Detection of *Apple Stem Grooving Virus* in Different Tissues of Apple Trees throughout the Year

Jiban K. KUNDU, Jiří SVOBODA and Jaroslav POLÁK

Department of Virology, Research Institute of Crop Production, Prague-Ruzyně, Czech Republic

Abstract

Kundu J.K., Svoboda J., Polák J. (2003): **Detection of *Apple stem grooving virus* in different tissues of apple trees throughout the year.** Plant Protect. Sci., **39**: 93–96.

The suitability of different apple tissues for *Apple stem grooving virus* (ASGV) detection throughout the year was checked by RT-PCR and ELISA. Detectable amounts of ASGV were generally found in all tissues (bark, dormant buds, petals and leaves) tested by RT-PCR from January to mid-June. Leaves during flowering (in May) were the most suitable tissues for the virus detection by both methods (RT-PCR and ELISA). The leaves collected in summer (June, July and August) or other tissues such as bark, dormant buds and petals were not reliable for ASGV detection by ELISA.

Keywords: *Apple stem grooving virus*; tissue sampling; RT-PCR; ELISA; reliability of detection

Apple stem grooving virus (ASGV), the type member of the genus *Capillovirus* (Yoshikawa *et al.* 1992) is the causal agent of decline and graft union necrosis diseases in apple cv. Virginia crab (Desvignes *et al.* 1990; Yanase *et al.* 1990). ASGV infection is frequently symptomless in apple cultivars (Németh 1986). The mixed infections with other pome fruit viruses are frequent in apple orchards and cause significant yield reduction (Campbell 1963). The virus is well distributed in the Czech apple orchards (Polák & Zieglerová 2001). Recently, 44% of the apple trees were recorded ASGV-infected in Czech and Moravian orchards (Kundu 2003).

The laboratory assays, including ELISA and RT-PCR, are widely used for the detection of ASGV (Fuchs 1981; MacKenzie *et al.* 1997; James 1999). However, little is known, whether the above detection assays are reliable using different tissues and during any time of the year. Usually, woody plant contain high amounts of components such as polyphenols and polysaccharides (Mitra & Kootstra 1993) which are believed to interfere with the sensitivity of the detection assays. It is likely that these amounts differ in the individual tissues in different periods of the year (Fuchs 1982; Stewart & Nassuth 2001).

This paper reports on the detection of ASGV by RT-PCR and ELISA using different apple tissues sampled during different periods of the year.

MATERIALS AND METHODS

Virus isolate and sampling. An apple isolate of ASGV (University of Halle, Wittenberg, Germany) maintained in *Chenopodium quinoa* was used as the positive control.

Four cultivars of field-grown *Malus domestica* Borkh. including Idared, Spartan, Vista Bella, and Stark Earliest without any artificial infection were selected for the test. Ten trees were tested per each cultivar. The sampling was done for each tree during the years 2001 and 2002. Bark, dormant buds, petals, and leaves from April to August were tested (Table 1).

Detection. Reverse transcription polymerase chain reaction (RT-PCR) (in 2002) and Double an-

Supported by the Ministry of Agriculture of the Czech Republic, Grant No. QD 1359.

tibody sandwich-enzyme linked immuno-sorbent assay (DAS-ELISA) (in 2001 and 2002) were used for the detection of ASGV.

Reverse transcription polymerase chain reaction (RT-PCR)

Extraction of total RNA. The extraction of total RNA from the tested apple tissues (*Malus domestica* Borkh.) or *C. quinoa* was performed by phenol-chloroform-isoamyl alcohol clarification and ethanol precipitation procedure as described by Kundu (2002, 2003).

Synthesis of cDNA, cDNA amplification and analysis of amplified RT-PCR products. Synthesis of cDNA and its amplification were performed by two-step RT-PCR as described by Kundu (2002). The primer pair ASGV-U & ASGV-2 (James 1999), specific for ASGV, was used. Aliquots of the PCR products were analysed by electrophoresis on agarose gel (1.5%) in TBE buffer (90mM Tris-borate, 2mM EDTA). The amplified DNA fragments were stained with ethidum bromide (0.5 μg/ml) and visualised under a UV transilluminator.

