BIOLOGICAL CHARACTERIZATION AND VARIABILITY IN THE COAT PROTEIN GENE OF AN ISOLATE OF *APRICOT LATENT VIRUS*

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SUMMARY

Virus-free accessions of peach, apricot, cherry and plum were graft-inoculated in 2004 with buds from a peach tree cv. Missour infected by *Apricot latent virus* (ApLV), then analysed by RT-PCR using a specific primer set. The expected DNA fragment of 200 bp in size was amplified from 33 different *Prunus* sources, thus extending the woody host range of ApLV to new cultivars of *Prunus persica*, *P. avium*, *P. armeniaca* and, for the first time, to European (*P. domestica*) and Japanese (*P. salicina*) plum cultivars. Several peach cultivars and apricot cv. Tirynthos were symptomatic. To determine the best source material for ApLV detection, RT-PCR assays were carried out during the growing season. ApLV was homogeneously distributed in flowers, leaves, petioles, barks and fruits of peach and apricot but was detected only in the leaves of cherry and plum. The genetic variability of the ApLV coat protein gene from graft-inoculated hosts and comparable sequences from databases ranged between 63.5 and 100%.

Key words: ApLV, stone fruit, symptoms, RT-PCR, sequence analysis.

INTRODUCTION

Apricot latent virus (ApLV) was first detected in Moldova in symptomless apricot cv. Silistra introduced from Bulgaria in 1993 (Zemtchik and Verderevskaya, 1993) and, following partial sequence of its genome (Nemchinov *et al.,* 2000), was assigned as definitive species to the genus *Foveavirus* (Martelli and Jelkmann, 1998). New ApLV records came later from France and Italy (Gentit *et al.,* 2001a), Turkey (Gumus *et al*., 2004), Iran (Sanchez-Navarro *et al.*, 2005), Palestine (Abou Ghanem-Sabanadzovic *et al*., 2005), Egypt (El Maghraby *et al*., 2006) and Lebanon (Jarrar *et al.*, 2007).

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ApLV induces yellow asteroid or sooty ringspots on the leaves of experimentally graft-inoculated peach seedlings, the most susceptible *Prunus* species (Zemtchik and Verderevskaya, 1993; Zemtchik *et al*., 1998; Grasseau *et al*., 1999; Nemchinov *et al*., 2000; Gentit *et al*., 2001b; Myrta *et al*., 2008). Occasional chlorotic spots, were also observed on the leaves of graft-inoculated *P. cerasifera* (Nemchinov and Hadidi, 1998), and red to purple rings and mottling on the leaves of *P. avium* (Abou Ghanem-Sabanadzovic *et al*., 2005). ApLV infects apricot trees without eliciting visible symptoms, with the exception of cvs Tirynthos and Haward, in which it induces chlorotic blotching and malformation of the leaves (Jarrar *et al.*, 2006).

Since information on the biological properties of ApLV is scanty, a study was conducted for identifying new potential woody hosts through indexing, and determining their symptomatological reactions. The variability of the ApLV coat protein (CP) gene from graft-inoculated hosts and corresponding sequences from databases was also investigated.

MATERIALS AND METHODS

Woody host range. Peach, apricot, cherry and plum accessions apparently free from *Apple chlorotic leaf spot virus* (ACSLV), *Apple mosaic virus* (ApMV), *Prune dwarf virus* (PDV), *Prunus necrotic ring spot virus* (PNRSV) and *Plum pox virus* (PPV) as determined by DAS and TAS-ELISA, were graft-inoculated in 2004 with buds from a peach tree cv. Missour infected by an ApLV isolate from Palestine (Apr-47, also referred to as 04PAL). Three inoculated and one healthy plant of each accession were planted in the experimental farm of the University of Bari at Valenzano (BA), Italy.

The appearance of symptoms was checked every month during the growing seasons of 2007 and 2008 and evaluated using an empirical scale ranging from: 0, no symptoms; 1, symptoms on one or two branches; 2, symptoms on more than two branches; 3, symptoms on the whole tree.

