Institute of Plant Protection, Poznań, Poland

BARLEY YELLOW DWARF VIRUS-MAV (BYDV-MAV) AND *BARLEY YELLOW DWARF VIRUS*-PAV (BYDV-PAV) ON MAIZE IN POLAND

K. Trzmiel

Key words: *Barley yellow dwarf virus*-MAV, *Barley yellow dwarf virus*-PAV, detection

Barley yellow dwarf is one of the most dangerous cereal diseases spread worldwide. Plant infection is displayed mainly by dwarfing and leaf discolourations: yellowing or reddening (Rochow et al. 1996). Disease symptoms are caused by a group of viruses, previously named *Barley yellow dwarf virus*. They have been recently classified as different virus species (Harrison 1999). The most common barley yellow dwarf viruses are: *Barley yellow dwarf virus*-MAV and *Barley yellow dwarf virus*-PAV (*Luteovirus, Luteoviridae*) and *Cereal yellow dwarf virus* (*Polerovirus, Luteoviridae*). Other barley yellow dwarf viruses have not been included to the *Luteoviridae* family (Syller 2001). The causal agents are vectored by aphids, mainly by *Sitobion avenae* and *Rhopalosiphum padi*. Barley yellow viruses infect plants in the family *Gramineae*. Maize is one of the natural hosts of the viruses. The first characteristic of maize disease which was probably induced by BYDV-PAV was published in 1952 (Loi et al. 2004). Members of the genus *Luteovirus* have linear, positive sense ssRNA about 6000 bp in length which is surrounded by the coat protein. Virions are icosahedral about 25 nm in diameter (Lapierre et al. 2004).

First report on barley yellow dwarf in Poland appeared in 1983 (Hoppe et al.). In 2001–2002 important outbreaks of barley yellow dwarf on winter wheat and winter barley were noticed (Jeżewska 2003), however, until 2005 there were no data concerning the occurrence of barley yellow dwarf viruses on maize in Poland. In 2005, for the first time BYDV-PAV was detected using the double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA test) in maize plants displaying symptoms of dwarfing, streaking or discolourations of leaves. Investigations on the occurrence of barley yellow dwarf viruses on maize were continued in 2006.

Field inspections were conducted in six locations, situated in western and southern part of Poland. Maize plants with symptoms of mosaic, streaking, stripping or yellowing, reddening leaves or stunting were collected for subsequent analyses.

For virus detection two kinds of ELISA tests were used: DAS-ELISA kit from Loewe (Germany) and TAS-ELISA (polyclonal/monoclonal antibodies) from Adgen (Great Britain). Extracts from healthy maize plants were used as negative controls. Samples with A_{405nm} values > three fold negative controls in the same

Phytopathol. Pol. 42: 53–56
© The Polish Phytopathological Society, Poznań 2006
ISSN 1230-0462

plate were considered positive (Clark and Adams 1977, D'Arcy et al. 1989, D'Arcy et al. 1992).

BYDV-PAV from field samples was transmitted by aphids onto healthy maize plants in greenhouse. Two lines of dent maize and two lines of flint maize were used. BYDV-PAV purification procedure was performed as described by D'Arcy et al. (1989). Purified preparations were observed in electron microscope Philips EM 201.

360 maize plants with disease symptoms were collected from six fields from southern and western Poland for further diagnostic assay. All of them were studied with DAS-ELISA and TAS-ELISA. BYDV-MAV and BYDV-PAV infections were revealed in tested plants. Four out of 30 *Barley yellow dwarf virus*-PAV infected maize plants were chosen for study with RT-PCR for confirmation of ELISA test.

The identification of BYDV-PAV isolated from diseased maize plants was confirmed using the reverse transcription (RT) polymerase chain reaction (PCR) with primers Lu 1 and Lu 4 (Robertson et al. 1991).

Total RNAs were isolated from 100-110 mg of fresh infected plant leaves 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 EB buffer.

One Step RT-PCR kit (Qiagen) was used to obtain and subsequently amplify cDNA.

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 entire volume of RT-PCR reagents was adjusted with water to 25 μl.

The reactions were conducted in Eppendorf Mastercycler personal.

The RT step was carried out under the conditions proposed by RT-PCR kit producer. The following PCR step included one cycle at 95°C (1 min), 41°C (2 min) and 72°C (20 min), followed by 40 cycles at 94°C (1 min), 41°C (1 min), 72°C (2 min), with a final cycle of 72°C (10 min).

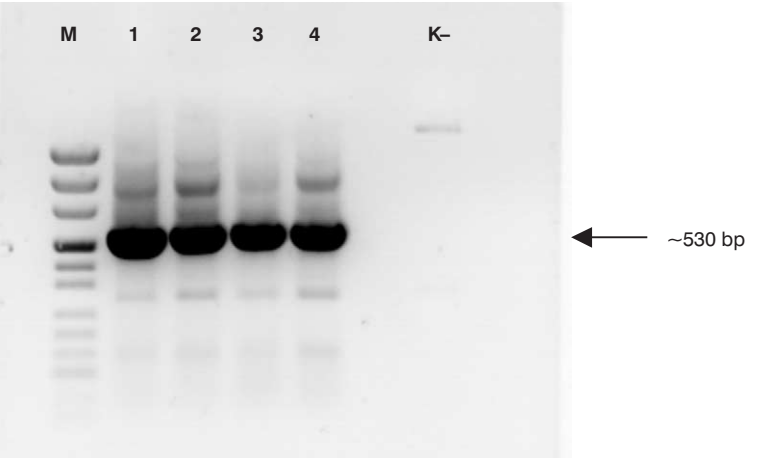
The RT-PCR products were separated electrophoretically in 1.5% agarose gel and visualized in UV light after staining with ethidium bromide.

