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# NEW RESULTS ON THE OCCURRENCE OF PLANT VIRUSES IN HUNGARIAN RIVERS AND LAKES

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In two year experiments a survey was carried out on the presence of plant viruses in Hungarian rivers and lakes. Water samples from 14 rivers and three lakes were collected and after concentrating by ultracentrifugation they were tested by ELISA method for the presence of 26 plant viruses. The results of the serological tests were confirmed by biological assay on herbaceous test plants. According to the results, out of 47 investigated water samples 17 proved to contain infectious plant viruses belonging to *carla-* (PVM, PVS), *cucumo-* (CMV), *monogemini-* (WDV), *necro-* (TNV), *nepo-* (ArMV), *poty-* (PPV, ZYMV), *tobra-* (TRV) and *tospovirus* (TSWV). CMV, PVM and PPV were found more often. ArMV, PVM, TSWV, WDV and ZYMV were found for the first time in natural waters.

**Key words:** plant viruses, natural waters, Hungary

## Introduction

Several human and animal viruses have been detected from natural waters, representing a potential danger to the respective organisms. The pollution of environmental (natural) waters with infectious viruses has attracted considerable attention for its possible role in virus disease epidemiology and because of the importance of the matter for public health (KOENIG 1986).

During nearly two past decades an increasing and considerable attention has been paid to the research on the occurrence of plant viruses in environmental waters. The plant viruses occurring in those waters remain in an active state for a fairly long period. The majority of plant viruses detected from natural waters are stable viruses; in addition, the presence of solid colloids also seems to have a protective effect on them (GIBBS and HARRISON 1976, JURETIĆ and HORVÁTH 1991, KEGLER et al., 1991). Adsorption to clay colloids or some organic materials probably protects these viruses against inactivation by various factors acting in the water. Several plant viruses occur in fairly high concentrations in rivers and lakes, which enables them to spread over long distances. Many reports have been published in different parts of the world on the occurrence of plant viruses in natural waters. First VAN DORST (1969) detected the presence of cucumber green mottle mosaic *tobamovirus* (CGMMV) in drainage and irrigation water. Later a very close connection was observed between the presence of CGMMV and the virus infection of cucumber plants in the Netherlands (VAN DORST1988). TOMLINSON et al. (1983b) isolated a distinctive strain of tobacco necrosis *necrovirus* (TNV), designated *Chenopodium* necrosis virus (TNV-CN), which was mechanically transmissible to *Chenopodium amaranticolor* and *Ch. quinoa* causing local lesions and systemic infection. KONTZOG et al. (1988) reported for the first time the occurrence of a plant pathogen virus in sea water. 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 (Table 1). In addition to this, further, but not completely identified viruses – some of them possibly being new ones – belonging to four definite virus groups were isolated (Table 2).

The three principle sources from which plant viruses come into environmental waters are: 1. undistributed roots, 2. injured or decaying plant material and 3. sewage (KOENIG 1986). The primary source of plant viruses in natural waters may be the infected plants living near or even in the water. The viruses may be released from the roots of such plants (TOMLINSON et al. 1983b). Another source of plant viruses in water could be the decaying virus-infected plant materials (LANTER et al. 1982, RAO and VARMA 1984). KEGLER et al. (1984) reported that a number of viruses present in infected leaves can pass through the alimentary tract of rabbits in an infective state, which means that plant viruses could be spread trough sewage, manure and liquid manure. Sewage is a very important source for occurrence of plant viruses in natural waters (KOENIG 1986). Regarding the role of "alimentary-resistant" plant viruses in their epidemiology it is known that the first plant virus found to be of such resistance was tomato bushy stunt *tombusvirus* (TBSV), when consumed in infected tomatoes by man (TOMLINSON et al. 1982, 1984).

