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AN UNUSUAL STRAIN OF *TOBACCO MOSAIC VIRUS* IN NATURAL INFECTIONS OF RYE AND TRITICALE PLANTS IN POLAND

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Key words: *Tobacco mosaic virus* – rakkyo strain, rye, triticale, RT-PCR

In the last years several new viruses belonging to the genus *Tobamovirus* have been described (Adkins et al. 2003, Lewandowski 2005, Rhie et al. 2007). Tobamoviruses are known as serious plant pathogens, in particular the type species, *Tobacco mosaic virus* (TMV), is considered one of the most dangerous plant viruses. Tobamoviruses are easily transmitted mechanically, without the help of vectors. This property enables their efficient transmission by seed, however not *sensu stricto*, as the viruses do not infect the embryo. Geographical distribution of tobamoviruses is world wide (Lewandowski 2005). There knowledge on tobamoviruses infecting cereals is poor, as only TMV was reported to have some monocotyledonous hosts (Dodds and Hamilton 1974, Hamilton and Dodds 1970, Jeżewska et al. 2004, Oxelfelt 1974).

In 2005 unusual symptoms of leaf narrowing and stunting in winter rye and winter triticale plants were observed in a plant breeding station in Wielkopolska region (central-west Poland). In electron microscope no virus-like particles were seen in the sap of diseased plants. Similarly, in routine ELISA tests no cereal virus commonly occurring in Poland (*Barley yellow dwarf virus-MAV*, *Barley yellow dwarf virus-PAV*, *Soil-borne cereal mosaic virus*, *Wheat dwarf virus* and *Wheat streak mosaic virus*) was detected. An unknown infective agent was transmitted mechanically in 0.05 M potassium phosphate buffer (pH 7.2) with carborundum to a set of test plants including *Chenopodium amaranticolor*, *C. quinoa*, *Nicotiana benthamiana*, *N. tabacum* cv. 'Samsun' and *N. tabacum* cv. 'Xanthi'. Inoculated plants developed symptoms typical of *Tobacco mosaic virus* (TMV). In the sap of plants with symptoms virus-like particles morphologically resembling TMV virions, i.e. rigid rods 300 nm long were observed in electron microscope. Back inoculations on rye and triticale plants induced characteristic disease symptoms. In spite of clear disease symptoms no virus particles were observed, consequently, in electron microscope in the plant sap. The virus presence could be confirmed directly only by means of reverse transcription-polymerase chain reaction (RT-PCR) assay using commercial Agdia primers detecting tobamoviruses.

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The tobamovirus isolate, tentatively named "cereal tobamovirus", was submitted to the identification procedure. Biological, serological and molecular properties of the virus were investigated.

Host range determination. An experimental host range for the cereal tobamovirus was determined by mechanical inoculation of 25 species from eight families. Following species were assayed:

Family *Brassicaceae*: *Brassica campestris, Sinapis alba*
Family *Chenopodiaceae*: *Chenopodium amaranticolor, C. quinoa*
Family *Cucurbitaceae*: *Cucumis sativus*
Family *Fabaceae*: *Phaseolus vulgaris* cv. 'Laura', *Pisum sativum* cv. 'Cud Kelvedonu'
Family *Liliaceae*: *Allium cepa, A. porrum*
Family *Plantaginaceae*: *Plantago maior, P. lanceolata*
Family *Poaceae*: *Avena sativa* cvs. 'Bohun' and 'Breton',
Hordeum vulgare cvs. 'Annabell', 'Antek' and 'Blask',
Panicum miliaceum,
Secale cereale cvs. 'Amilo', 'Bosmo', 'Dańkowskie Złote',
Sorghum vulgare,
Triticale sp. cvs. 'Moderato' and 'Sorento',
Triticum aestivum cvs. 'Grenada' and 'Kris',
Zea mays cvs. 'Blask', 'Cyrkon', 'Jubilee' and 'Płomyk'
Family *Solanaceae*: *Capsicum annuum, Lycopersicon esculentum*,
Nicotiana benthamiana,
N. tabacum cvs. 'Samsun', 'White Burley' and 'Xanthi',
Petunia hybrida,
Physalis floridana

No evidence of infection was detected in any of the tested species belonging to *Brassicaceae, Cucurbitaceae, Fabaceae* or *Plantaginaceae*. Symptoms were produced in all species from *Solanaceae* family, except for *L. esculentum*, and were consisted with those reported for TMV. Systemic mosaic and leaf narrowing were symptoms typical of *Allium* species as well as for all *Poaceae* species except maize. The characteristic feature of the virus isolate was high infectivity to cereals (rye, triticale, wheat, barley, oat and millet). Host range of the cereal tobamovirus strongly suggested its relationship with TMV, with special affinity to the *Monocotyledones* species.

Seed-transmission rate of the cereal tobamovirus was evaluated for rye cv. 'Amilo'. Young rye plants originating from seeds collected from virus-infected mother plants were tested for the virus presence. In this experiment typical leaf narrowing and mild dwarfing were observed in some rye plants. The infection was confirmed by RT-PCR with Agdia primers as well as by back inoculation on *N. tabacum* 'Xanthi' test plants. The efficiency of seed transmission in rye mounted up to 70%.