RT-PCR. Total RNAs were extracted from 100 mg

leaf tissues of all varieties using the silica-capture method (Rott and Jelkmann, 2001). RT-PCR tests were done using primers H-ALV1/C-ALV1 (Nemchinov and Hadidi, 1998) that amplify a fragment of the virus coat protein (CP) of 200 bp. Cycling parametrs were: denaturation at 94ºC for 30 sec, annealing at 58ºC for 30 sec, and extension at 72ºC for 45 sec, totalling 35 cycles, with a final extension at 72ºC for 7 min. Three randomly selected cultivars of each species were tested monthly for ApLV presence from April to October 2008, using leaf tissues, petioles, whole flowers, bark and fruits.

Sequence variability and phylogenetic analysis. The partial ApLV CP gene fragment was amplified from: (i) all hosts (peach, cherry, plum, apricot) that were graft-inoculated with the ApLV isolate from Palestine (Apr-47 or 04PAL); (ii) GF305 infected with the original virus source from Moldova; (iii) a peach isolate from Turkey.

PCR amplicons were directly sequenced in both orientations (Primm, Italy), sequence data were assembled with FinchTV 1.4 (Geospiza Inc., USA) and compared with database sequences using BLAST on the NCBI Web server. ORF analysis and translation of nucleotide sequences were done with the BioEdit 7.0.9 program (Ibis Biosciences, USA). Multiple aligments of partial CP nucleotide and amino acid sequences [from position 174 to 867, nt accession No. AF057035; (Nemchinov *et al*., 2000)] were obtained using the BioEdit 7.0.9 program. The data set was subjected to bootstrap by Neighbor-joining analysis with 1000 replicates (Saitou *et al*., 1987).

The following CP sequences retrieved from databases were included in the analysis: *Apple stem pitting virus* (ASPV, EU314950; Dong *et al*., 2007), *Peach asteroid spot virus* (01PASV, AF318061; Gentit *et al*., 2001), *Peach sooty ringspot virus* (01PSRSV, AF318062; Gentit *et al*., 2001), and the ApLV sequences AY697862 (04PAL; Abou Ghanem-Sabanadzovic *et al*., 2005) and AF057035 (00MOL; Nemchinov *et al*., 2000).

RESULTS

Woody host range. A 200 bp ApLV-specific amplicon was obtained from all 33 graft-inoculated *Prunus* species and cultivars (Table 1). This widens the ApLV woody host range to new cultivars of *P. persica* (Spring Gold, Baracca, Baby Gold 7, Baby Gold 9, Baby Gold 6, Anderson, Vivian-ppc-199, Armking, Nectared, O´Henry), *P. avium* (Fuciletta primizia, Adriana, Roma, Napolitana, Lapins, Moreau), *P. armeniaca* (Bulida, Orangered, Harcot) and, for the first time, to Japanese and European plum cultivars (Autumn Giant, Ozark Premium, Regina, St. Angeleno, Blue Free, Sangue di Drago, Friar, Burbank, Black Star, Stanley, President).

Symptoms associated with ApLV. All grafted cultivars of *P. persica* (Table 1) showed cholorotic spots on the leaves (Fig. 1 A, B), but not on flowers and fruits. Symptoms appeared at the end of May, their intensity reached a peak in June and July to decrease slowly in the following months (Table 2). The cvs Spring Gold, Missour and Nectared had the most severe symptoms, whereas all cherry and plum cultivars were symptomless, even though they gave positive RT-PCR reactions.

As to apricot, Tirynthos was the only symptomatic cultivar with chlorotic blotching of the leaves (Fig. 1, C) at the beginning of June, that became stronger by the end of the month through July and began to fade thereafter (Table 2). Flowers and fruit were symptomless.

RT-PCR detection. As shown in Fig. 2, ApLV was readily detected from April to October in peach and in

Table 1. Varieties and/or selections of stone fruit trees and sequences obtained in this study after grafting with an ApLV isolate from Palestine (Apr-47, also referred to as 04PAL).

P. persica		*P. avium*		*P. armeniaca*		*P. domestica, P. salicina*	
Cultivar	Sequence code	Cultivar	Sequence code	Cultivar	Sequence code	Cultivar	Sequence code
Spring Gold	09PALpe2	Fuciletta primizia		Orangered		Friar	
Baracca		Moreau		Harcot		Ozark Premier	09PALpl2
Baby Gold 7		Roma		Bulida		Burbank	
Baby Gold 9		Adriana	09PALche1	Tirynthos	09PALap1	Black Star	
Baby Gold 6		Lapins		Haward	09PALap2	Autumn Giant	09PALpl1
Anderson		Napolitana				St. Angeleno	
Vivian -ppc-199						Regina d´Italia	
Missour	09PALpe1					Blue Free	
Armking						Stanley	
Nectared						President	
O´Henry						Sangue di Drago	

Fig. 1. Symptoms caused by ApLV on chip budding-infected leaves of some hosts: A, B peach cv.Anderson, C apricot cv. Tirynthos.

