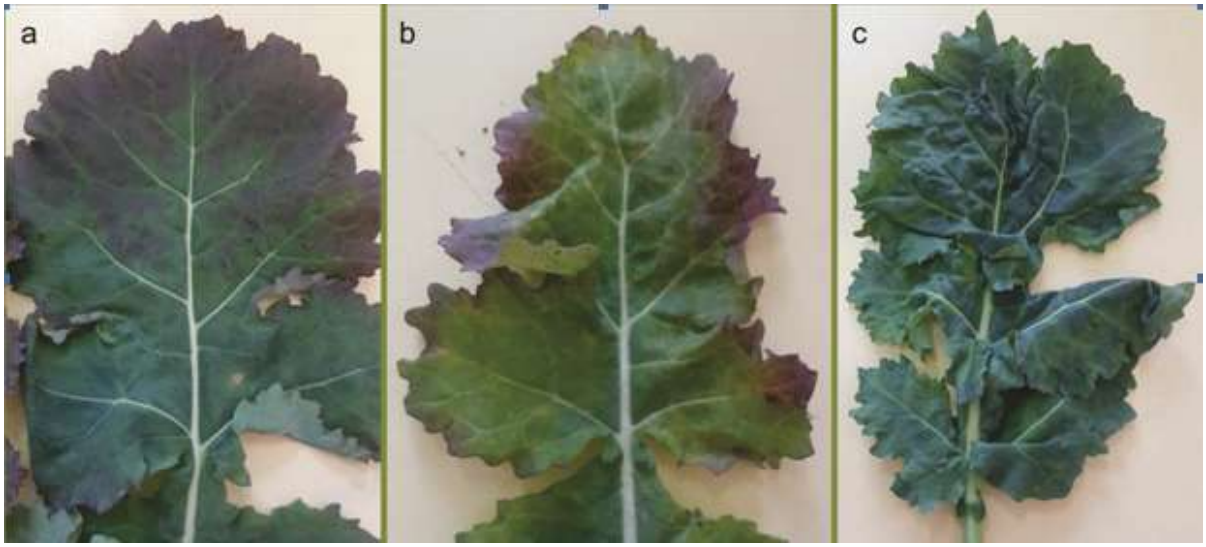

Figure 1. Symptoms of TuYV infection: **a** reddening of leaf margins; **b** reddening of leaf margins and interveinal yellowing; **c** leaf curling.

Serological analyses with commercial polyclonal antisera for the most common canola viruses revealed that TuYV was the only virus detected in almost all inspected crops (Table 2).

Table 2.
Number of canola samples from different Serbian districts analyzed and tested positive for the presence of turnip yellows virus by DAS-ELISA in 2019

District	Locations	No. of analyzed samples	ELISA positive samples
West Bačka	Sombor	10	10
	Apatin	6	6
	Stanišić	8	8
North Bačka	Kać	9	9
	Bačka Palanka	7	7
	Bačka Topola	10	10
	Njegoševo	7	7
	Mali Iđoš	9	9
	Donji Tavankut	6	6
	Mišićevo	8	8
	Đurđin	7	7
	Mala Pešta	8	8
	Novi Žednik	9	9
	Bač	5	0
South Bačka	Vrbas	10	8
North Banat	Kikinda	6	6
	Banatska Topola	10	10
	Tornjoš	7	7
	Senta	5	5
	Ada	8	8
	Gornji Breg	9	9
South Banat	Pančevo	7	7
Srem	Irig	8	8
	Rivica	5	0
	Jarak	9	9
Mačva	Jevremovac	8	8
	Slepčević	5	0
Total		206	189

None of the tested samples reacted with TuMV or CaMV antisera. TuYV was detected in 189 out of 206 symptomatic canola plants collected at 24 locations (Table 2). Many other studies (Stevens et al., 2008; Newbert, 2016; Orfanidou et al., 2021) have also shown that TuYV was the most widespread canola virus in different parts of Europe. TuYV is one of the most common detected virus in oilseed rape crops in the UK (Stevens et al., 2008). In Germany, the presence of TuYV in winter oilseed rape has caused great economic damage with regular epidemics (Graichen and Peterka, 1999). TuYV epidemics also occur regularly in different crops in Australia, including canola and pulse crops (Filardo et al., 2021).

However, a certain number (17) of collected samples did not react with antisera against any of the tested viruses even though they exhibited symptoms. Characteristic symptoms on diseased canola plants, such as reddening and yellowing of leaf margins and whole leaves, have often been associated with TuYV (Graichen and Peterka, 1999; Orfanidou et al., 2021), but these symptoms may also be a consequence of physiological and nutritional disorders, including phosphorus deficiency, as stated by Stevens et al. (2008).

