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RAPID COMMUNICATION

Detection of infectious tobamoviruses in irrigation and drainage canals in Greater Poland

Małgorzata Jeżewska, Katarzyna Trzmiel*, Aleksandra Zarzyńska-Nowak

Virology and Bacteriology, Institute of Plant Protection – National Research Institute, Władysława Węgorka 20, 60-318 Poznań, Poland

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*Corresponding address:
k.trzmiel@iorpib.poznan.pl

Abstract

Water samples were collected from irrigation ditches and drainage canals surrounding fields in southern Greater Poland. Initially, the samples were subjected to low and high-speed centrifugation and obtained pellets were used to perform biological assays. Viral identification involved biological, electron microscopic as well as molecular methods. The occurrence of *Tobacco mosaic virus* (TMV) and *Tomato mosaic virus* (ToMV) was demonstrated in 12 of the 17 examined water sources. The molecular analysis results showed TMV and ToMV co-infections in the analysed water samples. To our knowledge, this is the first report of tobamoviruses being found in environmental water in Poland.

Key words: RT-PCR, tobamoviruses, water-borne viruses

The aqueous environment is a good source of human, animal and plant infective viruses. Mehle and Ravnikar (2012), in an exhaustive review, listed 16 infective plant pathogenic virus species found in environmental waters, such as canals, rivers, streams, ponds, lakes and oceans. Tobamoviruses, especially *Tobacco mosaic virus* (TMV) and *Tomato mosaic virus* (ToMV), are the most commonly detected water-borne viruses worldwide (Tošić and Tošić 1984; Koenig 1986; Piazzolla *et al.* 1986; Jacobi and Castello 1991; Pares *et al.* 1992; Castello *et al.* 1995; Pleše *et al.* 1996; Fillhart *et al.* 1998; Boben *et al.* 2007). TMV, the type member of the genus *Tobamovirus* in the family *Virgaviridae,* being probably the most dangerous plant virus in the world, is known to infect about 200 species from 30 plant families, including cereals (Zaitlin 2000; Jeżewska and Trzmiel 2005). In contrast, ToMV, the virus closely related to TMV, also characterized by a wide range of hosts (Broadbent 1976), has not yet been reported to infect cereals.

There were no data on the occurrence of plant viruses in waters in Poland. In order to fulfil this gap, the aim of our research was to identify plant viruses found in irrigation ditches surrounding fields in the southern part of Greater Poland, a typical agricultural region. The sampling was performed in spring 2017. For collecting water from irrigation ditches and drainage canals surrounding cereal fields, new, 0.5 l plastic bottles were used. The material was collected from 17 locations. Two portions of water from each location were considered as one sample for further analysis.

Each sample, 1 liter of water, was submitted to a cycle of low-speed centrifugation (10,000 rpm for 10 min in a Beckman Coulter Avanti J-26 XPI centrifuge) and ultracentrifugation (Beckman Coulter Optima L-90K ultracentrifuge) (30,000 rpm, 2 h). The pellet obtained after low-speed centrifugation was re-suspended in 2 ml of 0.05 M phosphate buffer pH 7.0 and used for the inoculation of the tested plants while the supernatant was taken for ultracentrifugation. The subsequently obtained pellet was re-suspended in the same inoculation buffer and used for inoculation as well. The second supernatant was discarded.

The presence of plant viruses in sediments after low speed centrifugation and ultracentrifugation was

determined by infectivity assays and by electron microscopy. Sets of 3–7 plants of tobacco (*Nicotiana tabacum* cv. Xanthi), pigweed (*Chenopodium quinoa*), thorn apple (*Datura stramonium*), wheat (*Triticum aestivum* cv. Muszelka) and barley (*Hordeum vulgare* cv. Conchita) were mechanically inoculated with water samples. The plants were maintained in insect proof, closed compartments under standard greenhouse conditions (16 h of light and 8 h of darkness at 23°C). Local lesions of 2–4 mm in diameter, typical for tobamovirus infection, developed on tobacco cv. Xanthi, *C. quinoa* and *D. stramonium* leaves 3–5 days after inoculation (Fig. 1). No symptoms were noticed in barley and wheat plants. Infectivity tests suggested the possibility of the presence of tobamoviruses in 12 out of 17 analyzed water samples, occurring in different concentrations. In nine samples local lesions were noticed only on plant leaves inoculated with the pellet obtained after ultracentrifugation. This effect was probably caused by low virus concentrations. For three other samples, named R, N and S, the symptoms observed after inoculation with pellets obtained from both low-speed centrifugation and ultracentrifugation indicated higher virus concentrations. These samples were selected for further diagnostics and were propagated by mechanical inoculation to tobacco cv. Samsun plants. Observed systemic symptoms were typical for TMV, i.e. distinct leaf mosaic, rugosity and deformations (Fig. 1). The strongest symptoms were observed in sample S.

