## LETTER TO-THE-EDITOR

# IMPROVED DETECTION OF ARABIS MOSAIC VIRUS IN GRAPEVINE AND HOP PLANTS

P. KOMÍNEK[1], P. SVOBODA[2], N. ABOU GHANEM-SABANADZOVIC[3]

[1]Research Institute of Crop Production, Drnovská 507, 161 06 Prague 6, Czech Republic; [2]Hop Research Institute Žatec, Žatec, Czech Republic; [3]Department of Plant Pathology, University of California, One Shields Avenue, Davis, CA 95616, USA

*Received September 3, 2003; accepted September 12, 2003*

Arabis mosaic virus (ArMV) (the genus *Nepovirus)* has a wide host range, including the grapevine (*5*), hop (*4*), strawberry and raspberry. It causes on infected plants severe symptoms like stunting, deformation, leaf mottling and flecking. The virus is transmitted by the nematode *Xiphinema diversicaudatum* (*6*) and by seed transmission, but the most effective means of spread is a vegetatively propagated plant material.

ArMV is commonly detected in the grapevines and hop by an enzyme-linked immunosorbent assay (ELISA), but a successful detection is limited to spring time (*7*). We have attempted to increase the efficiency of detection of ArMV in prebasic propagation material of the grapevine and hop in the Czech Republic using a reverse transcription–polymerase chain reaction (RT-PCR) method instead of a double-antibody sandwich-ELISA (DAS-ELISA).

A prebasic material of grapevine clones from the Research Station of Viticulture Karlštejn, Czech Republic and hop plants from the Hop Research Institute Žatec, Žatec, Czech Republic, was tested by DAS-ELISA (*1*) to find plants infected with ArMV. Polyclonal antibodies to ArMV were purchased from Agritest, Italy and used according to the instructions of the manufacturer. The presence of ArMV in the grapevine was tested using dormant canes and young leaves, while that in hop leaves was tested during different stages of vegetation.

For RT-PCR detection, total nucleic acids were isolated from the grapevine (the leaves during vegetation and the phloem from dormant canes) and hop tissues (leaves) using the method of (*3*), modified for our needs. Plant tissue (0.5 g) was ground in liquid nitrogen and the obtained powder was homogenized in 6 ml of STE buffer (50 mmol/l Tris, 1 mmol/l ethylene diamine tetraacetate (EDTA), and 0.1 mol/l NaCl) containing 1% sodium dodecyl sulfate (SDS) and 1% β-mercaptoethanol. After homogenization, 3 ml of phenol (pH 7.5) and 3 ml of chloroform were added. The mixture was mixed vigorouly in a vortex for 1–2 mins and centrifuged at 10,000 x g for 5 mins. Five ml of the upper phase was mixed with 2.5 ml of ethanol and 0.2 g of cellulose Whatman CF-11 and stirred for 30 mins at room temperature. After centrifugation at 10,000 x g for 5 mins the cellulose pellet was washed with 6 ml of a washing buffer (STE 1x + 30% ethanol). The washing step was repeated three times. Then total nucleic acids were eluted from the cellulose pellet with 1 ml of RNase-free water. After stirring the pellet, the mixture was centrifuged at 10,000 x g for 8 mins. Finally the eluted nucleic acids were ethanol-precipitated overnight at -20°C. The day after the nucleic acids were centrifuged at 14,000 x g for 20 mins and the pellet was washed with 1 ml of 70% ethanol, dried and resuspended in 50 µl of sterile water.

E-mail: kominek@vurv.cz; fax: +4202-33311592

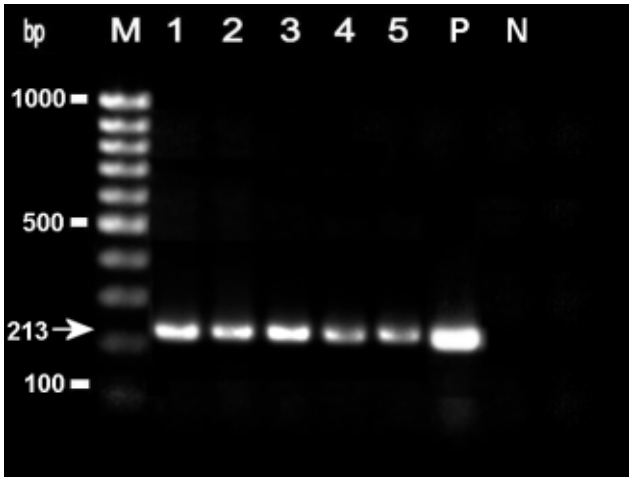
**Abbreviations:** ArMV = Arabis mosaic virus; ELISA = enzyme-linked immunosorbent assay; RT-PCR = reverse transcription–polymerase chain reaction; DAS-ELISA = double-antibody sandwich ELISA; EDTA = ethylenediamine tetraacetate; SDS = sodium dodecyl sulfate; CP = coat protein

