

## PLANT VIRUS CONTAMINATION OF NATURAL WATERS IN HUNGARY

Jozsef HORVÁTH<sup>1</sup>, Emil POCSAI<sup>2</sup>, Gabriella KAZINCZI<sup>3</sup>

<sup>1,3</sup>Pannon University of Agricultural Sciences, Georgikon Faculty of Agriculture,  
Keszthely, Hungary

<sup>2</sup>Laboratory of Virology, Plant Health and Soil Conservation Station of Fejér County,  
Velence, Hungary

### ABSTRACT

In Hungary natural waters are often used for irrigation. It means a potential danger for the crop production, because waters, as virus vectors may play important role in the transmission of plant viruses. In two years experiments a survey was carried out on the presence of plant viruses in Hungarian rivers and lakes. 47 water samples from 14 rivers and three lakes were collected and tested for the presence of plant viruses. After concentration by ultracentrifugation, the water samples were tested by ELISA method for the presence of 26 plant viruses. The results of the serological tests were confirmed by biological assays on herbaceous test plants. According to the results achieved in the serological and biological tests, out of the 47 water samples 17 proved to be infected by plant viruses. Cucumber mosaic *cucumovirus* (CMV), potato M *carlavirus* (PVM) and plum pox *potyvirus* (PPV) were detected in three water samples; arabis mosaic *nepovirus* (ArMV) in two samples; potato S *carlavirus* (PVS), tobacco necrosis *necrovirus* (TNV), tobacco rattle *tobravirus* (TRV), tomato spotted wilt *tospovirus* (TSWV), wheat dwarf *monogeminivirus* (WDV) and zucchini yellow mosaic *potyvirus* (ZYMV) in one water sample. Out of them five viruses (ArMV, PVM, TSWV, WDV and ZYMV) are new findings in natural waters, as there have been no previous reports about their occurrence in rivers and lakes.

### IZVLEČEK

#### KONTAMINACIJA NARAVNIH VODA NA MADŽARSKEM Z RASTLINSKIMI VIRUSI

Na Madžarskem naravne vode pogosto uporabljajo za namakanje. To pa pomeni potencialno nevarnost za rastlinsko pridelavo, ker imajo lahko vode kot vektorji virusov pomembno vlogo pri prenašanju rastlinskih virusov. V dvoletnih poskusih je bil izdelan pregled o zastopanosti rastlinskih virusov v madžarskih rekah in jezerih. Odvzetih je bilo 47 vzorcev vode iz 14 rek in treh jezer, ki so jih testirali na vsebnost rastlinskih virusov. Po koncentraciji z ultracentrifugiranjem, so vodne vzorce testirali z ELISA metodo na zastopanost 26 rastlinskih virusov. Rezultate seroloških testov so potrdili z biotičnimi testi na zelnatih indikatorskih rastlinah. Glede na rezultate, dobljene s serološkimi in biotičnimi testi, je bilo od 47 vzorcev 17 kontaminiranih z rastlinskimi virusi. Kumarni mozaik *cucumovirus* (CMV), krompirjev M *carlavirus* (PVM) in virus šarke *potyvirus* (PPV) so ugotovili v treh vodnih vzorcih, repnjakov mozaik *nepovirus* (ArMV) v dveh vzorcih, krompirjev S *carlavirus* (PVS), virus tobakove nekroze *necrovirus* (TNV), paradižnikov virus pisane uvelosti *tospovirus* (TSWV), virus pšenične pritikavosti *monogeminivirus* (WDV) in rumeni mozaik kukinjev *potyvirus* (ZYMV) v enem vodnem vzorcu. Poleg tega je bilo pet virusov (ArMV, PVM, TSWV, WDV in ZYMV) prvič najdeno v naravnih vodah, ker o njih v prejšnjih poročilih ni bilo nobenih navedb o najdbi v rekah in jezerih.

<sup>1</sup> Acad., Prof., H-8361, Keszthely

<sup>2</sup> PhD, H-2481, Velence

<sup>3</sup> PhD, H-8361, Keszthely

## 1 INTRODUCTION

Several human and animal viruses have been detected from natural waters, causing a potential danger to the living world. The pollution of environmental waters with infectious viruses and the importance of the epidemiology of virus disease for public health have attracted considerable attention (Koenig, 1986).

