Pestic. Phytomed. (Belgrade), 31(1-2), 2016, 37–44
DOI: 10.2298/PIF1602037M

UDC 632.38:635.12(497.11)
Original scientific paper

The presence of *Turnip yellows virus* in oilseed rape (*Brassica napus* L.) in Serbia

Dragana Milošević[1*], Maja Ignjatov[1], Zorica Nikolić[1], Ivana Stanković[2], Aleksandra Bulajić[2], Ana Marjanović-Jeromela[1] and Branka Krstić[2]

[1]Institute of Field and Vegetable Crops, Maksima Gorkog 30, 21 000 Novi Sad, Serbia
[2]University of Belgrade, Faculty of Agriculture, Nemanjina 6, 11080 Belgrade, Serbia
**Corresponding author: dragana.milosevic@nsseme.com*

Received: 30 December, 2015
Accepted: 30 January, 2016

SUMMARY

A total of 86 oilseed rape samples from six crops in different localities were collected during 2014 and analyzed for the presence of *Turnip yellows virus* (TuYV), *Cauliflower mosaic virus* (CaMV) and *Turnip mosaic virus* (TuMV) using commercial double-antibody sandwich (DAS)-ELISA kits. TuYV was serologically detected in 60 collected samples (69.77%), and none of the samples tested were positive for CaMV and TuMV. Six selected TuYV isolates were successfully transmitted by *Myzus persicae* to three test plants, confirming the infectious nature of the disease. In the selected ELISA-positive samples, the presence of TuYV was further confirmed by RT-PCR and sequencing. A comparison of the obtained sequence with those available in GenBank confirmed the presence of TuYV in oilseed rape samples. An analysis of P0 gene sequence data for a subset of these isolates showed they clustered with the known TuYV and were distinct from *Beet western yellows virus* (BWYV) isolates.

Keywords: Oilseed rape; *Turnip yellows virus*; Molecular detection; Serbia

INTRODUCTION

Oilseed rape (*Brassica napus* L), also known as rapeseed or canola, is a bright yellow flowering plant, a member of the *Brassicaceae* family. Due to its high oil and protein contents in seed, oilseed rape is mainly grown for the production of vegetable oil for human consumption, as an animal feed, and a biodiesel. The main producers of oilseed rape are China, the EU, Canada, and India. World production of oilseed rape is growing rapidly, increasing from 36 million tones in 2004 to an estimated 58.4 million tonnes in the 2010-2011 crop season (USDA, 2011). Furthermore, oilseed rape is a very profitable crop with very high oil yields per unit area, and for that reason the production of this crop is increasing in Serbia. The harvest area of this important oil-producing crop increased in Serbia from 12,012 ha in 2011/12 to 13,000 ha in 2015/16 (Association for the Promotion of Production and Exports of Grains and Oilseeds, www.zitasrbije.rs).

More than 12 viruses from different viral genera have been reported to infect oilseed rape and cause different

levels of losses in its production. However, economically the most important viruses are: *Beet western yellows virus* (BWYV), *Cauliflower mosaic virus* (CaMV), and *Turnip mosaic virus* (TuMV) (Latham et al., 2003; Shahraeen, 2012). There are reports of oilseed rape yields being severely affected by BWYV, CaMV and TuMV, sustaining reductions of 70-79% (Hardwick et al., 1994).

BWYV was first reported in Tasmania in the early 1980s and has been detected in a variety of crops, including legumes (Johnstone & Duffus 1984). Many of these hosts were asymptomatic (Johnstone et al., 1984). Some of the viruses previously described as BWYV, but shown not to infect sugarbeet (*Beta vulgaris* L.), have now been re-classified as a separate species in the genus *Polerovirus*, family *Luteoviridae*, under the name *Turnip yellows virus* (TuYV) (Graichen & Rabenstein, 1996; Hauser et al., 2000; Hauser et al., 2002). TuYV as a virus species has been ratified by the International Committee for the Taxonomy of Viruses (Mayo, 2002).