May to October in apricot. Virus detection in cherry and plum was less clear-cut because of differences between individual cultivars in the PCR signal, in terms of amplicon amount. In any case, the best period for virus detection in plum and cherry was July and September. ApLV was homogeneously distributed in flowers, leaves, petioles, barks and fruits of peach and apricot but was detected only in the leaves of cherry and plum (data not shown).

Sequence variability and phylogenetic analysis. Sequences of nine CP amplicons obtained from PCR analysis of graft-inoculated accessions (sequence codes in Table 1), Moldavian and Turkish isolates (sequence codes 09MOL and 09TUR, respectively) were deposited in the EBI website with the accession numbers from FN252232 to FN252240. Direct comparison of nucleotide and deduced amino acid sequences with those from GenBank showed identity ranging from as low as 63.5% (09PALpe2 vs. 01PSRSV) at the nucleotide level to up to 100% at the amino acid level for a subset of sequences obtained from different hosts after 04PAL grafting.

Nucleotide sequence from 09PALpe2 showed a somehow different behaviour if compared with the other sequences derived from the original graft-inoculation by 04PAL on the different species. While these sequences had a 78-89% nucleotide identity with 04PAL, 09PALpe2 ranged around 65% and had an unexpected increase up to 69.5% with the isolates from Moldova and Turkey.

Phylogenetic relationships established with the corresponding partial CP gene region of different ApLV isolates and ASPV (Fig. 3) clearly showed a coherent PAL-

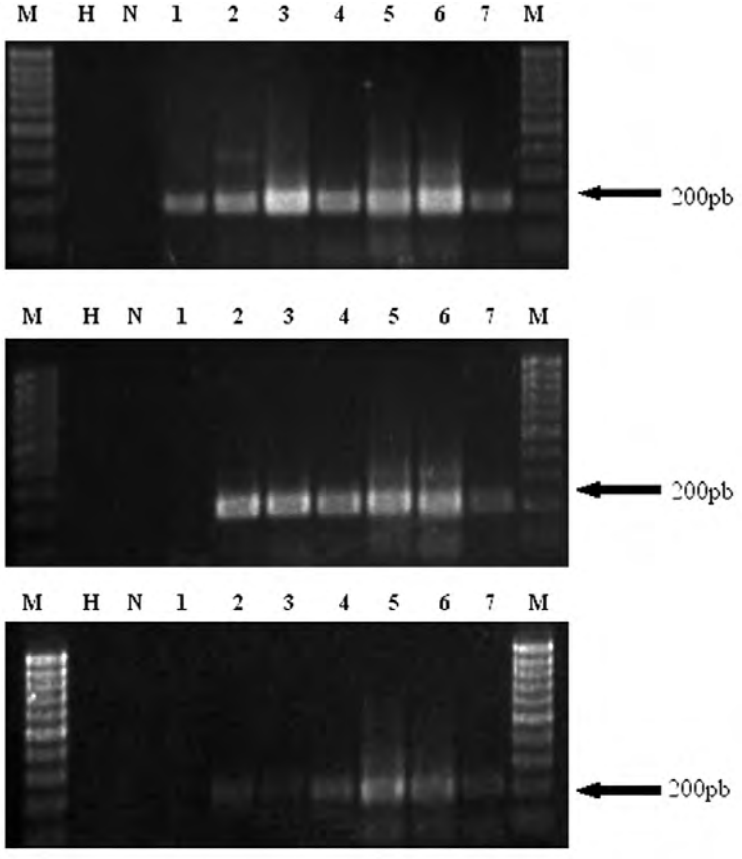

Fig. 2. ApLV detection by RT-PCR in different stone fruit species through the growing season. A: peach cv Spring Gold; B: apricot cv Tirynthos; C: cherry cv Adriana; D: plum cv Autumn Giant. Lane M: DNA marker (Mass ruler low range, Fermentas); H: water control; N: healthy control; 1, April; 2 , May; 3, June; 4, July; 5, August; 6, September; 7, October. Agarose gels stained by ethidium bromide.