Several viruses, including tobacco mosaic *tobamovirus* (TMV), cucumber mosaic *cucumovirus* (CMV) and TNV, are at least partially adsorbed to soil, whereas their concentration in the nearby waters can be below infectivity threshold. If this is so, sediments not only protect viruses but may exert an enrichment effect, trough a selective adsorption of virus particles. The epidemiological implication of this may be of great consequences for it is possible to envisage a

Tab. 1. Occurrence of viruses in natural water*

| Viruses | Country | References |
|---|---|---|
| Alfalfa mosaic *alfamovirus* (AMV) | Hungary | POCSAI and HORVÁTH (1996, 1997) |
| Barley yellow dwarf *luteovirus* (BYDV) | Hungary | POCSAI and HORVÁTH (1996, 1997) |
| Carnation Italian ringspot *tombusvirus* (CIRSV) | Germany | BÜTTNER et al. (1987) |
| Carnation mottle *carmovirus* (CarMV) | Germany | KOENIG and LESEMANN (1985), KONTZOG et al. (1988) |
| Carnation ringspot *dianthovirus* (CaRSV) | Germany | KOENIG et al. (1988, 1989) |
| | China | LI et al. (1992) |
| Cucumber green mottle mosaic *tobamovirus* (CGMMV) | The Netherlands | VAN DORST (1988) |
| Cucumber mosaic *cucumovirus* (CMV) | Italy | PIAZZOLLA et al. (1986), CANNIZZARO et al. (1990) |
| Grapevine Algerian latent *tombusvirus* (GALV) | Italy | CANNIZZARO et al. (1990) |
| | China | LI et al. (1992) |
| Maize dwarf mosaic *potyvirus* (MDMV) | Hungary | POCSAI and HORVÁTH (1996, 1997) |
| Pelargonium zonate spot *ourmiavirus* (PZSV)) | Italy | VOVLAS and DI FRANCO (1987) |
| Petunia asteroid mosaic *tombusvirus* (PeAMV) | Germany | KOENIG et al. (1989) |
| Plum pox *potyvirus* (PPV) | Hungary | POCSAI and HORVÁTH (1996, 1997) |
| Potato S *carlavirus* (PVS) | Hungary | POCSAI and HORVÁTH (1996, 1997) |
| Potato Y *potyvirus* (PVY) | Hungary | POCSAI and HORVÁTH (1996, 1997) |
| Ribgrass mosaic *tobamovirus* (RMV) | Hungary | JURETIĆ et al. (1986) |
| Tobacco etch *potyvirus* (TEV) | Hungary | POCSAI and HORVÁTH (1996, 1997) |
| Tobacco mosaic *tobamovirus* (TMV) | Yugoslavia | TOŠIĆ and TOŠIĆ (1984) |
| | Germany | KOENIG and LESEMANN (1985) |
| | Hungary | HORVÁTH et al. (1986), POCSAI et al. (1989), POCSAI and HORVÁTH (1996, 1997) |
| | Italy | PIAZZOLLA et al. (1986) |
| | Croatia | PLEŠE et al. (1996) |
| Tobacco necrosis *necrovirus* (TNV) | Germany | KONTZOG et al. (1988), KOENIG et al. (1989) |
| | England | TOMLINSON et al (1983b) |
| Tobacco rattle *tobravirus* (TRV) | Germany | KONTZOG et al.(1988), KEGLER et al. (1989) |
| | Czech Republic | POLAK (1994) |
| Tomato bushy stunt *tombusvirus* (TBSV) | England | TOMLINSON et al. (1982, 1983a,1983b), TOMLINSON and FAITHFULL (1984) |
| | Germany | KOENIG and LESEMANN (1985), KONTZOG et al. (1988) |
| | Brazil | TOMLINSON and FAITHFULL (1984) |
| | USA | TOMLINSON and FAITHFULL (1984) |
| | Italy | TOMLINSON and FAITHFULL (1984) |
| | India | TOMLINSON and FAITHFULL (1984) |
| Tomato mosaic *tobamovirus* (ToMV) | USA | JACOBI and CASTELLO (1991) |
| | Croatia | PLEŠE et al. (1996) |