TuYV has a wide range of hosts and can infect species from at least 13 plant families, including many important plant species (cauliflower, cabbage, spinach, lettuce, etc.), but the virus is particularly significant as a pathogen of oilseed rape (Jay et al., 1999; Hill et al., 1989). The diverse range of cultivated plants and weed species susceptible to TuYV complicates its epidemiology by expanding the potential reservoirs in which the virus can overwinter and thus providing sources for future viral outbreaks (Stevens et al., 1994; Latham et al., 2003).

Oilseed rape plants infected with TuYV produce a wide range of symptoms – most of which go unnoticed because they resemble those caused by stress and nutrient-deficiency. The most common symptoms are red discoloration along the edges of infected leaves, followed by an intense chlorosis of the whole blade, which becomes hard and brittle. Infected plants remain dwarfed, and have small roots (Duffus & Russell, 1972).

Considering the importance of oilseed rape, the increasing spread of TuYV on various types of fam. *Brassicae*, and the common presence of many aphids that are vectors of the virus, TuYV is potentially a limiting factor for successful production of oilseed rape in Serbia. After the first detection of TuYV infected oilseed rape (Milošević et al., 2015), a survey was conducted in the main oilseed rape-growing areas of Serbia in order to survey the incidence and distribution of TuYV, and possible presence of other important oilseed rape infecting viruses, TuMV and CaMV, and to perform molecular characterization of the obtained isolates by comparing them with isolates from all over the world.

MATERIAL AND METHODS

Survey and sample collection

During 2014, a total of 86 samples of oilseed rape plants showing virus-like symptoms were randomly collected in six localities in three districts of the Vojvodina Province: West Bačka (Sombor, Crvenka and Kula), Srem (Sremska Mitrovica) and North Banat (Kikinda and Zrenjanin). The samples consisted of symptomatic leaves from different parts of each plant, which were collected and placed in plastic bags, and stored at 4°C until ELISA testing (up to 5 days), or stored at -20°C until RNA extraction.

Serological detection

The collected samples were tested for the presence of the most common oilseed rape viruses: *Turnip yellows virus* (TuYV), *Cauliflower mosaic virus* (CaMV), and *Turnip mosaic virus* (TuMV), using commercial double-antibody sandwich (DAS)-ELISA diagnostic kits (Loewe Biochemica, Germany) according to the manufacturer's instructions. Plant tissues were prepared in an extraction buffer at a ratio of 1:10 (wt/vol). After incubation with p-nitrophenyl phosphate (Sigma-Aldrich, St. Louis, MO) at room temperature for 2 h in the dark, absorbance at 405 nm was measured with an ELISA microplate reader (Multiscan Ascent, Finland). The samples were considered positive if their absorbance value was twice as high as the negative control. Commercial positive and negative controls were included in each ELISA plate.

Aphid transmission

For aphid-borne TuYV inoculation, nymphs of *Myzus persicae* (Sulzer) were allowed to feed on the leaves of six selected serologically positive samples, i.e. one sample from each locality, over an acquisition access period (AAP) of 24 h. Groups of 5-7 aphids for each of the six isolates were then placed onto three plants of each *Capsella bursa-pastoris, Physalis floridana,* and *B. napus* cv. 'Banaćanka' for a 4-day inoculation access period (IAP). The plants with aphids were placed into

separate cages under controlled conditions, at 22°C, for a 16 h photoperiod and watered regularly.

