Short Communication

First Report of *Pepper mild mottle virus* in Pepper Seeds Produced in the Czech Republic

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Abstract

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Symptoms of viral infection were observed on plants of pepper, cv. OL 228, raised from commercial seeds of Czech origin in a greenhouse in the year 2002. Infected plants showed mosaic or mottling on leaves, and necrotic depressions on fruits. Straight, rod-shaped viral particles of about 300 nm, indicating a tobamovirus infection, were found by electron microscope. ELISA produced negative reactions for *Tobacco mosaic virus* (TMV) but positive reactions with an antiserum to *Pepper mild mottle virus* (PMMoV). In biological characterisation using pepper cultivars with the *L1*, *L2*, *L3* and *L4* tobamovirus resistance genes it was found that the Czech isolate of PMMoV belongs to pathotype P1.2. This is the first report of PMMoV in the Czech Republic. Its distribution, however, may still be limited as a survey did not reveal other infections in the main pepper producing areas. As PMMoV spreads with infected seeds, the possibility of its chemical deactivation by NaOH was tested and confirmed.

Keywords: *Pepper mild mottle virus*; characterisation; pathotype P1.2; *Capsicum annuum;* ELISA; electron microscopy; seed transmission; virus deactivation; sodium hydroxide

Pepper mild mottle virus (PMMoV) had so far not been detected in the Czech Republic, although it had been found in several other European countries, i.e. Belgium (Verhoyen 1994), Denmark (Paludan 1982), France (Gebre Selassie *et al.* 1981), Greece (Avgelis 1986), Hungary (Kalman *et al.* 2001), Italy (Wetter *et al.* 1984), Spain (Marte & Wetter 1986), the Netherlands (Rast 1979) and the United Kingdom (Brunt 1986).

PMMoV belongs to the genus *Tobamovirus*, ssRNA+ viruses. It was first described by McKinney (1952). The virus is transmissible by contact

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between plants, plant sap and seeds originating from infected plants (Brunt *et al*. 1996). Two pathotypes, P1.2 and P1.2.3, can be discriminated (Rast 1988).

The aim of this work was to identify a viral disease of pepper plants in the Czech Republic, and to confirm whether chemical disinfection of seeds by 2% sodium hydroxide (NaOH) water solution (Střelec *et al*. 1978) is feasible.

MATERIAL AND METHODS

Plant material. Seeds of *Capsicum annuum*, cv. OL 228, had been produced in the Czech Republic in the year 2001. Part of the seed (twelve seeds) was sown in a greenhouse in spring 2002. Grown up plants developed symptoms of viral infection. Then, the rest of seed (twelve seeds) was used for tests on chemical disinfection.The plants were raised separately from other pepper plants and were transferred to an unheated greenhouse in late April. In May, mottling and mild mosaic were observed on leaves of all plants. The mosaic became stronger with the aging of leaves. Some leaves were deformed. Diseased plants were stunted and their fruits were malformed with necrotic depressions (Figure 1), compared to healthy plants.

Biological characterisation. The studied virus isolate was maintained on systemically infected plants of *Nicotiana tabacum* cv. Samsun. To prepare inoculum, 1 g of infected leaves was homogenised with 5 ml of 1% phosphate buffer (K_2HPO_4), pH 9.0. Cellite was added to the homogenate at a concentration of 3% (w/v) and the first three true leaves of pepper plants were inoculated by rubbing. For the biological characterisation the following *Capsicum* species and cultivars with the tobamovirus resistance genes *L1*, *L2*, *L3* and *L4* were inoculated: *Capsicum annuum* cvs. Sivria and Doux des Landes with the *L*+ gene, *C. annuum* cvs. Sivria-RY and Yolo Wonder (*L1*), *C. frutescens* cv. Tabasco (*L2*), *C. chinense* accession P.I. 152225 (*L3*) and *C. chacoense* accession P.I. 260429 (*L4*).

The Dutch isolate P8 of PMMoV pathotype P1.2 (Rast 1979) was used as a reference. Symptoms on inoculated plants were recorded during the following 3 weeks.

The inoculation experiments were conducted two times, using a pair of plants for each inoculation. The plants were maintained in a growth chamber at temperatures of 24°C/day and 22°C/night, with a 16h light period.

Electron microscopy. Leaf samples of pepper cv. OL 228 with mosaic symptoms were ground in a mortar with 0.01M HEPES buffer, pH 8.2 (1 g leaves + 2 ml buffer). The homogenate was filtered through a silon sieve and negatively stained by phosphotungstic acid in ratio 1:1, pH 6.9. Then the mixture was adsorbed to a grid. Grids were examined with a Philips 2085 transmission electron microscope.

ELISA. For the tests of pepper plants, *Cucumber mosaic virus* (CMV), *Broad bean wilt virus* (BBWV), *Potato virus Y* (PVY), *Tobacco mosaic virus* (TMV), *Tomato spotted wilt virus* (TSWV) and PMMoV polyclonal antibodies (Loewe Biochemica, Germany) were used in the direct double antibody sandwich ELISA (DAS-ELISA) described by Clark and Adams (1977). Samples for ELISA were prepared by grinding 0.2 g of leaf tissue in phosphate buffered saline, pH 7.4 with 2% polyvinylpyrrolidone and 0.2% of egg albumin, in ratio 1:20. ELISA microtiter plates were incubated one hour at 20°C after pipetting the substrate solution. Colour reaction was screened using the MR 5000 Dynatech reader at 405 nm. Samples with $A_{405} > 0.50$ were considered as positive, while samples with $A_{405} < 0.03$ were rated as negative. The value of A_{405} of the negative control was 0.005.