At first time Van Dorst (1969) detected the presence of cucumber green mottle mosaic *tobamovirus* (CGMMV) in drainage and irrigation water. Later very close connection was observed between the presence of CGMMV and the virus infection of cucumber plants in the Netherlands (Van Dorst, 1988). Tomlinson *et al.* (1983a) isolated a distinctive strain of tobacco necrosis *necrovirus* (TNV), designated *Chenopodium necrosis* (TNV-CN), which was mechanically transmissible to *Chenopodium amaranticolor* and *C. quinoa* caused local lesions and systemic infection. Pocsai and Horváth (1997) detected eight plant viruses from the waters of Hungarian rivers and lakes. So far 21 viruses from twelve countries were identified from natural waters in the world. In addition to this, further new, but not exactly identified viruses were isolated belonging to four virus groups.

The waters of rivers and lakes are generally used for irrigation. Therefore, a knowledge of the infection of natural waters with plant viruses is important from the point of view of crop production and protection. The objective of our investigations was to determine the further presence of plant viruses in Hungarian rivers and lakes.

## 2 MATERIALS AND METHODS

47 water samples from 14 rivers (Berettyó, Bodrog, Danube, Hármas-Körös, Eastern Channel, Maros, Rába, Rábca, Répce, Sajó, Sió Channel, Tisza, Zagyva, Zala) and three lakes (Balaton, Velence, Fertő) were collected in Hungary. After storing in refrigerator at 4 °C the water samples were shaken vigorously and 500 ml of each was concentrated by ultracentrifugation, in 3170/b type ultracentrifuge (P-40 rotor) at 30 000 rpm (97000 g) for two hours. After ultracentrifugation, one half of the pellets was resuspended in 3 ml of sample buffer solution for the ELISA test. The second half of the pellets was resuspended in 3 ml 0.02 M phosphate buffer solution at 7.2 pH for biological assay on herbaceous plants.

The concentrated water samples were tested by ELISA method after Clark and Adams (1977) for the presence of the following plant viruses: alfalfa mosaic *alfamovirus* (AMV), arabis mosaic *nepovirus* (ArMV), barley stripe mosaic *hordeivirus* (BSMV), beet necrotic yellow vein *benyvirus* (BNYVV), brome mosaic *bromovirus* (BMV), CMV, impatiens necrotic spot *tospovirus* (INSV), maize dwarf mosaic *potyvirus* (MDMV), plum pox *potyvirus* (PPV), potato A *potyvirus* (PVA), potato leafroll *luteovirus* (PLRV), potato M *carlavirus* (PVM), potato S *carlavirus* (PVS), potato X *potexvirus* (PVX), potato Y *potyvirus* (PVY), prune dwarf *ilarvirus* (PDV), prunus necrotic ringspot *ilarvirus* (PNRSV), raspberry ringspot *nepovirus* (RpRSV), soyabeane mosaic *sobemovirus* (SoMV), tobacco etch *potyvirus* (TEV), tobacco mosaic *tobamovirus* (TMV), TNV, tobacco rattle *tobravirus* (TRV), tobacco streak *ilarvirus* (TSV), tomato spotted wilt *tospovirus* (TSWV), wheat dwarf *monogeminivirus* (WDV), wheat soil-borne mosaic *fuovirus* (WSBMV), wheat spindle streak mosaic *bymovirus* (WSSMV), zucchini yellow mosaic *potyvirus* (ZYMV). The evaluation of the serological reactions was carried out using a LabSystems Multiscan Plus photometer at 405 nm and 492 nm, depending on the type of diagnostics. The results of the serological tests were confirmed by biological tests. Two plants of each test species (*Chenopodium quinoa*, *C. foetidum*, *C. murale*, *Gomphrena globosa*, *Nicotiana debneyi*, *N. rustica*, *N. tabacum* cv. Xanthi, *N. tabacum* cv. Samsun, *N. glutinosa*) were mechanically inoculated with concentrated and resolved pellets of the water samples.