RT-PCR detection

The presence of TuYV in oilseed rape plants was further confirmed by the conventional reverse transcription (RT)-PCR. Total RNAs were extracted by the RNeasy Plant Mini Kit (Qiagen, Hilden, Germany) from 100 mg of leaf tissue of six selected samples originating from different localities. RT-PCR was performed using the One-Step RT-PCR Kit (Qiagen) with primers TuYVorf0F and TuYVorf0R amplifying the P0 gene, the most useful for delineation of *Poleroviruses* (Schubert et al., 1998). The RT-PCR reaction mixture included 400 µM of each of the four dNTPs, 1 µl of RT-PCR enzyme mix, 0.6 µM of each primer, and 1 µl of extracted RNA in a final volume of 25 µl. Amplifications were performed in a thermal cycler (Eppendorf, Germany) with the following cycling parameters: reverse transcription at 50°C for 30 min and an initial PCR denaturation step at 95°C for 15 min, followed by 35 cycles consisting of a denaturation step for 30 s at 94°C, primer annealing for 30 s at 55°C, and extension for 60 s at 72°C. The final extension was performed at 72°C for 10 min. The amplified products were analyzed by 1% agarose gel electrophoresis and visualized under a UV transilluminator. A tissue sample from the healthy oilseed rape leaf was used as a negative control in RT-PCR assays.

Sequencing and phylogenetic analysis

After purification with a QIAquick PCR Purification Kit (Qiagen), the amplified products from one selected isolate (119-TuYV) were sequenced directly in both directions, using the same primers as in RT-PCR. Additionally, a previously identified oilseed rape TuYV isolate 114-TYuV (Milošević et al., 2015) was also included in the investigation. The sequence of the Serbian TuYV isolate was compared with the previously reported isolates available in GenBank (http://www.ncbi.nlm.nih.gov/BLAST/), using the ClustalW program (Thompson et al., 1994) and MEGA5 software (Tamura et al., 2011). A p-distance model was applied for nucleotide (nt) and deduced amino acid (aa) sequence analyses.

A phylogenetic tree was constructed using the TuYV P0 gene sequence generated in this study, one generated in another study by Milošević et al. (2015) and 11 P0 sequences of TuYV, *Beet western yellows virus*, *Beet chlorosis virus*, and *Cucurbit aphid-borne yellows virus* isolates retrieved from GenBank (Table 1), using the Maximum parsimony method implemented in MEGA5. Intra- and inter-group diversity values were calculated as the average genetic distance using Kimura 2-parameter model Gamma distributed (K2+G), which was chosen as the best-fitting model of nt substitution.

Table 1. P0 gene sequences of *Turnip yellows virus*, *Beet western yellows virus*, *Beet chlorosis virus*, and *Cucurbit aphid-borne yellows virus* isolates used for phylogenetic analysis

Virus	Isolate	Geographical origin	Host	GenBank Acc. No.
Turnip yellows virus (TuYV)	119-TuYV*	Serbia	Oilseed rape	KU351664
	114-TuYV*	Serbia	Oilseed rape	KR351306
	TuYV-BN5	Germany	Oilseed rape	AF168606
	TuYV-GB	England	Oilseed rape	AF168608
	FL1	France	Lettuce	X13063
Beet chlorosis virus (BChV)	BChV-CR	unknown	unknown	AF352025
	BChV-GW	California	unknown	AF167485
Beet western yellows virus (BWYV)	USA	USA	unknown	AF473561
	BJ-B	China	Sugar beet	HM804472
	BJ-A	China	Sugar beet	HM804471
Cucurbit aphid-borne yellows virus (CABYV)	N	France	unknown	X76931
	Xinjiang	China	Cantaloupe	EU636992
	Beijing	China	Cucurbit	EU000535

* isolates from oilseed rape from Serbia

RESULTS

Virus detection and symptomatology in the field

During our visual inspection of oilseed rape fields in 2014, similar symptoms were observed in all inspected localities with disease incidence ranging from 10 to 70%. Oilseed rape plants exhibited virus-like symptoms including the reddening of leaf margins (Figure 1a) and interveinal yellowing (Figure 1b).