Survey of field occurrence of viruses on pepper plants. Pepper plants were inspected for symptoms of viral infection in selected farming areas in the Czech Republic. Leaf samples were collected and examined by ELISA for the presence of CMV, BBWV, PMMoV, PVY, TMV and TSWV. Positive findings were confirmed by transmission onto indicator plants and by electron microscopy.

Chemical disinfection of infected seeds. Part of the original seeds of the pepper variety OL 228, from the same package where the PMMoV infection was found, was treated by soaking them in 2% NaOH water solution for 2 min at 20°C. Seeds were then washed with tap water, dried and immediately sowed.

RESULTS

Symptomatic leaf tissue was examined by electron microscopy, and straight rod-shaped tobamovirus-like particles of about 300 nm in length were found (Figure 2).

In ELISA a clear reaction was obtained with the antiserum to PMMoV (absorbance values over 1.7),

Figure 1. Symptoms of PMMoV on the pepper cv. OL 228

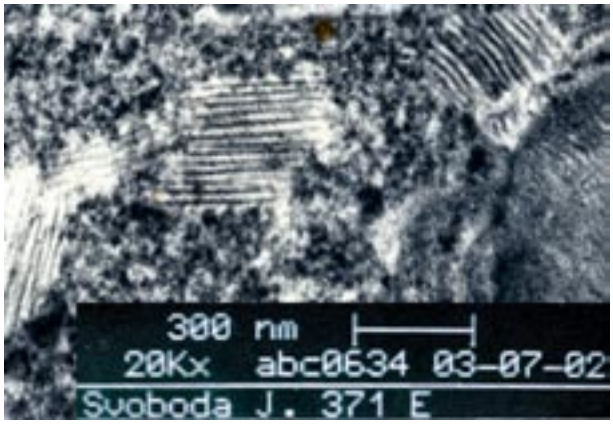

Figure 2. Observed viral particles of PMMoV

while the reaction with the antiserum to TMV was negative (absorbance values below 0.006).

The pathotype of PMMoV was determined by evaluating the symptoms on inoculated *Capsicum* plants with tobamovirus resistance genes. Systemic symptoms were observed on plants of *C. annuum* cvs. Sivria and Doux des Landes with resistance gene *L+*, *C. annuum* cvs. Sivria-RY and Yolo Wonder with gene *L1*, and on *C. frutescens* cv. Tabasco with gene *L2*. Only local necrotic lesions were observed on plants of *C. chinense* P.I. 152225 and *C. chacoense* P.I. 260429 containing the *L3* and *L4* resistance genes, respectively. The Dutch isolate P8 of PMMoV pathotype P1.2 that was used as reference produced similar symptoms on these cultivars and lines (Table 1). These observations show that the studied virus isolate belongs to the pathotype P1.2 of PMMoV.

During the years 2003 and 2004, pepper plants were inspected in two areas of the Czech Republic, i.e. Polabí and South Moravia. Six fields and greenhouses were inspected and 50 more or less symptomatic leaf samples were taken and tested by ELISA. The presence of CMV, BBWV and PVY was shown in thirteen, two and one samples, respectively. However, neither TMV, TSWV nor PMMoV were detected.

The germinability of seeds treated with NaOH solution before sowing was 90%. None of the germinated plants showed mosaic symptoms or fruit deformation (Figure 2) and all samples were negative both in ELISA and electron microscopy.

DISCUSSION

This is the first report of an isolate of PMMoV in the Czech Republic. Infection was found only on plants *C. annuum* cv. OL 228. Seeds were assumed to be the only possible source of infection. However, no infection was found on pepper plants

Table 1. Symptoms caused by the Czech isolate of PMMoV and the Dutch isolate P8 on test plants of various *Capsicum* spp.

Test plant (with resistance gene)	Local/systemic reaction	
	Czech isolate	Dutch P8 isolate
Capsicum annuum cv. Sivria (*L+*)	–/m, l	–/m, l
C. annuum cv. Doux des Landes (*L+*)	–/m, l	–/m
C. annuum cv. Sivria – RY (*L1*)	–/m, l	–/m, l
C. annuum cv. Yolo Wonder (*L1*)	–/m, l	–/m, l
C. frutescens cv. Tabasco (*L2*)	cl, nl/m, l, d	cl, nl/m, l, d
C. chinense P.I. 152225 (*L3*)	nl/–	nl/–
C. chacoense P.I. 260429 (*L4*)	nl/–	nl/–

cl = chlorotic lesions; d = dwarfing; l = leaf malformation; m = mosaic or mottle; nl = necrotic lesions; – = no symptoms

grown from seeds of other varieties from the same producer. Unfortunately, further tests for PMMoV on the seed we used for our experiments are no longer possible as no more seeds are available from that seed lot of *C. annuum,* cv. OL 228. This seed is no longer available on the market.

The occurrence of PMMoV in the Czech Republic still seems limited, as the virus was not found in a survey in 2003 and 2004. PMMoV can easily be transmitted over long distances by both infected seeds and plantlets. In this way the virus can be introduced into non-infected cultivations of pepper. Once present, the virus is easily spread by contact during crop handling.

To prevent introductions of PMMoV, seeds can be desinfected by 2% NaOH (Salamon & Kaszta 2000) or by 10% Na_3PO_4 (Rast & Stijger 1987). Heating seeds at 76°C for 3 days also may disinfect seeds, but this treatment may affect viability and germination of the seeds (Rast & Stijger 1987). It is necessary to note that chemical treatment should not be repeated, and treated seed should be sown immediately.

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