Serological properties of the cereal tobamovirus were initially examined with TMV-antiserum in immunospecific electron microscopic tests according to Derrick (1973) and ELISA (Clark and Adams 1977). Initially an own TMV-antiserum produced in the Department of Virology and Bacteriology (Institute of Plant Protection) was used, subsequently commercial kits for TMV and *Tomato mosaic virus*

(ToMV) detection were also applied (Loewe, Germany). No serological reaction was noted in any assay. Serological relationships were studied with following other antisera: *Bell pepper mottle virus* (BPeMV), *Odontoglossum ring spot virus* (ORSV), *Paprika mild mottle virus* (PaMMV), *Pepper mild mottle virus* (PMMoV-Kr), *Tobacco mosaic virus* – rakkyo strain (TMV-R). Only in the case of TMV-R-antiserum clear positive serological reaction was observed.

The cereal tobamovirus was purified from extracts of infected leaves of *N. tabacum* 'Xanthi' according to the method adapted from Shirako and Brakke (1984).

Viral RNA was isolated from fresh infected plant leaves or from purified virus preparations. Total RNA extraction from 100–120 mg of fresh infected plant leaves was carried out with the use of RNeasy Mini Kit manufactured by Qiagen, according to the procedure supplied by the producer. The RNA was eluted with 40 μl Rnase-free water.

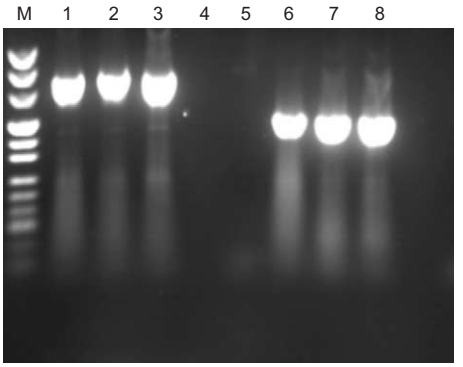
The size of RNA genome of the cereal tobamovirus, as evaluated in gel electrophoresis was the same as RNA of TMV, i.e. about 6400 nucleotides.

One step RT-PCR kit (Qiagen) was used to obtain and subsequently amplify cDNA fragments. RT-PCR test was performed with following primer pairs: Tobamovirus Group PCR Primer Mix (Agdia, USA) – only for the detection of any tobamovirus, Tob3end/TobCP (Adkins et al. 2003), PS1/PAS1 and PS2/PAS1 (Alexandre et al. 2000), 1P/1M and RS1/RS2 (Chen et al. 1996). 1 μl of target RNA was added to RT-PCR components in standard concentrations recommended by manufacturer (with 10 μM final concentration of primers). The reactions were conducted in Whatman Biometra Tpersonal thermocycler. The RT-PCR products were separated electrophoretically in 1.5% agarose gel and visualized in UV light after staining with ethidium bromide.

No product appeared after RT-PCR with Tob3end/TobCP primers. Primers PS1/PAS1 and PS2/PAS1 as well as 1P/1M generated products of expected sizes: 700, 500 and 1400 bp, respectively.

Products of PS1/PAS1 and PS2/PAS1 were excised from the gel and eluted using QIAEX II Kit (Qiagen). The amplified fragments DNA were ligated to the vector pGEM-T Easy (Promega) and transformed into *Escherichia coli* DH5α cells. Clones were verified using X-Gal + IPTG system.

Positively verified plasmid DNA was obtained only with using PS2/PAS1 primers pair. It was isolated after overnight cultures using QiaPrepPlazmid Miniprep Kit (Qiagen) following the



Phot. 1. Electrophoresis of RT-PCR products of tobamoviruses with two pairs of primers: PS1/PAS1 (lanes 1–4) and PS2/PAS1 (lanes 5–8). Lane M – marker DNA, lane 1 – ToMV, lane 2 – TMV, lane 3 – cereal tobamovirus, lane 4 – water, lane 5 – water, lane 6 – ToMV, lane 7 – TMV, lane 8 – cereal tobamovirus (photo by M. Jeżewska and K. Trzmiel)

manufacturer's protocol. DNA was sequenced with standard primers for cloning vectors. Sequence analysis was performed in Molecular Biology Techniques Laboratory at the Adam Mickiewicz University in Poznań. Amplified fragment of genome consisted of a part of the CP region. Sequence analysis of obtained 484 bp genome fragment indicated that the cereal tobamovirus isolate shared 98% nucleotide sequence identity with ToMV1-2 strain (DQ873692) and with TMV strains (X02144, AF155507, AJ243571, AF103779).

Product 1P/1M, corresponding to the conservative part of viral genome, was not submitted to the procedure of cloning and sequencing.

Surprisingly, primer pair specific for TMV-R, RS1/RS2, did not generate any PCR product, in spite of examining different variants of PCR thermal profiles in Whatman Biometra TProfessional gradient thermocycler.

The identification of the cereal tobamovirus was not fully completed. Its biological, serological properties suggested that it was an isolate of TMV-R (Kwon and Sako 1994). However, the lack of reaction with primers specific for TMV-R, as reported by Chen et al. (1996), indicated that such classification would be unjustified. Therefore it was concluded, taking into consideration its serological specificity as well as special affinity to *Monocotyledones* plants, that the cereal tobamovirus should be recognised as TMV-R-like tobamovirus.

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