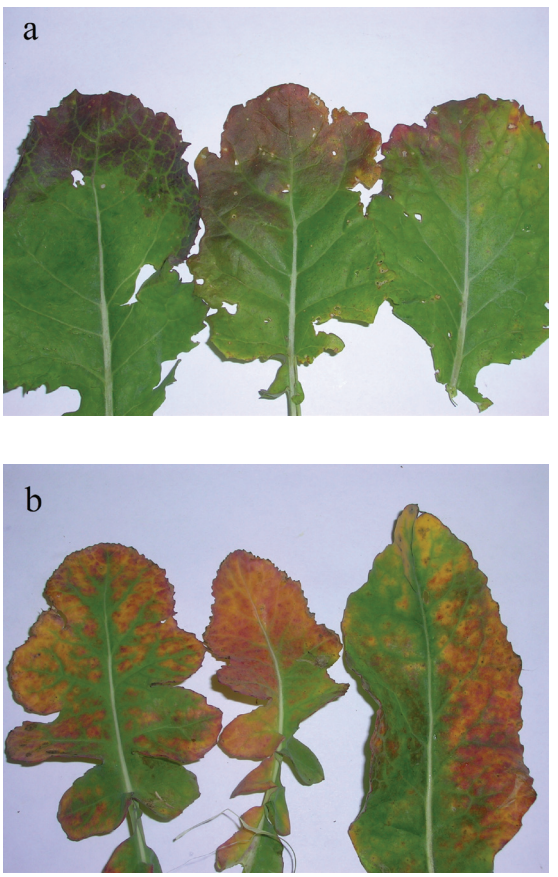

Figure 1. Oilseed rape leaves showing the symptoms of reddening of leaf margins (a) and interveinal yellowing (b)

Serological analysis of oilseed rape samples revealed the presence of TuYV in all inspected localities in the Vojvodina Province. The presence of TuYV was serologically detected in 69.77% of the tested samples and all were negative for CaMV and TuMV (Table 2). After the first detection of TuYV in 20 tested oilseed rape samples originating from Crvenka locality, the virus was serologically detected in additional 40 oilseed rape samples collected from another five localities: Sombor, Kula, Sremska Mitrovica, Kikinda and Zrenjanin. The highest incidence of TuYV was in Crvenka (100% samples tested positive) and Sremska Mitrovica localities, where the virus was confirmed in 11 out of 15 tested samples (73.33%). In Kikinda locality, TuYV was detected in 70% of all tested samples, while the virus was confirmed in 66.66% of the tested samples in Zrenjanin locality. In Kula locality, the virus was detected in 9 out of 15 oilseed rape samples (60%), while the presence of the virus was confirmed in only 5 out of 14 tested samples (35.71%) in Sombor locality.

Table 2. Presence and incidence of *Turnip yellows virus* in oilseed rape in 2014

Locality	District	Tested samples	Positive samples
Sombor	West Bačka	14	5 (35.71%) *
Crvenka		20	20 (100%)
Kula		15	9 (60%)
Sremska Mitrovica	Srem	15	11 (73.33%)
Kikinda	North Banat	10	7 (70%)
Zrenjanin		12	8 (66.66%)
Total		86	60 (69.77%)

* - Number of infected samples (% infected samples calculated over the total number of tested samples).

Host range

The virus isolates from naturally infected oilseed rape plants, one from each locality including the isolate described by Milošević et al. (2015), were successfully transmitted by *M. persicae* to the test plants. All inoculated *C. bursa-pastoris* plants exhibited leaf reddening and stunting, while all inoculated *P. floridana* plants showed a very mild intervenial chlorosis 5 weeks post-inoculation (wpi). The virus was successfully transmitted to *B. napus* cv. 'Banaćanka', which reacted with a mild yellowing symptom 6 wpi. All inoculated plants of each species tested positive for TuYV using ELISA test.

Molecular detection, identification and phylogenetic analysis

The results of serological analyses of TuYV presence in oilseed rape in Serbia was further confirmed by the molecular RT-PCR method using the specific primers TuYVorf0F and TuYVorf0R, which amplify a fragment

of the TuYV P0 gene. These primers successfully detected the presence of TuYV in all tested samples and amplified cDNA fragments of predicted size. One clear band of 780 bp was visible in all oilseed rape plants assayed, while no amplification products were observed in the healthy controls.