Double antibody sandwich-enzyme linked immunosorbent assay (DAS-ELISA). DAS-ELISA was performed according to Cocktail ELISA procedure described by Flegg and Clark (1979). ASGV-specific polyclonal (antibody and antibody conjugate) ELISA kit (Loewe Phytodignostica) was used for the test. The microtiter plates were coated for 4 h at 37°C with 200 μl per well of IgG diluted 1:200

Table 1. Comparison of ASGV detection in different apple tissues by DAS-ELISA and RT-PCR

Apple cultivars	Testing		ASGV positive plants/tested plants		
	tissue	period	ELISA ^{2001}	ELISA ^{2002}	RT-PCR^{2002}
Idared	bark	January	–	0/10	3/10
	dormant buds	January	5/10	4/10	6/10
	leaves	April	4/10	–	–
	leaves	May	–	5/10	6/10
	petals	May	–	0/10	6/10
	leaves	June	0/10	3/10	6/10
	leaves	August	0/10	–	–
Spartan	bark	January	–	0/10	8/10
	dormant buds	January	9/10	5/10	9/10
	leaves	April	9/10	–	–
	leaves	May	–	9/10	9/10
	petals	May	–	4/10	9/10
	leaves	June	0/10	4/10	8/10
	leaves	August	0/10	–	–
Stark Earliest	bark	January	–	3/10	5/10
	dormant buds	January	4/10	8/10	9/10
	leaves	April	6/10	–	–
	leaves	May	–	9/10	10/10
	petals	May	–	0/10	10/10
	leaves	June	0/10	5/10	10/10
	leaves	August	0/10	–	–
Vista Bella	bark	January	–	0/10	1/10
	dormant buds	January	1/10	0/10	2/10
	leaves	April	1/10	–	–
	leaves	May	–	3/10	2/10
	petals	May	–	0/10	2/10
	leaves	June	0/10	0/10	2/10
	leaves	August	0/10	–	–

– not tested

in 50mM carbonate buffer (pH 9.6). The plates were then washed three times with phosphate-buffer saline-Tween (PBS-T: 10mM phosphate buffer, pH 7.2, containing 0.8% NaCl, 0.02% KCl and 0.05% Tween-20). Samples (0.2 g tissue) were homogenised in 4 ml of extraction buffer (PBS-T containing 2 % polyvinylpyrolidone 40K and 0.2% egg albumin) and 100 µl of supernatant was added to each well. Immediately thereafter, 100 µl of antibody-alkaline-phospatase-conjugate (diluted 1: 200 in extraction buffer) was added to each well, mixed gently and incubated overnight at 4°C. The plates were washed again 5 times with PBS-T and an aliquot of 200 µl of *p*-nitrophenylphosphate (1 mg/ml) in 10% diethanolamine (pH 9.8) was then added to each well and the plates were conserved at room-temperature for 1–2 h in a dark place. The absorbance was read at 405 nm in a MR 5000 reader (Dynatech).

RESULTS AND DISCUSSION

The results are summarised in Table 1. ASGV was detected by ELISA in 2001 only in dormant buds (January) and leaves (April). The leaves collected in June and August were all negative in ELISA, where the virus concentration was low. During 2002 the same samples were tested by ELISA and RT-PCR. The virus was undetectable by ELISA in bark samples in all cultivars except cv. Stark Earliest. Although some trees were positive in dormant buds, petals and leaves in June, the highest number of ASGV-infected trees was recorded with leaves collected in May. Our ELISA results correspond with the previous findings of Fuchs (1982) about the higher detection efficiency from April to the mid-May. From the end of May the leaf tissue was no more reliable for ASGV detection by ELISA. The apple leaves were a more suitable tissue than petals, using RT-PCR detectable amounts of ASGV were generally found in all tissues tested from January (bark and dormant buds) to mid-June (leaves). The leaf material in general proved to be the most suitable tissue for RNA isolation and giving the highest detection efficiency in comparison with bark or petals by RT-PCR. Previously, petals were reported as the most effective tissue for *Plum pox virus* detection in peach (Polák 1995) which was not proved in ASGV detection by both assays, RT-PCR and ELISA. Moreover, in most cases (cvs. Idared and Stark Earliest) the virus failed in ELISA-detection in petals.

In comparison with ELISA, RT-PCR appeared more effective and reliable for ASGV detection and the assay can be used for apple tissue testing throughout the year. However, ELISA is more accessible for routine detection of this virus and leaf tissues in spring are recommended. In general, the leaf tissues are suggested for the effective ASGV detection by both methods (RT-PCR and ELISA).

Acknowledgments: We are grateful to Mgr. Katarina Čiháková for her excellent technical assistance and Dr. Arben Myrta for critical reading of the manuscript.