3.2. Sequence analysis and phylogenetic tree

The result of RT-PCR analysis revealed that all seven selected canola samples yielded an amplicon of expected size (780 bp) that confirmed the presence of TuYV. No amplification product was recorded in the healthy control.

To characterize selected isolates they were directly sequenced and obtained sequences were deposited in GenBank under the accession numbers shown in Table 1. BLAST results revealed that P0 sequences of the new Serbian TuYV isolates showed the highest nt identity of 98.17–99.5% with those available in GenBank, which is consistent with species demarcation criteria for the genus *Polerovirus* (King *et al*. 2011), and showed that the selected isolates belong to TuYV species. All TuYV sequences generated in this study were also compared with each other, as well as with all previously identified sequences of Serbian TuYV isolates in order to characterize the genetic variability among them. The P0 gene sequences of Serbian TuYV isolates collected in 2019 shared nt identities of 96.5% to 100% (94.5–100% aa identity), while their homology with previously identified Serbia TuYV isolates ranged from 92.5% to 98.9% at the nt level (88.5% to 99.1% aa identities). Also, this study showed that the identity among confirmed Serbian canola TuYV isolates was lower (88.5–91.1% aa identities) than the identity among those collected in 2019 and previously identified cabbage and mustard TuYV isolates (93.6–97.9% and 94.9–99.1% aa identities, respectively).

A maximum parsimony tree (Figure 2) based on the partial sequences of the P0 gene generated in this study and previously sequenced TuYV isolates from Serbia, as well as sequences of TuYV, brassica yellows virus (BrYV), beet western yellows virus (BWYV), beet chlorosis virus (BChV), and cucurbit aphid-borne yellows virus (CAVYV) isolates retrieved from GenBank showed clustering of the selected isolates into four well-defined groups with high bootstrap values (100%): TuYV/BrYV, BChV, CABYV, and BWYV. Genetic diversity among the group ranged from 0.672±0.060 to 3.087±0.616, whereas the diversity within each group was: 0.074±0.007 (TuYV/BrYV), 0.003±0.002 (BChV), 0.147±0.018 (CABYV), and 0.134±0.012 (BWYV). All isolates were grouped according to virus species except for TuYV/BrYV group, as previously noticed by Filardo et al. (2021). Despite the differences in amino acid sequence between TuYV and BrYV isolates, phylogenetic analysis based on P0 gene sequences revealed that all TuYV and BrYV isolates were clustered together into one monophyletic group indicating that they share a common ancestor for this genomic region. Phylogenetic tree also showed four subgroups of isolates belonging to the TuYV/BrYV group (subgroups 1–4) with bootstrap support of 100% (Figure 2). Subgroup 1 contained 11 isolates from Serbia (348-19, 351-19, 358-19, 361-19, 367-19, 371-19, 374-19, 98Bni, 99Sal, 364Cb, and 345Cb), seven TuYV isolates from Australia and two TuYV isolates from Greece. Subgroup 2 comprised two isolates from Serbia (114-TuYV and 119-TuYV), two TuYV isolates from Australia and one BrYV isolate each from China and Japan. Subgroup 3 included one TuYV isolate from China and one BrYV isolate each from China and Japan, while subgroup 4 included only one BrYV isolate from Japan. Genetic diversity among the four subgroups of isolates ranged from 0.079±0.009 to 0.136±0.014, while it was lower within each subgroup: 0.059±0.006 (1), 0.014±0.003 (2), 0.044±0.007 (3). BrYV was first identified in China and it shared less than 90% aa identity with TuYV in almost all ORFs (Xiang et al., 2011). Another study revealed the occurrence of three distinct BrYV genotypes named BrYV-A to -C (Zhang et al., 2014), while Orfanidou et al. (2021) reported the existence of a TuYV/BrYV recombinant isolate in canola crops in Greece. However, Filardo et al. (2021) revealed that TuYV and BrYV belong to the same species based on their entire genome sequences and that BrYV is only a strain of TuYV. In addition, they suggested that the species demarcation criteria for poleroviruses should include a similarity of less than 83% nt identity in the whole coding region of the genome, as well as any marked host range, vector, and serological difference. The seven canola isolates originating from 2019, as well as the previously characterized Serbian TuYV isolates, were grouped into the TuYV/BrYV group, but in two different subgroups. Canola isolates collected in 2019, as well as cabbage and mustard TuYV isolates originating in Serbia, were different from the previously identified Serbian canola isolates and formed another subgroup within the TuYV/BrYV group, indicating the existence of two different subpopulations of the TuYV in Serbia. Further research should focus on determining the variability within TuYV population in canola and other brassica crops, based on whole-genome sequencing, which will provide more detailed information on its population structure in Serbia. Such analyses would help develop resistant varieties with better performance in controlling the disease caused by this virus.