After purification, the RT-PCR product derived from the isolate 119-TuYV was directly sequenced in both directions using the same primer pair as in RT-PCR, and deposited in GenBank (GenBank Accession No. KU351664). Sequence analysis of the P0 gene, conducted with MEGA5 software, revealed 99.7% nt identity (100% aa identity) between the two Serbian TuYV isolates from oilseed rape. One Serbian isolate (119-TuYV) showed the highest nucleotide identity of 99% (100% amino acid identity) with TuYV-GB isolates from England (AF168608).

A maximum parsimony tree (Figure 2), reconstructed using partial sequences of the P0 gene isolates, revealed that the TuYV isolate characterized in this study and the previously identified Serbian TuYV isolate (Milošević et al., 2015), as well as the selected sequences of 11 characterized TuYV, BWYV, BChV, and CABYV isolates retrieved from GenBank database clustered into four groups depending on virus species, with high bootstrap values (100%). Genetic diversity among the four molecular groups of isolates ranged from 0.516±0.038 to 0.583±0.212, while diversity within each group was: 0.068±0.008 (TuYV), 0.119±0.013 (BWYV), 0.002±0.002 (BChV), and 0.119±0.013 (CABYV). An analysis of P0 gene sequence data for a subset of these isolates showed that they clustered with known TuYV and were distinct from BWYV isolates.

DISCUSSION

Oilseed rape is now the second most important source of vegetable oil in the world (Raymer, 2002). Oilseed rape oil is of high quality, rich in proteins (over 23%) and with a suitable composition of unsaturated fatty acids and low percentage of saturated fatty acids (Ebrahim-Ghomi, 2014). Accordingly, the production of oilseed rape and especially its limiting factors, including viruses, are attracting great attention in all growing regions. Due to its importance, cultivation is increasing annually in Serbia and oilseed rape is grown on close to 10,000 ha with a production volume of over 31,000 t in 2014 (Statistical Office of the Republic of Serbia, 2014). The emergence of new and destructive pathogens, such is TuYV, is of great importance. As information about the variety of symptoms in oilseed rape, virus distribution, and above all its incidence were not available, a survey was conducted in the main oilseed rape-producing areas in the Province of Vojvodina. After the first reported outbreak of TuYV in oilseed rape in the locality of Crvenka (Milošević et al., 2015), the virus was in this study detected serologically in five other localities in 2014. The presence of TuYV was serologically detected in 69.77% of the tested samples and all samples were negative for CaMV and TuMV. The highest incidence of TuYV was found in the localities Crvenka (100%) and Sremska Mitrovica (73.33%). The incidence was lowest in Sombor locality, but still with a significant percentage of samples (35.71%). In almost all localities, a small number of samples tested negative for the presence of TuYV, and other tested viruses, CaMV and TuMV, and remained with unknown ethiology. Further research focusing on the presence of possible other viruses is ongoing.