References

Campbell A.I. (1963): The effect of some latent virus infections on the growth and cropping of apples. J. Hort. Sci., **38**: 15–19.

Desvignes C.J., Boyé R., Cornaggia D., Grasseau N. (1990): Maladies a virus des arbres fruitiers. Ctifl, Paris: 127.

Flegg C.L., Clark M.F. (1979): The detection of apple chlorotic leaf spot virus by a modified procedure enzyme-linked immunosorbent assay (ELISA). Ann. Appl. Biol., **91**: 61–65.

Fuchs E. (1981): Serological detection of *Apple chlorotic leaf spot virus* (ACLSV) and *Apple stem grooving virus* (ASGV) in apple trees. Acta Hort., **94**: 69–73.

Fuchs E. (1982): Studies of the development of concentration of Apple chlorotic leaf spot virus (CLSV) and Apple stem grooving virus (SGV) in apple trees. Acta Phytopath. Acad. Sci. Hung., **17**: 23–27.

James D. (1999): A simple and reliable protocol for the detection of *Apple stem grooving virus* by RT-PCR and in a multiplex PCR assay. J. Virol. Methods, **83**: 1–9.

Kundu J.K. (2002): The application of RT-PCR assay for the detection of *Apple stem pitting virus* and *Apple stem grooving virus* in four apple cultivars. Plant Protect. Sci., **38**:13–17.

Kundu J.K. (2003): The occurrence of *Apple stem pitting virus* and *Apple stem grooving virus* within field-grown apple cultivars evaluated by RT-PCR. Plant Protect. Sci., **39**: 88–92.

MacKenzie D.J., McLean M.A., Mukerji S., Green M. (1997): Improved RNA extraction from woody plants for the detection of viral pathogens by reverse transcription-polymerase chain reaction. Plant Dis., **81**: 222–226.

Mitra D., Kootstra A. (1993): Isolation of RNA from apple skin. Plant Mol. Biol. Reptr., **11**: 326–332.

Németh M. (1986): Virus, Mycoplasma and Rickettsia Diseases of Fruit Trees. Akademiai Kiado, Budapest.

Polák J. (1995): Reliability of detection and relative concentration of plum pox virus determined by ELISA in an infected peach tree during the vegetation period. J Plant Dis. Protect., **102** :16–22.

Polák J., Zieglerová J. (2001): Distribution of *Apple stem grooving virus* in apple trees in the Czech Republic. Plant Protect. Sci., **37**: 1–4.

Stewart S., Nassuth A. (2001): RT-PCR based detection of *Rupestris stem pitting associated virus* within field-grown grapevines throughout the year. Plant Dis., **85**: 617–620.

Yanase H., Mink G.I., Sawamura K., Yamaguchi A. (1990): Apple topworking disease. In: Jones A.L., Aldwinckle H.S. (eds): Compendium of Apple and Pear Diseases. APS, St. Paul, MN: 74–75.

Yoshikawa N., Sasaki E., Kato M., Takahashi T. (1992): The nucleotide sequence of *apple stem grooving capillovirus* genome. Virology, **191**: 98–105.

Received for publication May 6, 2003
Accepted after corrections July 31, 2003

Souhrn

Kundu J.K., Svoboda J., Polák J. (2003): **Detekce *Apple stem grooving virus* v rozdílných pletivech jabloně během vegetace.** Plant Protect. Sci., **39**: 93–96.

Pro zjištění vhodnosti různých pletiv jabloní pro detekci *Apple stem grooving virus* (ASGV) byly použity metody RT-PCR a ELISA. ASGV byl pomocí RT-PCR detekován u všech testovaných pletiv (kůra, dormantní pupeny, okvětní lístky a listy) v období od ledna do června. Listy během kvetení (v květnu) se ukázaly jako nejspolehlivější pletiva pro detekci ASGV pomocí obou metod (RT-PCR a ELISA). Detekce ASGV metodou ELISA nebyla spolehlivá u listů odebraných v létě (v červnu až srpnu) a rovněž u jiných pletiv jako je kůra, dormantní pupeny a korunní plátky.

Klíčová slova: *Apple stem grooving virus*; odběr vzorků pletiv; RT-PCR; ELISA; spolehlivost detekce

Corresponding author:

Dr. Jiban K. Kundu, Výzkumný ústav rostlinné výroby, odbor rostlinolékařství, 161 06 Praha 6-Ruzyně, Česká republika
tel.: + 420 233 022 212, fax: + 420 233 311 592, e-mail: jiban@vurv.cz