Institute of Plant Protection, Poznań, Poland

TOBACCO MOSAIC VIRUS (TMV) IN WINTER BARLEY – TRANSMISSION WITH SEEDS

M. Jeżewska and K. Trzmiel

Key words: Tobacco mosaic virus, barley, seed-transmission, RT-PCR

Tobacco mosaic virus (TMV) is probably the best known virus in the world. Its particles, rigid rods 300 nm long, are very stable and are present in big quantities in many of its hosts. TMV has wide host range and is transmitted by means not involving vectors; it is easily transmitted by mechanical inoculation, by grafting, by contact between plants and through the testa. The virus is distributed world-wide. Virions contain 5% nucleic acid and 95% protein. Genome consists of single molecule of positive sense linear ssRNA (64 kb) (Zaitlin 1996). Although TMV is known as a pathogen of dicotyledonous plants, it may infect also monocotyledonous hosts (Hamilton and Dodds 1970, Dodds and Hamilton 1974, Oxelfelt 1974). In 2003 TMV was identified as causal agent of serious disease symptoms in winter barley (Jeżewska et al. 2004). TMV is not a seed-transmitted virus, as it cannot be carried within the embryo. However, it is so readily infecting its host that it can successfully invade young seedlings from testa during germination (tomato, pepper) (Mink 1993).

The aim of the work presented below was to demonstrate that TMV is able to infect barley seedlings from testa during germination.

Material for experiments: seeds of winter barley cv. ‘Kroton’ collected from plants mechanically inoculated with TMV. TMV inoculum originated from the virus isolate identified initially in barley and maintained in Nicotiana species (N. benthamiana L. and N. tabacum L. cv. ‘Xanthi’). In spite of the lack of clear disease symptoms on the inoculated plants the seeds were considered the potential source of TMV for infection in next generation at the moment of germinating. They were germinated in sterile Perlit granules. At three-leaf-stage seedlings were assayed for TMV presence by means of biological, serological, electron microscopic and RT-PCR tests. Biological method consisted of mechanical inoculation of N. benthamiana and N. tabacum ‘Xanthi’ plants with crude sap of barley seedlings. Electron microscopy included dip preparations and ISEM assays (Derrick 1973). Antiserum for ISEM was obtained from Prof. dr hab. H. Pospieszny (Institute of Plant Protection, Poznań, Poland). ELISA tests (Clark and Adams 1977) were performed with TMV ELISA kit produced by Loewe Biochemica GmbH (Sauerlach, Ger-
many). Total RNA was isolated using RNeasy Plant Mini Kit (Qiagen GmbH, Hilden, Germany). The kit was used according to manufacturer’s recommendations. RT-PCR procedure was performed using Qiagen One Step RT-PCR kit with Tobamovirus Group PCR Primer Mix (Agdia Inc., Elkhart, USA). Primer mix was added to reaction tubes to achieve final concentration 0.4 μM (5 μL 2 μM primer mix per reaction). The reactions were carried out in Eppendorf Thermo Cycler with following thermal conditions: a) reverse transcription: 50°C (30 min), b) initial PCR activation step: 95°C (1 min), c) three-step cycling including denaturation: 94°C (1 min), annealing: 50°C (3 min) and extension: 72°C (1 min), 35 cycles, d) final extension: 72°C (10 min). Samples were stored at 4°C until needed. PCR mixtures (5 μL) were electrophoresed on a 1.5% agarose gel in TBE buffer at 50 V for 1 h with pUC Mix Marker, 8 (Fermentas, Lithuania). The bands were visualised by ethidium bromide staining. The expected PCR product size is about 370 to 400 bp.

Studies of TMV occurrence in barley seedlings after germination under in vitro conditions (in Perlit) were performed in following experiments.

In the first experiment the virus was detected by means of biological tests, electron microscopy and ELISA.

100 seeds were allowed to germinate but only 75 seedlings grown up to the third–fourth leaf stage could be taken for analyses. No clear symptoms were noticed. No virus particles were seen in electron microscope even in ISFM tests. ELISA did not detect the virus, either. However, in 15 seedlings TMV presence was revealed by biological method thus indicating the rate of TMV transmission about 20%.

The second experiment was aimed at verification of the relatively high rate of TMV transmission after longer period of storage – 10 months, versus five months in the 1st series of assays. Biological method was used to evaluate TMV presence. 85 seedlings were tested and only two were found infected. In the second experiment TMV diagnostics was completed with RT-PCR technique. 10 seedlings were chosen for tests; two of them were already found infected with TMV by biological method and others were taken randomly. Electrophoretic pattern of PCR products is presented in Photograph 1. Results of RT-PCR for nine barley seedlings, out of 10 tested, are displayed. As could be seen in Photograph, only one barley seedling appeared virus free (in lane 3). Other seedlings were found carrying TMV, in contrast to the results of biological tests which detected TMV in two seedlings (samples in the lanes 6 and 11). In the 10th seedling tested by RT-PCR, lacking in the Photograph, PCR product indicating TMV presence was also observed.

Experimental plants found containing TMV at the third leaf stage by biological method and/or RT-PCR were observed during further development under glasshouse conditions for three months. No clear symptom expression occurred in any of them.

Already first results concerning detection of TMV in barley seedlings confirmed our presumption that the virus was able to infect this cereal host from testa during germination of seeds. However, the concentration of virions was very low and therefore routine diagnostics, except for biological method, yielded unreliable re-
On the other hand, the rate of infections via seeds revealed by means of biological tests appeared surprisingly high.

Results of the second experiment provided further evidence on the occurrence of TMV in barley seedlings due to the use of RT-PCR technique. Another very important observation was the decrease of the percentage of TMV-infected seedlings found by biological method after longer period of seed storage; in the 1st term it was 20% and in the 2nd one only 2.3%. It suggests that during storage inactivation of the virus is going on, thus diminishing the risk of subsequent TMV transmission into next generation.

The discrepancy between the results of detection by biological tests and RT-PCR may be explained by high sensitivity of the latter (Maroon and Zavriev 2002). In fact, RT-PCR technique with primers from Agdia proved appropriate diagnostic tool for investigation of TMV incidence in barley seedlings.

However, very high frequency of TMV incidence in barley seedlings demonstrated by RT-PCR was not reflected by correspondingly high rate of disease symptom development. Undoubtedly, there are many factors involved in symptom expression and one of them is probably the concentration of TMV particles.

Summarising the above presented data it may be concluded that:

– infection of barley plants with TMV may result in the virus transmission to the progeny,
– diagnostics of TMV in barley seedlings requires a very sensitive method; biological method is not fully reliable,
– during storage of barley seeds the risk of TMV transmission to young seedlings decreases considerably,
– disease symptom expression is not necessary for TMV transmission via seeds,
the problem of barley infection with TMV is perhaps of limited economical importance; nevertheless, it may consist a link in TMV circulation chain in the environment.

Acknowledgements

The authors wish to thank Maria Lubik, Eng., for excellent technical assistance.

Literature


Authors’ address: Dr Małgorzata Jeżewska, Katarzyna Trzmiel, M.Sc., Institute of Plant Protection, ul. Miczurina 20, 60-318 Poznań, Poland e-mail: m.jezewska@ior.poznan.pl

Accepted for publication: 20.11.2005