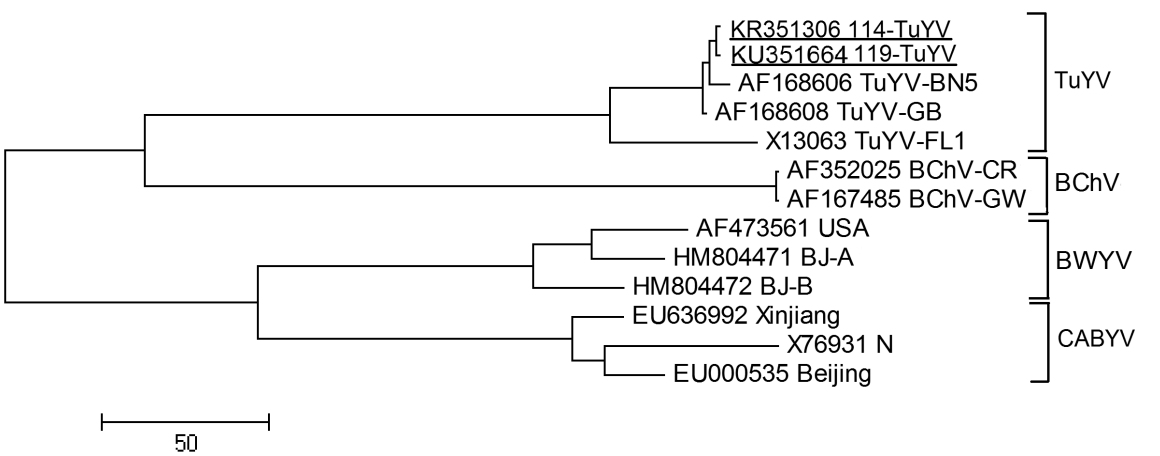

Figure 2. Maximum parsimony tree based on partial sequences of the P0 gene of *Turnip yellows virus*, *Beet western yellows virus*, *Beet chlorosis virus* and *Cucurbit aphid-borne yellows virus* isolates. The phylogram was generated with MEGA5 using bootstrap analysis with 1000 replicates and bootstrap values (>50%) are shown next to relevant branches. The *Turnip yellows virus* isolates from oilseed rape from Serbia are underlined.

In major oilseed rape growing regions, incidence reports for TuYV have been variable and ranging from 10 to 85% (Walsh et al., 1989; Hardwick et al. 1994; Jay et al., 1999). In Australia, the presence of TuYV in oilseed rape crops has led to yield decreases of up to 46% (Jones et al., 2007), while TuYV-infected crops in Germany have been reported to yield between 12% and 34% fewer seeds than virus-free plants (Graichen & Schliephake, 1999). Most probably, the high incidence of TuYV infection in oilseed rape over a period of several years is related to its wide host range and a great number of aphid species which are able to transmit TuYV to oilseed rape plants. In the latest host range studies, a TuYV isolate from oilseed rape was able to infect 65 out of a total of 130 species, among them many common weeds and several cultural crops (Graichen & Schliephake, 1999). Under experimental conditions, 17 of 24 tested aphid species were able to transmit the TuYV virus (Schliephake et al., 2000). Epidemiological data for TuYV in Serbia are still to be investigated and are of great importance considering the virus incidence detected in this research.

Efficient spreading of TuYV by several aphid vectors, and such high incidence and distribution in Serbia, imply that TuYV could become an impediment to successful production of oilseed rape in our country. Moreover, considering that the virus is infective to other species of the family Brassicaceae, as well as numerous weeds, further research is needed to detect the span of this virus and inoculum sources in nature.

Red discolouration at the margins can be the first symptom of TuYV infection of oilseed rape, followed later by conspicuous discolouration of the whole leaf (Graichen & Peterka, 1999), and both symptoms were observed on oilseed rape plants in Serbia. The incidence of TuYV in oilseed rape crops is closely related with the activity of flying aphid vectors, while virus spreading depends on the number and movement of vectors in the crop (Walsh & Tomlinson, 1985). In Germany, a high intensity of TuYV infection was observed in oilseed rape crops during 1995-1996, followed by a high activity of aphids in the autumn of 1995.

The Serbian isolate (119-TuYV) from oilseed rape showed the highest nucleotide identity of 99% (100% amino acid identity) with a TuYV-GB isolate from England (AF168608), which is consistent with the official criterion of separation of species in the genus. According to King et al. (2011) the species demarcation criteria for the genus *Polerovirus* include differences in amino acid sequence identity of any gene product greater than 10 %.

TuYV has been shown to be widespread in all major crop-producing areas in Vojvodina at high incidence, and it is the only virus detected in a large number of samples. This study provides data which were missing about viruses in oilseed rape crops in Serbia. Considering that the production of oilseed rape is growing rapidly in Serbia, the occurrence of TuYV could become an impediment to successful production of that crop. As TuYV is often found in various crops (Graichen & Rabenstein, 1996; Stevens et al., 1994), and is readily transmitted in a non-persistent manner by aphids, constant monitoring of TuYV status and its presence in Serbia is necessary. Therefore, future research of epidemiology and formation of natural reservoirs of major viruses and the most efficient aphid species is of utmost importance for determining and implementing effective control measures.