### 3 RESULTS AND CONCLUSION

Out of the 47 water samples collected from three lakes and 14 rivers 17 samples were infected with plant viruses on the basis of serological tests. Ten plant viruses were detected from the water samples (Table 1). Out of them five viruses (ArMV, PVM, TSWV, WDV and ZYMV) are new findings in natural waters, as there have been no previous reports about their occurrence in rivers and lakes. The detected viruses belong to the *carla*-, *cucumo*-, *monogemini*-, *necro*-, *nepo*-, *poty*-, *tobra*- and *tospovirus* groups. The most frequent virus was PVM occurring in three of the rivers tested. No explanation can be given for the frequent occurrence of PVM in rivers, which might be connected with the susceptible crop and weed species. In our previous research on the occurrence of plant viruses in Hungarian rivers and lakes, the most frequent viruses were PVY and PPV. PPV occurred in the waters of Lake Fertő and Lake Velence, too (Pocsaí and Horváth 1996, 1997).

Table 1: Results of serological tests of water samples

Name of lake or river	Sampling sites	Viruses present in water samples <sup>1</sup>
Lake Balaton	Keszthely	TSWV, CMV
Lake Velence	Velence	PPV
Lake Fertő	Fertőrákos	PPV
River Danube	Komárom	PVS
River Hármas-Körös	Magyartés	PVM
Eastern Channel	Balmazújváros	ArMV, PVM
River Maros	Makó	WDV
River Rába	Rum	ArMV, CMV, PPV, TNV, TRV, ZYMV
River Zala	Fenekpuszta	CMV, PVM

<sup>1</sup>ArMV, *Arabis mosaic nepovirus*; CMV, cucumber mosaic *cucumovirus*; PPV, plum pox *potyvirus*; PVM, potato *M carlavirus*; PVS, potato *S carlavirus*; TNV, tobacco necrosis *neerovirus*; TRV, tobacco rattle *tobravirus*; TSWV, tomato spotted wilt *tospovirus*; WDV, wheat dwarf *monogeminivirus*; ZYMV, zucchini yellow mosaic *potyvirus*

The presence of WDV in the river Maros is the first report on the occurrence of a *monogeminivirus* in natural waters. The incidence of WDV in cereals has increased in Hungary last years. It is very likely that this virus may derive from the decaying cereal roots in river water. Rába proved to be the most infected river. On the basis of serological tests six viruses (ArMV, CMV, PPV, TNV, TRV, ZYMV) were present in this river.

The presence of plant viruses in the concentrated water samples was also confirmed by biological tests on herbaceous plants. The results of the biological tests are presented in Table 2. On the basis of biological tests river Danube at Baja and river Répce at Répcevis was infected with - so far not identified- plant viruses although the results of serological test were negative in this respect. Virus contamination of natural waters and water plants can give the answer the following questions: (i) what is the virus contamination of natural waters and water plants in Hungary, (ii) what is the role of these viruses in the virus infection of the weeds and cultivated plants and opposite, (iii) what etiological relations are there in the studied ecosystems. Regarding to the occurrence of economical important

viruses in natural waters and water plants, it is very important to study the virus infection chain and virus ecological relations in future.

Table 2: Results of biological tests of herbaceous plants

Name of lake or river	Sampling sites	Reaction on herbaceous plants*			
		<i>C. foetidum</i>	<i>C. quinoa</i>	<i>G. globosa</i>	<i>N. rustica</i>
Lake Balaton	Keszthely		NI/-	PI/-	
Lake Velence	Velence	NI/-			
Lake Fertő	Fertőrákos	CI, NI/-			
River Danube	Komárom		CI/-		
River Danube	Baja		NI/Y, Led		
River Hármas-Körös	Magyartés		CI/-		
Eastern Channel	Balmazújváros		CI/-		
River Maros	Makó				Nsp/-
River Rába	Rum		CI/-		
River Zala	Fenekpuszta		CI, NI/Tn	Psp/-	
River Répce	Répevis		CI, Vc/-		

\*local/systemic symptoms; CI, chlorotic lesions; Led, leaf deformation; NI, necrotic lesions; Nsp, necrotic spots; PI, purple lesions; Psp, purple spots; Tn, top necrosis; Y, yellowing; Vc, vein clearing

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