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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, JURETIC and HORVATH 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. Pocsat 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	POCSA1 and HORVÁTH (1996, 1997)
Barley yellow dwarf-luteovirus (BYDV)	Hungary	POCSAI and HORVÁTH (1996, 1997)
Camation Italian ringspot tombusvirus (CIRSV)	Germany	BÜTTNER et al. (1987)
Camation mottle carmovirus (CarMV)	Germany	KOENIG and LESEMANN (1985), KONTZOG et al. (1988)
Camation 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	Lı et al. (1992)
Maize dwarf mosaic potyvirus (MDMV)	Hungary	POCSAI and HORVÁTII (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 HORVATH (1996, 1997)
Potato S carlavirus (PVS)	Hungary	POCSAI and HORVÁTH (1996, 1997)
Potato Y potyvirus (PVY)	Hungary	POCSAI and HORVATH (1996, 1997)
Rib grass mosaic tobamovirus (RMV)	Hungary	JURETIĆ et al. (1986)
Tobacco etch potyvirus (TEV)	Hungary	POCSAI and HORVA III (1996, 1997)
Tobacco mosaic tobamovirus (TMV)	Yugoslavia	Tošic and Tošic (1984)
	Germany	KOENIG and LESEMANN (1985)
	Hungary	HORVATH et al. (1986), POCSAI et al. (1989), POCSAI and HORVATH (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	KOENG and LESEMANN (1985), KONTZOG et al. (1988)
	Brazil	TOMLINSON and FAITHFULL (1934)
	USA	TOMLINSON and FATTHFULL (1984)
	Italy	TOMLINSON and FAITHFULL (1984)
	India	TOMLINSON and FAITHFULL (1984)
Tomato mosaic tobamovirus (ToMV)	USA	JACOBI and CASTELLO (1991)
	Croatia	PLLSE et al. (1996)
*mainly rivers and lakes		

Genus of virus	Country	References
Carmovirus	China	Lt 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	TOMLINSON and FAITHFULL (1984)
	Africa	
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)

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

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 Fertö	Fertörákos		
River Berettyo	Pocsaj		
River Bodrog	Bodrogkeresztúr		
River Danube	Komárom, Dunaföldvár, Baja		
River Hármas-Körös	Magyartés		
Eastern Channel	Balmazújváros		
River Maros	Makó		
River Rába	Rum		
River Råbea	Lébénymiklós		
River Repce	Répcevis		
River Sajó	Kesznyéten		
Sio Channel	Simontornya		
River Tisza	Tiszabecs, Polgár, Szolnok, Tápe		
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 scrological 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 Fertő and Lake Velence, too (Pocsal and Horvath 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 scrological 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 Hannas-Körös	Magyartés	PVM		
Eastern Channel	Balmazújváros	ArMV, PVM		
River Maros	Mako	WDV		
River Råba	Rum	ArMV, CMV, PPV, TNV, TRV, ZYMV		
River Zala	Fenékpuszta	CMV, PVM		

Tab. 5. Results of biological tests on herbaceous plants

NI 0.1 1.1	6 1: :	Reaction on herbaceous plants*				
Name of river or lake	Sampling site	Ch. foetidum	Ch. quinoa	G. globosa	N. rustica	
Lake Balaton	Kesztheley		NI/-*	PI/-		
Lake Velence	Velence	NI/-				
Lake Fertő	Fertörákos	Cl, NI/-				
River Danube	Komárom		CI/-			
River Danube	Baja		NI/Y, Led			
River Hármas-Körös	Magyartés		CI/-			
Eastern Channel	Balmazújváros		C1/-			
River Maros	Makó				Nsp/-	
River Råba	Rum		C1/-			
River Zala	Fenékpuszta		CI, NI/Tn	Psp/-		
River Répce	Répcevis		CI, Ve/-			

^{*} local/systemic symptoms on leaves; CI, chlorotic lesions; Led, leaf deformation; NI, necrotic lesions; Nsp, necrotic spots; PI, purple lesions; Psp, purple spots; Tn, top necrosis; Y, yellowing; Ve, 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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