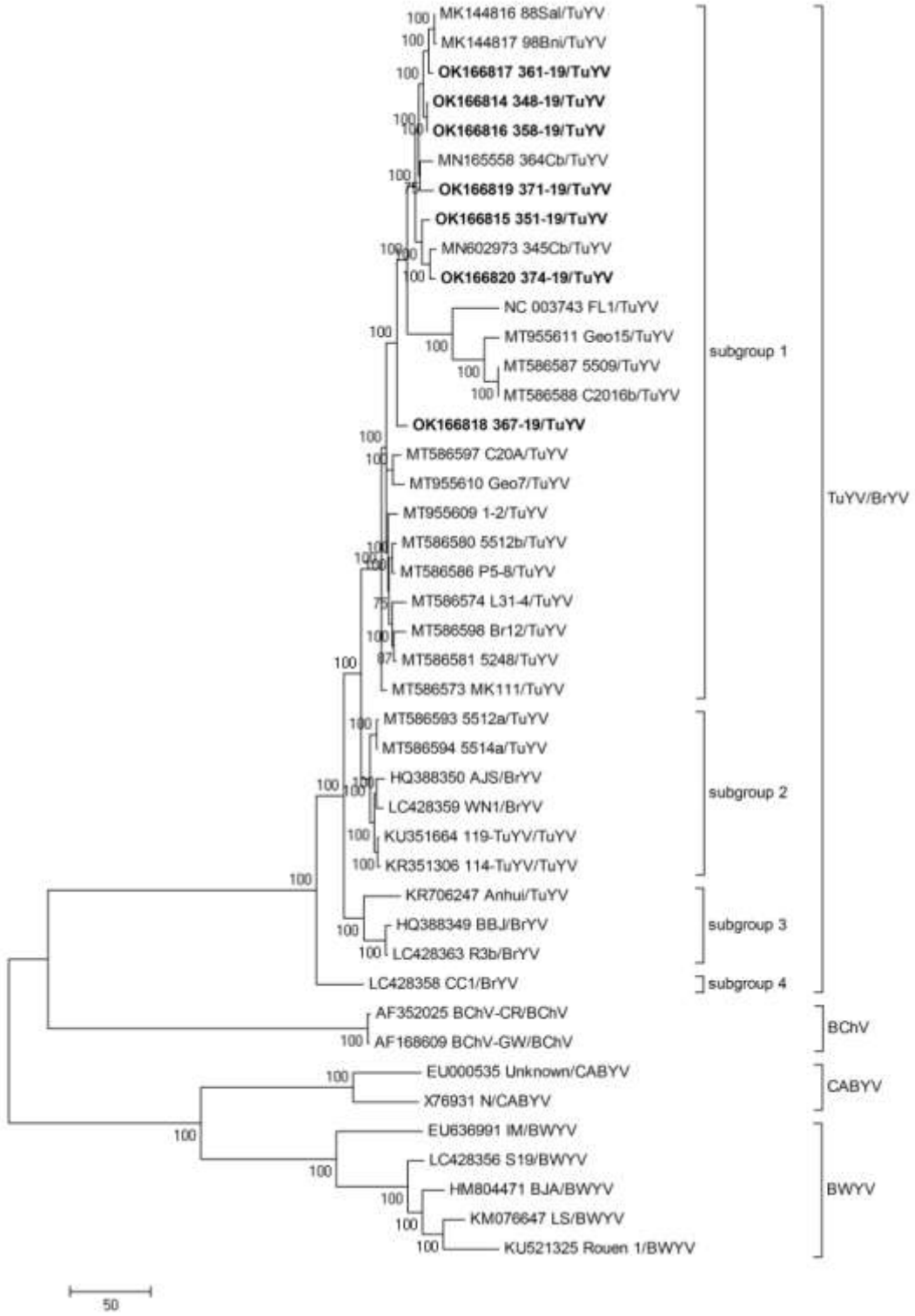

Figure 2. Maximum parsimony tree based on 43 partial P0 nucleotide sequences of polerovirus isolates using MEGA7 and bootstrap analysis with 1000 replicates. Bootstrap values of 50% and above are shown at nodes. The turnip yellows virus isolates from this study are bolded.