Samples for the electron microscopy observations were prepared directly from sediments after both centrifugations as well as from symptomatic leaves and

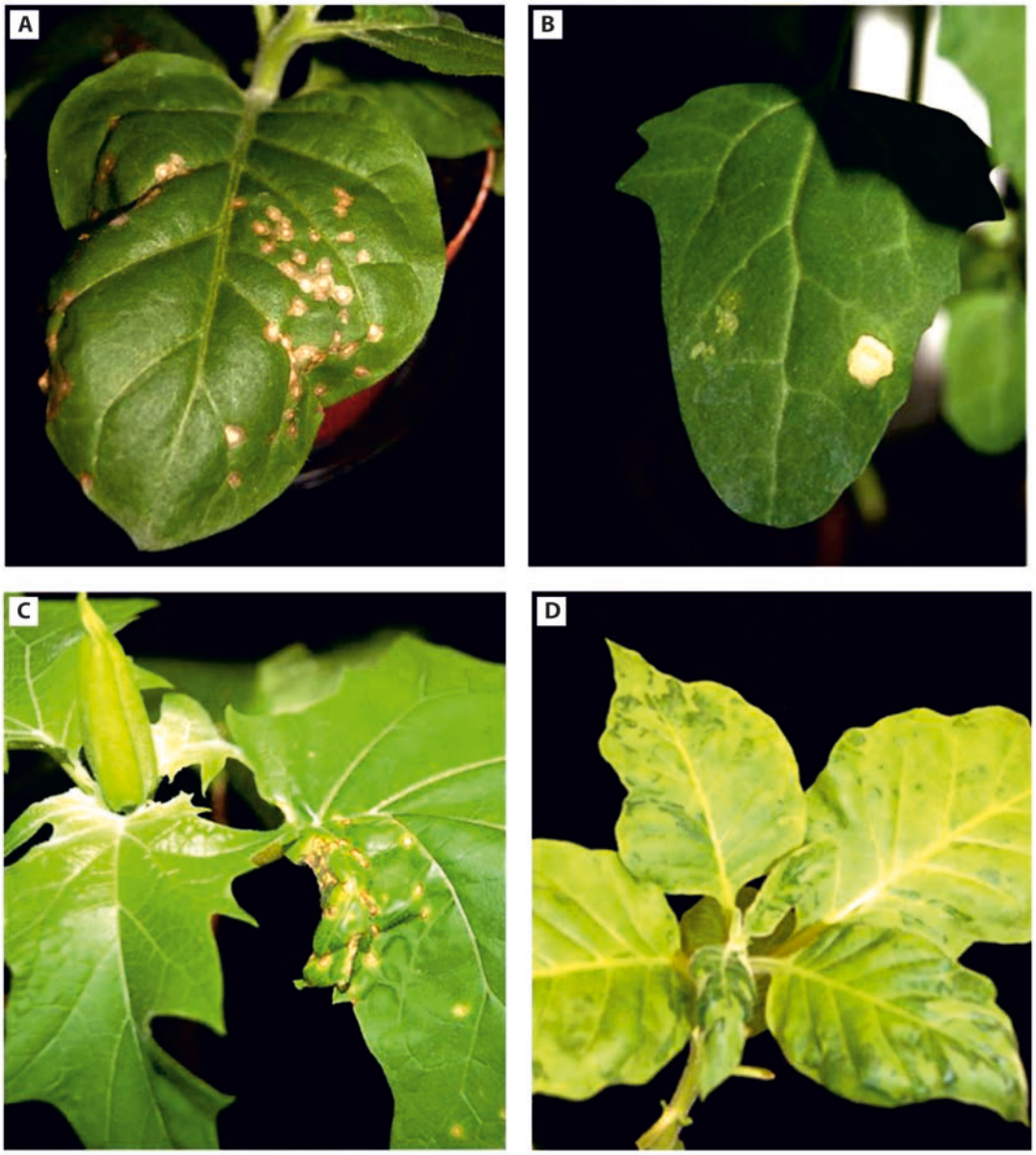

Fig. 1. Local symptoms of *Tomato mosaic virus* (TMV) and *Tobacco mosaic virus* (ToMV) in *Nicotiana tabacum* cv. Xanthi (A) and *Chenopodium quinoa* (B), local necrotic lesions caused by ToMV on *Datura stramonium* leaf (C), systemic symptoms in *N. tabacum* cv. Samsun co-infected with TMV and ToMV (D)

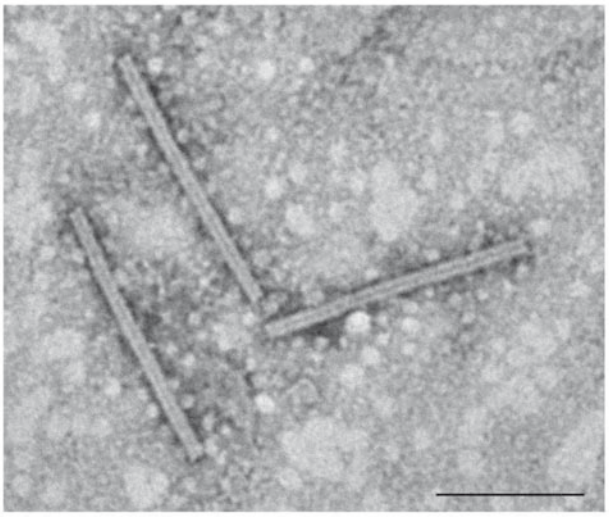

Fig. 2. Electron micrograph depicting tobamo-like virus particles from *Chenopodium quinoa* plants inoculated with R sample. Bar scale = 200 nm

stained with phosphotungstic acid (pH 7.4). Preparations were examinedin in a Hitachi HT7700 transmission electron microscope at an accelerating voltage of 100 kV (Hitachi, Tokyo, Japan). Electron microscopy examination of the dip preparations of sediments as well as the sap of symptomatic leaves of inoculated tobacco cv. Xanthi and cv. Samsun, and *C. quinoa* plants revealed the occurrence of rod-shaped virus particles of about 300 nm in length, typical for tobamoviruses (Fig. 2).

The identification of tobamoviruses was further conducted by molecular methods. RNA extraction was performed from a set of six symptomatic plants (2 *C. quinoa*, 2 tobacco cv. Xanthi, 2 tobacco cv. Samsun) inoculated with the selected R, N and S samples. Total RNA Purification Kit (Novazym Polska, Poznań, Poland) was used according to the manufacturer's instructions. DNA was amplified with the OneStep RT-PCR Kit (Qiagen, Hilden, Germany). Reactions were conducted in a final volume of 10 µl, which consisted of 1 µl template RNA, 1 µl each forward and reverse primers (10 µM), 2 µl 5×OneStep RT-PCR Buffer, 0.4 µl dNTP mix (10 mM), 0.4 µl OneStep RT-PCR Enzyme Mix and 10 U RiboLock RNase inhibitor (Thermo Fisher Scientific, Waltham, MA, USA). Reverse transcription-polymerase chain reaction (RT-PCR) were initially carried out with a Tobamovirus Group PCR Primer Mix (Agdia, Elkhart, Indiana, USA) and subsequently with primers for the simultaneous detection and differentiation of TMV and ToMV (Kumar *et al.* 2011). The commercial primers are based on the conserved genome region of viruses belonging to the *Tobamovirus* genus and the expected product size is about 370 to 400 bp. In order to distinguish between TMV and ToMV two specific primer pairs were used which amplify the part of polymerase gene of 880 nt and 318 nt, respectively (Kumar *et al.* 2011). The RT-PCR reactions were performed following the producer's instructions and under the thermal conditions described by the authors. DNA amplicons were separated by 1% agarose gel electrophoresis and stained with the Midori Green DNA stain (Nippon Genetics Europe GmbH, Düren, Germany) for UV light visualization. Preliminary RT-PCR assays performed with a commercial primer pair confirmed the infection with tobamoviruses in all 18 tested plants displaying disease symptoms (data not shown). A specific amplification product of the expected size, i.e. 880 bp, for TMV, was obtained for 12 samples (inoculated with R and S) while the product specific for ToMV (318 bp) was generated for all 18 tested samples (inoculated with R, N and S). Partial results are presented in Figure 3. The specificity of RT-PCR products obtained with diagnostic and differentiating primers was confirmed by sequencing. RT-PCR products of expected sizes were excised from agarose gels and purified using the Wizard® SV Gel and PCR Clean-Up System (Promega Corp., Madison, WI, USA).