*mainly rivers and lakes

Tab. 2. Some new, but not definitely identified viruses (virus isolates) from natural water

| Genus of virus | Country | References |
|---|---|---|
| *Carmovirus* | China | LI et al. (1992) |
| *Potexvirus* | Germany | KOENIG and LESEMANN (1985) |
| *Tobamovirus* | Germany | TOMLINSON and FAITHFULL (1984), KOENIG and LESEMANN (1985), KONTZOG et al. (1988) |
| | Italy | TOMLINSON and FAITHFULL (1984), VOVLAS AND DI FRANCO (1987) |
| | Egypt | TOMLINSON and FAITHFULL (1984) |
| | Brazil | TOMLINSON and FAITHFULL (1984) |
| | South Africa | TOMLINSON and FAITHFULL (1984) |
| *Tombusvirus* | China | LI et al. (1992) |
| | Germany | KOENIG and LESEMANN (1985) |
| | Italy | VOVLAS AND DI FRANCO (1987), GALLITELLI et al. (1989) |
| Ungrouped isometric viruses | Germany | KOENIG and LESEMANN (1985) |
| | Italy | PIAZZOLLA et al. (1986) |

mechanism whereby many different viruses, including unstable ones, can be spread through an ecological system comprising water (vector), sediments (protector) and virus (TOMLINSON et al. 1983a, PIAZZOLLA et al. 1986).

VAN DORST (1988) reported the changing of virus concentration in waters during the year. The rapid increase of the virus concentration in spring is probably caused by the fast-growing number of diseased plants as a concequence of the many activities in the crop such as pruning and harvesting. The virus concentration reached a maximum in June and July. In August the concentration was low. In September, when the crop was removed and the soil leached, the virus concentration increased again.

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

## Materials and methods

In two year experiments 47 water samples from 14 rivers and three lakes were collected in Hungary (Table 3). The 1000 mL water samples were taken from the rivers in midstream and transported in cooling boxes to the Laboratory of Virology, where they were stored in refrigerator at 4 °C for two weeks. After storing the water samples were shaken vigorously and a 500 mL sample of each was concentrated by ultracentrifugation in 3170/b type ultracentrifuge (P-40 rotor) at 30 000 rpm (97 000 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 dissolved in 3 mL of 20 mM phosphate buffer solution at pH 7.2 for biological assay on herbaceous plants.

Tab. 3. Origin of water samples collected in Hungarian rivers and lakes

| Name of river or lake | Sampling site |
|---|---|
| Lake Balaton | Keszthely, Siófok |
| Lake Velence | Agárd, Velence |
| Lake Fertő | Fertőrákos |
| River Berettyó | Pocsaj |
| River Bodrog | Bodrogkeresztúr |
| River Danube | Komárom, Dunaföldvár, Baja |
| River Hármas-Körös | Magyartés |
| Eastern Channel | Balmazújváros |
| River Maros | Makó |
| River Rába | Rum |
| River Rábca | Lébénymiklós |
| River Répce | Répcevis |
| River Sajó | Kesznyéten |
| Sió Channel | Simontornya |
| River Tisza | Tiszabecs, Polgár, Szolnok, Tápé |
| River Zagyva | Újszász |
| River Zala | Fenékpuszta |

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), sowbane mosaic *sobemovirus* (SoMV), tobacco etch *potyvirus* (TEV), TMV, TNV, tobacco rattle *tobravirus* (TRV), tobacco streak *ilarvirus* (TSV), tomato spotted wilt *tospovirus* (TSWV), wheat dwarf *monogeminivirus* (WDV), wheat soil-borne mosaic *furovirus* (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 diagnostic. The results of the serological tests were confirmed by biological tests. Two plants of each test species (*Chenopodium quinoa*, *Ch. foetidum*, *Ch. murale*, *Gomphrena globosa*, *Nicotiana debneyi*, *N. rustica*, *N. tabacum* cv. Xanthi, *N. tabacum* cv. Samsun, *N. glutinosa*) were mechanically inoculated with concentrated pellet suspensions of the water samples. Isolates showing virus symptoms were maintained on *Ch. quinoa* and *G. globosa* plants under glasshouse conditions. Viruses in the leaves were preserved and stored in Petri dishes over calcium chloride at 4 °C.