4. Conclusions

The data obtained in this study clearly show that TuYV is present at high incidence in almost all inspected canola crops, and that it represents a serious threat to successful canola production in Serbia. Therefore, it is necessary to improve growers' awareness of management options of the virus and its vectors, in particular control measures aimed at limiting early virus infection. Further epidemiological investigation is also needed to identify and understand the factors affecting the emergence, severity and consequences of the disease caused by TuYV in Serbia.

Acknowledgment

This research was supported by grants 451-03-68/2022-14/200116 and 451-03-68/2022-14/200032

of the Ministry of Education, Science and Technological Development, Republic of Serbia.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

Brault, V., Périgon, S., Reinbold, C., Erdinger, M., Scheidecker, D., Herrbach, E., Richards, K., Ziegler-Graff, V. (2005). The polerovirus minor capsid protein determines vector specificity and intestinal tropism in the aphid. *Journal of Virology*, 79(15), 9685–9693.

D'Arcy, C.J., Domier, L.L. (2005). Luteoviridae. In: Fauquet C.M., Mayo M.A., Maniloff J., Desselberger U., Ball L.A. (Eds.), *Virus Taxonomy, VIIIth Report of the ICTV,* Elsevier/Academic Press, London, pp. 891–900.

Daun, J. (2011).Origin, distribution and production. In: Daun J., Eskin N., Hickling D. (Eds.), *Canola: Chemistry, production, processing and utilization,* AOCS Press, Urbana, pp. 1–27.

Duffus, J.E., Russell, G.E. (1970). Serological and host range evidence for the occurrence of *beet western yellows virus* in Europe. *Phytopathology*, 60(8), 1199–1202.

Farzadfa, S., Ahoonmanes, A., Hossein Mosaheb, G., Pourrahi, R., Reza Golnaragh, A. (2007). Occurrence and distribution of *Cauloflower mosaic virus* on Cruciferous plant in Iran. *Plant Pathology Journal*, 6(1), 22–29.

Filardo, F., Nancarrow, N., Kehoe, M., McTaggart, A.R., Congdon, B., Kumari, S., Aftab, M., Trębicki, P., Rodoni, B., Thomas, J., Sharman, M. (2021). Genetic diversity and recombination between turnip yellows virus strains in Australia. *Archives of Virology*, 166(3), 813–829.

Graichen, K., Peterka, H. (1999). Generation of oilseed rape with resistance to turnip yellows luteovirus. *10th International Rapeseed Congress - New Horizons from an Old Crop*, *Canberra,* 26-29 September, pp. 148.

Hauser, S., Stevens, M., Beuve, M., Lemaire, O. (2002). Biological properties and molecular characterization of *Beet chlorosis virus* (BChV). *Archives of Virology*, 147(4), 745–762.

Jay, C.N., Rossall, S., Smith, H.G. (1999). Effects of *Beet western yellows virus* on growth and yield of oilseed rape (*Brassica napus*). *Journal of Agricultural Science*, 133(2), 131–139.

King, A.M.Q., Adams, M.J., Carstens, E.B., Lefkowitz, E.J. (2011). *Virus Taxonomy: Ninth report of the International Committee on Taxonomy of Viruses*. Elsevier Academic Press, San Diego, 1327 p.

Kumar, S., Stecher, G., Tamura, K. (2016). MEGA7: Molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Molecular Biology Evolution*, 33(7), 1870–1874.

Lin, L., Allemekinders, H., Dansby, A., Campbell, L., Durance-Tod, S., Berger, A. (2013). Evidence of health benefits of canola oil. *Nutrition Reviews*, 71, 370–385.

Marjanović Jeromela, A., Atlagić, J., Stojanović, D., Terzić, S., Mitrović, P., Milovac, Ž., Dedić, D. (2016). Dostignuća u oplemenjivanju NS hibrida uljane repice. *Selekcija i semenarstvo*, 22(2), 49–60.

Mayo, M. A. (2002). ICTV at the Paris ICV: results of the plenary session and the binomial ballot. *Archives od Virology*, 147, 2254–2260.

Mayo, M.A., Miller, W.A. (1999). The structure and expression of luteovirus genomes. In: Smith H.G., Baker H. (Eds.), *The Luteoviridae*, CAB International, Wallingford, pp. 23–43.

Mayo, M.A., Robinson, D.J., Jolly, C.A., Hyman, L. (1989). Nucleotide sequence of *Potato leafroll luteovirus* RNA. *Journal of General Virology*, 70, 1037–1051.

Milošević, D., Ignjatov, M., Vlajić, S., Nikolić, Z., Gvozdanović Varga, J., Stanković, I., Krstić, B. (2020). First Report of *Turnip Yellows Virus* on Cabbage in Serbia. *Plant Disease*, 104(3), 997.