ACKNOWLEDGMENTS

This research was supported by grants TR 31025 and III-43001 of the Ministry of Education, Science and Technological Development of the Republic of Serbia.

REFERENCES

Association for the Promotion of Production and Exports of Grains and Oilseeds (n.d.). Retrieved from "Cereals Serbia" at www.zitasrbije.rs

Duffus, J.E. & Russell, G.E. (1972). Serological relationship between *Beet western yellows* and *Turnip Yellows Virus*. *Phytopathology*, *62*, 1274-1277.

Ebrahim-Ghomi, M. (2014). Study on distribution and detection of cauliflower mosaic virus (CaMV) in Dezful region of Iran. *International Journal of Biosciences*, *4*(11), 271-275. doi:10.12692/ijb/4.11.271-275

Graichen, K. & Peterka, H. (1999). Generation of oilseed rape with resistance to turnip yellows luteovirus (p 148). In *10th International Rapeseed Congress - New Horizons from an Old Crop*. Canberra, Australia: GCIRC.

Graichen, K. & Rabenstein, F. (1996). European isolates of *Beet western yellows virus* (BWYV) from oilseed rape (*Brassica napus* L. ssp. *napus*) are non-pathogenic on sugar beet (*Beta vulgaris* L. var. *altissima*) but represent isolates of Turnip yellows virus (TuYV). *Zeitschrift für Pflanzenkrankheiten und Pflanzenschutz – Journal of Plant Diseases and Protection*, *103*, 233-245.

Graichen, K. & Schliephake, E. (1999). Infestation of winter oilseed rape by Turnip yellows luteovirus and its effect on yield in Germany (p131). In *10th International Rapeseed Congres - New Horizons from an Old Crop.* Canberra, Australia: GCIRC.

Hardwick, N.V., Davies J.M.L. & Wright D.M. (1994). The incidence of three virus diseases of winter oilseed rape in England and Wales in the 1991/92 and 1992/93 growing seasons. *Plant Pathotogy, 43*, 1045-1049. doi:10.1111/j.1365-3059.1994.tb01656.x

Hauser, S., Stevens, M., Beuve, M. & Lemaire, O. (2002). Biological properties and molecular characterization of *Beet chlorosis virus* (BChV). *Archives of Virology, 147*, 745-762. doi:10.1007/s007050200023

Hauser, S., Stevens, M., Mougel, C., Smith, H. G., Fritsch, C., Herrbach, E. & Lemaire, O. (2000). Biological, serological and molecular variability suggest three distinct polerovirus species infecting beet or rape. *Phytopathology, 90*(5), 460-466. doi:10.1094/PHYTO.2000.90.5.460

Hill, S.A., Lane, A. & Hardwick, N.V. (1989). The incidence and importance of *Beet western yellows virus* in oilseed rape. *Aspects of Applied Biology*, *23,* 311-318.

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*,131-139.

Johnstone, G.R., Ashby, J.W., Gibbs, A.J., Duffus, J.E., Thottapilly, G. & Fletcher J. D. (1984). The host ranges, classification and identification of eight persistent aphid-transmitted viruses causing diseases in legumes. *Netherlands Journal of Plant Pathology, 90*(6), 225-245. doi:10.1007/BF01976381

Johnstone, G.R., & Duffus, J.E. (1984). Some luteovirus diseases in Tasmania caused by *Beet western yellows* and subterranean clover red leaf viruses. *Australian Journal of Agricultural Research, 35*, 821-830.