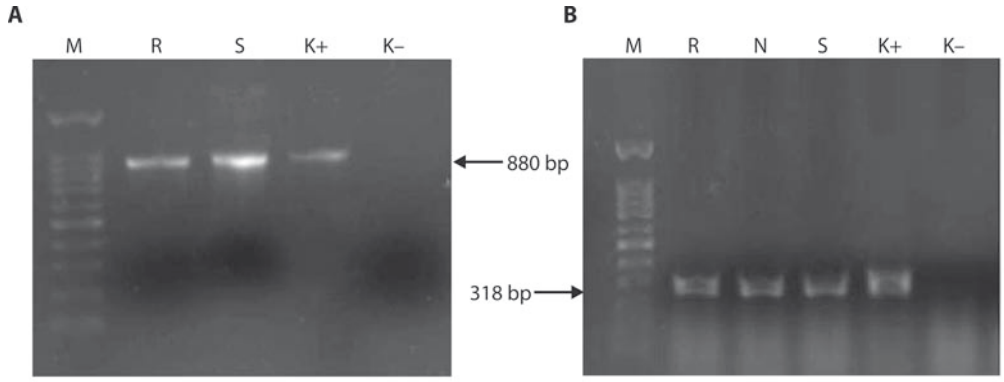

Fig. 3. Electrophoretic separation of RT-PCR products from plants inoculated with R, S and N samples in 1% agarose gel. (A) 880 bp TMV amplification products, (B) 318 bp ToMV amplification products. M: Nova 100-bp DNA ladder (Novazym), "K+" – positive control, "K–" – no-template control

Each amplicon was subsequently sequenced in triplicate by Genomed S.A. (Warsaw, Poland) using specific primers. The nucleotide sequences were analyzed using Standard Nucleotide BLAST (BLAST, http://blast.cnbi.nlm.nih.gov/Blast.cgi), compiled and edited using the BioEdit software (Hall 1999). Obtained partial coding sequences of RNA dependent RNA polymerase were deposited in the National Center for Biotechnology Information (NCBI) GenBank database with the following accession numbers: TMV-S (MF476188) and ToMV-S (MF476189). The obtained nucleotide sequences of TMV-S and TMV-R were identical. The analysis revealed 100% identity to the corresponding regions of TMV variants 1 and 2 (V01408, V01409) and 99% identity to e.g. the Chinese TMV isolates –152 and –Jimo (AF395129, HE818433) and South Korean TMV-IM (AB369276). Analogical analysis of DNA amplicons obtained with ToMV-F/ToMV-R primers for N, S and R samples confirmed nucleotide sequence identity. A high level of similarity (99% identity) was indicated to other known ToMV isolates e.g. Taiwanese ToMV-Penghu (KJ207374), Chinese ToMV-N5 (GQ280794), Australian ToMV-Queensland (AF332868) and Kazaks ToMV-K1 (AJ243571). The molecular analysis results demonstrated TMV and ToMV co-infections in analysed water samples.

The results presented above are consistent with numerous communications concerning the common spread of water-borne tobamoviruses. The epidemiological consequences of this fact may be important because they create a risk of infection for a broad range of crops. Further research on the detection of water-borne plant viruses which are important in agriculture should be continued.

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