Milošević, D., Ignjatov, M., Marjanović-Jeromela, A., Stanković, I., Nikolić, Z., Timindžić, G., Krstić, B. (2019). Molecular characterization of *yellow beet virus* - a new pathogen of mustard in Serbia. *Ratarstvo i povrtarstvo*, 56(3), 82–87.

Milošević, D., Ignjatov, M., Nikolić, Z., Stanković, I., Bulajić, A., Marjanović-Jeromela, A., Krstić, B. (2016). The presence of *Turnip yellows virus* in oilseed rape (*Brassica napus* L.) in Serbia. *Pesticidi i fitomedicina*, 31(1-2), 37–44.

Milošević, D., Marjanović-Jeromela, A., Ignjatov, M., Jovičić, D., Stanković, I., Bulajić, A., Krstić, B. (2015). First report of *Turnip yellows virus* on oilseed rape in Serbia. *Plant Disease*, 99(12), 1869.

Newbert, M.J. (2016). The genetic diversity of *Turnip yellows virus* in oilseed rape (*Brassica napus*) in Europe, pathogenic determinants, new sources of resistance and host range. Doctoral dissertation, University of Warwick, Coventry.

Nooh, S. (2012). An overview of oilseed rape (canola) virus diseases in Iran. *International Research Journal of Microbiology*, 3(1), 24–28.

Orfanidou, C.G., Lotos, L., Tsiolakis, G., Stefanidis, S.K., Tsialtas, J.T., Katis, N.I., Maliogka, V.I. (2021). Molecular characterization of poleroviruses isolated from oilseed rape in Greece. *Virus Genes*, 57(3), 289–292.

Pfeffer, S., Dunoyer, P., Heim, F., Richards, K.E., Jonard, G., Ziegler-Graff, V. (2002). P0 of *Beet western yellows virus* is a suppressor of posttranscriptional gene silencing. *Journal of Virology*, 76(13), 6815–6824.

Sakač, M.B., Filipović, S.S., Borojević, Č.M., Ristić, M.D., Kormanjoš, Š.M. (2006). The influence of extrusion on total glucosinolates and total phenols in rapeseed. *Acta Agriculturae Serbica,* 11(21), 19–27.

Schliephake, E., Graichen, K., Rabenstein, F. (2000). Investigations on the vector transmission of the *Beet mild yellowing virus* (BMYV) and the *Turnip yellows virus* (TuYV). *Journal of Plant Diseases and Protection*, 107(1), 81–87.

Schubert, J., Rabenstein, F., Graichen, K., Richter, K. (1998). Comparison of the 5´-end nucleotide sequences of luteoviruses from oilseed rape and sugarbeet. *Archives of Phytopathology and Plant Protection*, 31(6), 519–530.

Sharma, P., Verma, R.K., Mishra, R., Choudhary, D.K., Gaur, R.K. (2013). First report of *Turnip yellow virus* (TuYV) in *Brassica juncea* (Indian mustard) in India. *New Disease Reports,* 27(1), 21.

Smirnova, E., Firth, A.E. Miller, W.A. Scheidecker, D., Brault, V., Reinbold, C., Rakotondrafara, A.M., Chung, B.Y.W., Ziegler-Graff, V. (2015). Discovery of a small non-AUG-initiated ORF in Poleroviruses and Luteoviruses that is required for long-distance movement. *PLoS Pathogens*, 11(5), e1004868.

Stevens, M., McGrann, G., Clark, B., Authority, H. (2008). Turnip yellows virus (syn Beet western yellows virus): an emerging threat to European oilseed rape production? *HGCA Research Review,* 69, 1–37.

Thompson, J.D., Higgins, D.G., Gibson, T.J. (1994). CLUSTAL W: Improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position–specific gap penalties and weight matrix choice. *Nucleic Acids Research*, 22(22), 4673–4680.

Wang, F., Wu, Q.F., Zhou, B.G., Gao, Z.L., Xu, D.F. (2015). First Report of *Turnip yellows virus* in Tobacco in China. *Plant Disease*, 99(12), 1870–1870.

Xiang, H.-Y., Dong, S.-W., Shang, Q.-X., Zhou, C.-J., Li, D.-W., Yu, J.-L., Han, C.-G. (2011). Molecular characterization of two genotypes of a new polerovirus infecting brassicas in China. *Archives of Virology*, 156(12), 2251–2255.

Zhang, X.-Y., Xiang, H.-Y., Zhou, C.-J., Li, D.-W., Yu, J.-L., Han, C.-G. (2014). Complete genome sequence analysis identifies a new genotype of Brassica yellows virus that infects cabbage and radish in China. *Archives of Virology*, 159(8), 2177–2180.