Jones, R.A.C., Coutts, B.A. & Hawkes, J. (2007). Yield-limiting potential of *Beet western yellows virus* in *Brassica napus*. *Australian Journal of Agricultural Research, 58*, 788-801. doi:10.1071/AR06391

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

Latham, L.J., Smith, L.J. & Jones, R.A.C. (2003). Incidence of three viruses in vegetable brassica plantings and associated wild radish weeds in south-west Australia. *Australasian Plant Pathology*, *32*, 387-391. doi:10.1071/AP03031

Mayo, M.A. (2002). ICTV at the Paris ICV: results of the plenary session and the binomial ballot. *Archives of Virology*, *147*, 2254-2260. doi:10.1007/s007050200052

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. doi:10.1094/PDIS-05-15-0503-PDN

Raymer, P.L. (2002). Canola: An emerging oilseed crop (pp 122-126). In J. Janick and A. Whipkey (eds.), *Trends in new crops and new uses*. Alexandria, VA: ASHS Press.

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). *Zeitschrift für Pflanzenkrankheiten und Pflanzenschutz – Journal of Plant Diseases and Protection*, *107*, 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*, 519-530. doi:10.1080/03235409809383265

Shahraeen, N., (2012). An overview of oilseed rape (canola) virus diseases in Iran. *International Research Journal of Microbiology*, *3*(1), 024-028.

Statistical Office of the Republic of Serbia (2014). Retrieved from www.stat.gov.rs

Stevens, M., Smith, H.G. & Hallsworth, P.B. (1994). The host-range of beet yellowing viruses among common arable weed species. *Plant Pathology*, *43*, 579-588. doi:10.1111/j.1365-3059.1994.tb01593.x

Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M. & Kumar, S. (2011). MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Molecular Biology and Evolution*, *28*(10), 2731-9. doi:10.1093/molbev/msr121

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*, 4673-4680. doi:10.1093/nar/22.22.4673

USDA (2011). *Oilseeds: World markets and trade*. Retrieved from USDA Foreign Agricultural Service at http://www.fas.usda.gov/data/oilseeds-world-markets-and-trade.

Walsh, J.A., Perrin, R.M., Miller, A. & Laycock, D.S. (1989). Studies of beet western yellows virus in winter oilseed rape (Brassica-napus ssp oleifera) and the effect of insecticidal treatment on its spread. *Crop Protection*, *8*, 137-143. doi:10.1016/0261-2194(89)90090-2

Walsh, J.A. & Tomlinson, J.A. (1985). Viruses infecting winter oilseed rape (*Brassica napus* ssp. *oleifera*). *Annals of Applied Biology*, *107*, 485-495. doi:10.1111/j.1744-7348.1985.tb03165.x

Prisustvo virusa žutice postrne repe na uljanoj repici (*Brassica napus* L.) u Srbiji

REZIME

Tokom 2014. godine, sa šest lokaliteta gajenja uljane repice, ukupno je sakupljeno 86 uzorka koji su serološki testirani na prisustvo virusa žutice postrne repe [*Turnip yellow virus* (TuYV)], virusa mozaika karfiola [*Cauliflower mosaic virus* (CaMV)] i virusa mozaika postrne repe [*Turnip mosaic virus* (TuMV)], korišćenjem komercijalno dostupnih kitova za DAS-ELISA test. Prisustvo TuYV dokazano je u 60 (69.77%) prikupljenih uzoraka, dok prisustvo CaMV i TuMV nije dokazano ni u jednom od testiranih uzoraka. Za dalja istraživanja odabrano je šest uzoraka prirodno zaraženih biljaka uljane repice poreklom iz različitih lokaliteta koji su uspešno preneti vašima na tri različite test biljke, čime je potvrđena infektivna priroda oboljenja. Prisustvo TuYV u ELISA pozitivnim uzorcima je potvrđeno korišćenjem RT-PCR i sekvencioniranjem. Poređenjem dobijenih sekvenci sa sekvencama dostupnih u GenBank bazi podataka, potvrđena je autentičnost serološki detektovanih virusa. Na osnovu sekvenci P0 gena izolata TuYV, utvrđena je pripadnost ispitivanih izolata iz uljane repice grupi sa ostalim TuYV izolatima, koji su jasno razdvojeni od *Beet western yellows virus* (BWYV) izolata.

Ključne reči: Uljana repica; Virus žutice postrne repe; Molekularna detekcija; Srbija