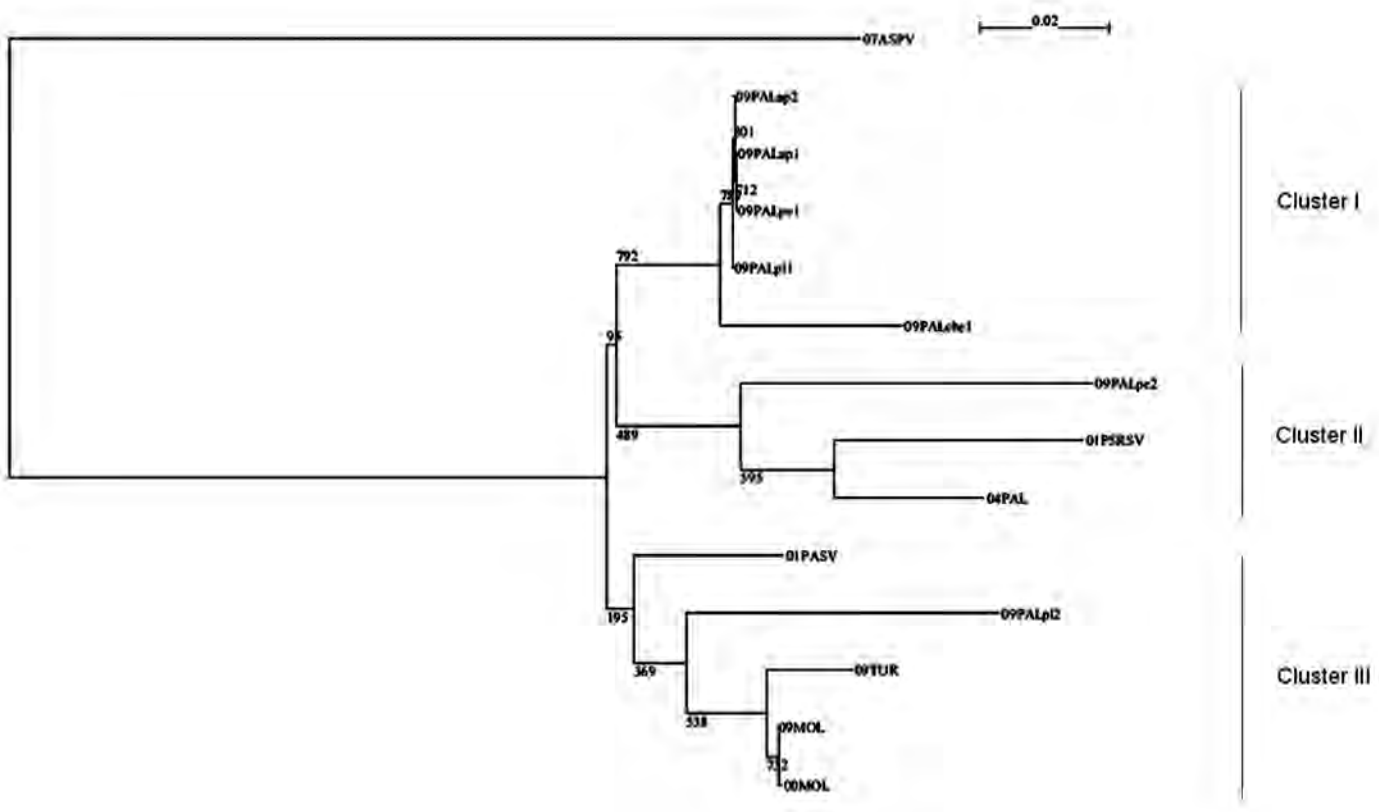

Fig. 3. Phylogram constructed with the sequence of part of the viral CP of different ApLV isolates and sequences obtained after 04PAL graft-inoculation to different hosts. Isolate 07ASPV of *Apple stem pitting virus* is the outgroup reference.

derived branch (cluster I) compared to the 04PAL sequence (Abou Ghanem-Sabanadzovic *et al*., 2005) as well as 09PALpe2 (from Spring Gold peach; cluster II) and 09PALpl2 (Ozark premium plum; cluster III).