The fragments of the expected size were purified using the Qiagen Gel Extraction Kit and cloned into pGEM-T Easy (Promega) following the manufacturers' protocols. Sequence analysis was performed in Molecular Biology Techniques Laboratory at the Adam Mickiewicz University in Poznań.

BYDV-MAV and BYDV-PAV were detected in surveyed plants. Out of 360 maize plants tested, 24 were found BYDV-MAV infected (6.7%) and 30 infected with BYDV-PAV (8.3%). In four cases mixed infection BYDV-MAV and BYDV-PAV occurred.

The result of purification *Barley yellow dwarf virus*-PAV from infected maize plants was not satisfactory. Preparations which were observed in electron microscopy contained only a few virus particles.

Four maize samples found infected with BYDV-PAV by ELISA were chosen for subsequent analysis by RT-PCR. A major RT-PCR product of about 530 bp was present in all samples of diseased plants but not in healthy control (Phot. 1).



Phot. 1. Electrophoresis separation in 1.5% agarose gel of the RT-PCR products with using primer sets: Lu 1 and Lu 4. M – pUC Mix Marker, lanes: 1, 2, 3, 4 – infected maize plants, K– – negative control (photo by K. Trzmiel)

Results of sequencing reaction confirmed the identity of BYDV-PAV. The RT-PCR product sequence alignments showed that it shared identities of 98% with *Barley yellow dwarf virus*-PAV WA95 coat protein (ORF3) gene, complete cds.

DAS-ELISA test did not specifically detect BYDV-PAV. However, the use of the primer pair Lu 1 and Lu 4 for RT-PCR allowed to detect BYDV-PAV but not BYDV-MAV, thus being a good tool to differentiate both viruses. In the case of TAS-ELISA, the monoclonal antibodies enabled to detect specifically only BYDV-MAV.

Maize proved to be a good host for luteoviruses: BYDV-MAV and BYDV-PAV also in Poland. However, no specific disease symptoms on infected maize plants were noticeable. In addition, very frequently after infections with the viruses the surveyed plants were symptomless. Such observations are consistent with reports of other authors (Ivanović et al. 1995, Loi et al. 2004).

The finding that BYDV-PAV was dominant is in agreement with previously reported data (Jeżewska 2003, Miller et al. 2004).

Literature

Clark M.F., Adams A.N., 1977: Characteristics of the microplate method of enzyme-linked immunosorbent assay for the detection of plant viruses. J. Gen. Virol. 34: 475–483.

D'Arcy C.J., Hewings A.D., Eastman C.E., 1992: Reliable detection of barley yellow dwarf viruses in field samples by monoclonal antibodies. Plant Dis. 76: 273– 276.

D'Arcy C.J., Martin R.R., Spiegel S., 1989: A comparative study of luteovirus purification methods. Can. J. Plant Pathol. 11: 251–255.

D'Arcy C.J., Torrance L., Martin R.R., 1989: Discrimination among luteoviruses and their strains by monoclonal antibodies and identification of common epitopes. Phytopathology 79: 869–873.

Harrison B.D., 1999: Steps in the development of luteovirology. In: The Luteoviridae. Eds. H.G. Smith, H. Barker. CAB Int., Wallingford: 1–14.
Hoppe W., Ruszkiewicz M., Zielińska L., 1983: Occurrence of barley yellow dwarf virus on oat cultures in Poland. Zesz. Probl. Post. Nauk Roln. 291: 119–129.
Ivanović D., Osler R., Katis N., Ivanović M., Ignjatović D., 1995: Principal maize viruses in Mediterranean countries. Agronomie 15: 443–446.
Jeżewska M., 2003: Choroby wirusowe zbóż, diagnostyka i zwalczanie. Progr. Plant Prot. / Post. Ochr. Rośl. 43: 164–171.
Lapierre H., 2004: Barley yellow dwarf associated with BYDV-MAV. In: Viruses and virus diseases of *Poaceae* (*Gramineae*). Eds. H. Lapierre, P.A. Signoret. INRA, Paris: 460–463.
Loi N., Osler R., Lapierre H., 2004: Barley yellow dwarf associated to BYDV-PAV virus. In: Viruses and virus diseases of *Poaceae* (*Gramineae*). Eds. H. Lapierre, P.A. Signoret. INRA, Paris: 618–620.
Miller W.A., Becket R., Ordon F., Friedt W., Lapierre H., 2004: Barley yellow dwarf associated with BYDV-PAV. In: Viruses and virus diseases of *Poaceae* (*Gramineae*). Eds. H. Lapierre, P.A. Signoret. INRA, Paris: 465–471.
Robertson N.L., French R., Gray S.M., 1991: Use of group-specific primers and the polymerase chain reaction for the detection and identification of luteoviruses. J. Gen. Virol. 72: 1473–1477.
Rochow W.F., Sward R.J., Waterhouse P.M., 1996: Barley yellow dwarf *luteovirus*. In: Viruses of plants descriptions and lists from the VIDE database. Eds. A.A. Brunt, K. Crabtree, M.J. Dallwitz, A.J. Gibbs, L. Watson. CAB Int., Cambridge: 162–167.
Syller J., 2001: Luteowirusy – charakterystyka i aktualne zasady klasyfikacji. Post. Mikrobiol. 40: 171–185.

Author's address:
Katarzyna Trzmiel M.Sc., Institute of Plant Protection, ul. Miczurina 20, 60-318 Poznań, Poland, e-mail: K.Trzmiel@ior.poznan.pl

Accepted for publication: 20.12.2006