## Results and discussion

Out of the 47 water samples collected from 14 rivers and three lakes altogether 17 samples were found polluted with plant viruses, based on the results of the serological tests. Among them ten different viruses were identified (Table 4). They belong to the *carla-, cucumo-, monogemini-, necro-, nepo-, poty-, tobra-* and *tospovirus* groups. The most frequent virus in rivers was PVM occurring in three of the rivers tested. No definite 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 Fertö and Lake Velence, too (POCSAI and HORVÁTH 1996, 1997). 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

Tab. 4. Results of serological tests with water samples

| Name of river or lake | Sampling site | Virus present in water samples |
|---|---|---|
| Lake Balaton | Keszthely | TSWV, CMV |
| Lake Velence | Velence | PPV |
| Lake Fertö | Fertörákos | 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 | Fenékpuszta | CMV, PVM |

Tab. 5. Results of biological tests on herbaceous plants

| Name of river or lake | Sampling site | Reaction on herbaceous plants* | | | |
|---|---|---|---|---|---|
| | | *Ch. foetidum* | *Ch. quinoa* | *G. globosa* | *N. rustica* |
| Lake Balaton | Keszthely | | Nl/-* | Pl/- | |
| Lake Velence | Velence | Nl/- | | | |
| Lake Fertö | Fertörákos | Cl, Nl/- | | | |
| River Danube | Komárom | | Cl/- | | |
| River Danube | Baja | | Nl/Y, Led | | |
| River Hármas-Körös | Magyartés | | Cl/- | | |
| Eastern Channel | Balmazújváros | | Cl/- | | |
| River Maros | Makó | | | | Nsp/- |
| River Rába | Rum | | Cl/- | | |
| River Zala | Fenékpuszta | | Cl, Nl/Tn | Psp/- | |
| River Répce | Répcevis | | Cl, Vc/- | | |

* local/systemic symptoms on leaves; Cl, chlorotic lesions; Led, leaf deformation; Nl, necrotic lesions; Nsp, necrotic spots; Pl, purple lesions; Psp, purple spots; Tn, top necrosis; Y, yellowing; Vc, vein clearing.

be the most virus-contaminated river. On the basis of serological tests six viruses (ArMV, CMV, PPV, TNV, TRV, ZYMV) were found 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 are presented in Table 5. On the basis of biological tests river Danube at Baja and river Répce at Répcevis were polluted with plant viruses but the ones so far not identified.

Out of 10 plant viruses identified during this work from the waters of Hungarian rivers and lakes, five viruses (ArMV, PVM, TSWV, WDV and ZYMV) present new findings for natural waters.There have been no previous reports about their occurrence in such media.

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## Sažetak

### NOVI PODACI O NAZOČNOSTI BILJNIH VIRUSA U RIJEKAMA I JEZERIMA MAĐARSKE

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Tijekom dviju godina istraživana je nazočnost biljnih virusa u površinskim vodama Mađarske, u 14 rijeka i tri jezera. Sadržaj 47 prikupljenih uzoraka vode, svaki volumena 500 mL, bio je najprije koncentriran visokookretajnim centrifugiranjem. Nastali talozi bili su zatim otopljeni zasebno u dva odgovarajuća pufera i istraženi osjetljivom serološkom metodom ELISA pomoću antiseruma od 26 virusa i na osnovi reakcije pokusnih biljaka nakon njihove mehaničke inokulacije. Nazočnost infekcioznih virusa dokazana je u 17 uzoraka vode, i to ukupno njih 10: M virus krumpira (PVM), S virus krumpira (PVS) (rod: *Carlavirus*), virus mozaika krastavca (CMV) (rod: *Cucumovirus*), virus kržljavosti pšenice (WDV) (rod: *Monogeminivirus*), virus nekroze duhana (TNV) (rod. *Necrovirus*), virus mozaika gušarke (ArMV) (rod: *Nepovirus*), virus šarke šljive (PPV) i virus žutog mozaika tikvice (ZYMV) (rod: *Potyvirus*), virus šuštavosti duhana (TRV) (rod: *Tobravirus*) i virus pjegavosti i mozaika rajčice (TSWV) (rod: *Tospovirus*). Nađena su i daljna dva virusna izolata koja zasad još nisu identificirana. Nešto češće od drugih virusa bili su nađeni CMV, PVM i PPV. Od naprijed navedenih virusa po prvi puta uopće otkriveni su u prirodi kao kontaminantni u vodama ArMV, PVM, TSWV, WDV i ZYMV.

U radu se daje pregled dosadašnjih nalaza biljnih virusa u površinskim vodama (uglavnom vodotoci i jezera) zemalja svijeta.

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