The nucleotide distance among clusters was not higher than the average distance between isolates (not shown) and this was further confirmed by the comparison with the outgroup ASPV.

Table 2. Symptom response on graft-inoculated peach cultivars and apricot cv. Tirynthos (verified during two growing seasons) as assessed by the arbitrary scale: 0, no symptoms; 1, symptoms on one or two branches; 2, symptoms on more than two branches; 3, symptoms on the whole tree.

Peach cultivars	April	May	June	July	August	September	October
Armking	0	1	3	3	2	2	1
Missour	0	1	3	3	2	2	1
Spring Gold	0	1	3	3	2	2	1
Anderson	0	1	2	2	1	0	0
Baracca	0	1	2	2	1	0	0
Vivian-ppc-199	0	1	2	2	1	0	0
Baby Gold 7	0	1	2	1	1	0	0
Baby Gold 9	0	1	2	1	1	0	0
Baby Gold 6	0	1	2	1	0	0	0
Nectared	0	1	1	1	0	0	0
O´Henry	0	0	1	1	0	0	0
Apricot cultivar	April	May	June	July	August	September	October
Tirynthos	0	1	2	2	1	0	0

DISCUSSION

Grafting experiments showed that ApLV can infect a wide range of *Prunus* species although only peach cultivar and apricot cv. Tirynthos showed symptoms. Thus, in the absence of a natural vector, infected but symptomless plum, apricot and cherry cultivars constitute a virus reservoir for unwanted ApLV spread through nursery productions. This likelihood should be taken into serious consideration when designing and implementing stone fruit certification schemes so as to restrain, or better, rule out potential contamination of the stocks with this virus.

A phylogenetic analysis (Fig. 3) showed grouping of five out of seven sequences (09PALap2, 09PALap1, 09PALpl1 and 09PALpe1) separately from 09PALche1 (cluster I). The two remaining sequences and the original 04PAL used for inoculation branched with sequences of isolates from Turkey and Moldova (cluster II and III). It is known that the N-terminus of the CP gene is highly variable among foveaviruses, while the C-terminus is strongly conserved (Gentit *et al*., 2001a; Martelli and Jelkmann, 1998). The primer set used in the present work was designed in the N-terminal portion of the CP gene and was able to specifically recognize ApLV. The position of 04PAL and 09PALpe2, which are closer to PSRSV, and the position of 09PALpl2, which is more related to PASV, may indicate the existence of a wide complex of molecular variants originally present in the inoculum and revealed after analysis of graft-inoculated tissue. PASV and PSRSV isolates were detemined to be variants of ApLV (Gentit *et al.*, 2001a, 2001b) and the differentiation in terms of phylogenetic distance from the PAL-derived sequences did not exceed the level of their diversity. Similar patterns of variants, which may also show different biological behaviours, have been reported for other foveaviruses. i.e. ASPV (Jelkmann, 1994; Schwarz and Jelkmann, 1998; Yoshikawa *et al*., 2001) and GRSPaV (Nolasco *et al*., 2005; Meng *et al*., 2006). To investigate whether this situation stems from a host-driven differential selection or enrichment of variants, further work is needed.

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REFERENCES

Abou Ghanem-Sabanadzovic N., Abbadi H., Al Rwahnih A., Castellano M.A., Myrta A., 2005. Identification and partial characterization of an isolate of *Apricot latent virus* from Palestine. *Journal of Plant Pathology* **87**: 33-37.

El Maghraby I., Sanchez-Navarro J., Matic S., Myrta A., Pallas V., 2006. First report of two filamentous particles from stone fruit trees in Egypt. *Journal of Plant Pathology* **88**: S69.

Gentit P., Foissac X., Svanella-Dumas L., Peypelut M., Candresse T., 2001a. Characterization of two different *Apricot latent virus* variants associated with peach asteroid spot and peach sooty ringspot diseases. *Archives of Virology* **146**: 1453-1464.

Gentit P., Foissac X., Svanella-Dumas L., Peypelut M., Candresse T., 2001b. Variants of *Apricot latent foveavirus* (ApLV) isolated from south European orchards associated with peach asteroid spot and peach sooty ringspot diseases. *Acta Horticulturae* **550**: 213-219.