Primers for ArMV detection were selected after comparing four sequences available at the NCBI database (acc. Nos. D10086, X55460, X81814, and X81815). The region with high homology was found in the coat protein (CP) gene of ARMV RNA 2; the primers were selected to amplify a 213 bp long fragment of viral genome. The forward primer: 5´-ACC AGT GCC TAC AAG AGT GTG TCC-3´, nt 581-604 in the sequence of Acc. No. X55460. The reverse primer: 5´-TTG ATT CCA GTT GTT AGT GAC CCC-3´, nt 770-793 of Acc. No. X55460.

The SuperScript™ II enzyme (Invitrogen) was used for RT with the reverse primer. The reaction was run for 1.5 hr at 42°C. Taq polymerase (Promega) was used in the PCR with specific primers under following conditions: 94°C for 10 mins (pre-heating), followed by 35 cycles of 94°C/45 secs (template denaturation), 60°C for 45 secs (primer annealing), and 72°C/45 secs (DNA synthesis). The reaction was completed by elongation at 72°C for 10 mins. Amplification products were analyzed by electrophoresis in 1.5% agarose gels.

During four years, 150 individual plants from prebasic propagation material were tested by DAS-ELISA for the presence of ArMV. Ten plants were found to be positive for ArMV, but only when cortical scrapings were tested. During vegetation, the same plants gave negative results when tested from leaves or shoot tops. These plants were tested by RT-PCR, which, in the case of infected plants, yielded a product of expected size of 213 bp. The figure shows agarose gel electrophoresis of RT-PCR products from: grapevines infected with ArMV (lanes 1, 2, 4, and 5), hops infected with ArMV (lane 3), a positive control represented by *Nicotiana benthamiana* infected with ArMV (lane P) and a negative control represented by non-infected grapevine (lane N). Specificity of the PCR product was further ascertained by cloning into pCR4-TOPO (Invitrogen) and nucleotide sequencing. The obtained sequence is available at GenBank under Acc.No. AY376395. ArMV in grapevines was easily detected by RT-PCR not only from cortical scrapings during winter, but also from leaves during vegetation period.

In the Hop Research Institute Žatec ArMV was found during ELISA screening only in one hop plant cv. Early Prolific from collection of hops maintained at the Institute. ArMV was detected by ELISA in young leaves of this plant sampled early at the beginning of vegetation period only. By RT-PCR, ArMV was successfully detected throughout the vegetation period in this hop plant and its clone maintained in a greenhouse.




The main advantage of using cellulose CF-11 for nucleic acids extraction is perfect elimination of various inhibiting plant compounds like polysaccharides and polyphenols, which contaminate RNA extracts prepared by conventional methods (*2*). The described procedure of RT-PCR increased the chance to detect ArMV, a serious pathogen of grapevine and hop in the Czech Republic.

**Acknowledgement.** This work was supported by the grant NAZV I-C-04, QC 0048 from the Ministry of Agriculture, Czech Republic.

## References

*1.* Clark MF, Adams AN, *J. Gen. Virol.* **34**, 1977, 475–483.
*2.* Demeke T, Adams RP, *Biotechniques* **12**, 1992, 332–334.
*3.* Dodds JA, Morris TJ, Jordan RI, *Ann. Rev. Phytopathol.* **22**, 1984, 161–168.
*4.* Eppler A, *Meded Rijksuniv Gent Fak Landbouwkd Toegep Biol Wet*. **66**, 2001, 133–138.
*5.* Gölles R, da Câmara Machado A, Minafra A, Savino V, Saldarelli G, Martelli GP, Pühringer H, Katinger H, Laimer M, *Acta Hort*. **528**, 2000, 305–311.
*6.* Trudgill DL, Brown DJF, McNamara DG, *Revue de Nématologie* **6**, 1983, 133–141.
*7.* Wetzel T, Jardak R, Meunier L, Ghorbel A, Reustle GM, Krczal G, *J. Virol. Methods* **101**, 2002, 63–69.