Grasseau N., Macquaire G., Boye R., Cornaggia D., Desvignes J.C., 1999. Peach red marbling and peach sooty ringspot, two new virus-like degenerative diseases of *Prunus*. *Plant Pathology* **48**: 395-401.

Gumus M., Al-Rwahnih M., Myrta A., 2004. First report of *Apricot latent virus* in Turkey. *Journal of Plant Pathology* **86**: 92.

Jarrar S., Boscia D., Myrta A., Minafra A., Lonconsole G., Savino V., 2006. Studies on the biological and molecular diagnosis of *Apricot latent virus*. *Journal of Plant Patology* **88**: S46.

Jarrar S., Choueiri E., Sanchez-Navarro J.A., Myrta A., El Zammar S., Savino V., Pallas V., 2007. First report of *Apricot latent virus* in Lebanon. *Journal of Plant Pathology* **89**: 303.

Jelkmann W., 1994. Nucleotide sequences of *Apple stem pitting virus* and of the coat protein gene of similar virus from pear associated with vein yellows disease and their relationship with potex- and carlaviruses. *Journal of General Virology* **75**: 1535-1542.

Martelli G.P., Jelkmann W., 1998. *Foveavirus*, a new plant virus genus. *Archives of Virology* **143**: 1245-1249.

Meng B., Li C., Wang W., Goszczynski D., Gonsalves D., 2005. The complete genome sequences of two new variants of *Grapevine rupestris stem pitting-associated virus* and comparative analyses. *Journal of General Virology* **86**: 1555-1560.

Myrta A., Minafra A., Castellano M.A., 2008. *Apricot Latent Virus* and Peach Asteroid Spot. In: Rao G.P., Myrta A., Ling K.S. (eds.). Disease, Characterization, Diagnosis and Management of Plant Viruses. Horticultural Crops Vol. 2, pp. 249-258. Studium Press, USA.

Nemchinov L., Hadidi A., 1998. *Apricot latent virus*: a novel stone fruit pathogen and its relationship to *Apple stem pitting virus*. *Acta Horticulturae* **472**: 159-173.

Nemchinov L.G., Shamloul A.M., Zemtchik E.Z., Verderevskaya T.D., Hadidi A., 2000. *Apricot latent virus*: a new species in the genus *Foveavirus*. *Archives of Virology* **145**: 1801-1813.

Nolasco G., Santos C., Petrovic N., Teixeira Santos M., Cortez I., Fonseca F., Boben J., Nazare Pereira A.M., Sequeria O., 2006. *Rupestris stem pitting associated virus* (RSPaV) isolates

are composed by mixtures of genomic variants which share a highly conserved coat protein. *Archives of Virology* **15**: 83-96.

Rott M.E., Jelkmann W., 2001. Characterization and detection of several viruses of cherry: adaptation of an alternative cloning method (DOP-PCR) and modification of RNA extraction protocol. *European Journal of Plant Patology* **107**: 411-420.

Saitou N., Nei M., 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Molecular Biology and Evolution* **4**: 406-425.

Sanchez-Navarro J.A., Aparicio F., Herranz M.C., Minafra A., Myrta A., Pallas V., 2005. Simultaneous detection and identification of eight stone fruit viruses by one step RT-PCR. *European Journal of Plant Pathology* **111**: 77-84.

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Schwarz K. and Jelkmann W., 1998. Detection and characterization of European *Apple Stem pitting virus* sources from apple and pear by PCR and partial sequence analysis. *Acta Horticulturae* **472**: 75-86.

Yoshikawa N., Matsuda H., Oda Y., Isogai M., Takahashi T., Ito T., Yoshida K., 2001. Genome heterogenity of *Apple stem pitting virus* in apple trees. *Acta Horticulturae* **550**: 285-290.

Zemtchik E.Z., Verderevskaya T.D., 1993. Latent virus on apricot unknown under Moldavian conditions. *Selskohozeaystvennaya Biologia (Russian Agricultural Biology)* **3**: 130-133.

Zemtchik E.Z., Verderevskaya T.D., Kalashian Y.A., 1998. *Apricot latent virus*: transmission, purification and serology. *Acta Horticulturae* **472**: 153-158.