Turk J Agric For
29 (2005) 251-257

Apple Chlorotic Leaf Spot Virus (ACLSV) Status in Turkey and Sensitive Detection Using Advanced Techniques

Çiğdem ULUBAŞ*
Mustafa Kemal University, Plant Protection Department 31034 Antakya, Hatay - TURKEY
Filiz ERTUNÇ
Ankara University, Plant Protection Department 06100 Dışkapı, Ankara - TURKEY

Received: 14.09.2004

Abstract: The prevalence of apple chlorotic leaf spot virus (ACLSV) was investigated in all the main stone fruit growing areas of Turkey except for East Anatolia. Three hundred and sixty-nine plant samples showing virus related symptoms were collected from sweet and sour cherry, peach, nectarine, apricot and plum trees. All samples were tested using DAS-ELISA and reverse transcription-polymerase chain reaction (RT-PCR). Thirteen samples were detected as infected with ACLSV using ELISA while RT-PCR revealed 51 positive samples. The highest ACLSV incidence was in Bursa (64%), followed by Yalova (20.51%), Burdur (18.18%), Amasya (13.43%), Afyon (12.07%), Isparta (6.25%), Çanakkale (4.76%) and İzmir (2.72%). Among the collected stone fruit species, nectarine was the most infected species, followed by apricot, peach, sour cherry, plum and sweet cherry. Some ACLSV isolates were used for the adoption of an immuno capture (IC) RT-PCR system. ACLSV infected plant sap diluted up to 1:10 was detected by IC-RT-PCR. The successful application of RT-PCR and IC-RT-PCR for the accurate and sensitive detection of ACLSV was reported in stone fruit trees in Turkey.

Key Words: Apple chlorotic leaf spot virus, Reverse transcription-polymerase chain reaction, Turkey

Elma Klorotik Leke Virüsü (ACLSV)'nün Türkiye'deki Durumu ve İleri Tekniklerle Hassas Teşhisi

Özet: Sert çekirdekli meyve yetiştiriciliğinin Türkiye'de, Doğu Anadolu Bölgesi dışında, yoğun olduğu bölgelerde Elma klorotik yaprak lekesi virüsü (ACLSV)'nün yaygınlığı araştırılmıştır. Toplanan tüm virüs simptomu gösteren kiraz, şeftali, nektarin, kayısı ve erik örnekleri DAS-ELISA ve revers transkripsiyon polimeraz zincir reaksiyonu (RT-PCR) tekniği ile test edilmiştir. 369 bitki örneğinin arasından 13 ağacın ACLSV ile enfekteli olduğu ELISA ile tespit edilmiştir. Diğer taraftan, RT-PCR 51 pozitif ortaya koymuştur. En yüksek ACLSV infeksiyon oranı Bursa'dan temin edilen örneklerde (% 64) tespit edilmiş, bunu Yalova (% 20.51), Burdur (% 18.18), Amasya (% 13.43), Afyon (% 12.07), Isparta (% 6.25), Çanakkale (% 4.76) ve İzmir (% 2.72)'den toplanan örnekler izlemiştir. Araştırmada kullanılan sert çekirdekli meyve türleri arasında, nektarin en çok enfektelenen tür olarak tespit edilmiştir, bunu kayısı, şeftali, vişne, erik ve kiraz takip etmiştir. Bazı ACLSV izolatları immun olarak yakalanmış (IC) RT-PCR sisteminin uygulanmasında kullanılmıştır. Bitki ekstraktının 1:10 oranına kadar sulandırılmasında IC-RT-PCR ile teşhis yapılmıştır. Bu çalışmada, ülkemizdeki sert çekirdekli meyve ağaçlarındaki ACLSV'nün doğru ve hızlı teşhisinde RT-PCR ve IC-RT-PCR'ın başarılı bir şekilde uygulaması ortaya konmuştur.

Anahtar Sözcükler: Apple chlorotic leaf spot virus, Revers transkripsiyon-polimeraz zincir reaksiyonu, Türkiye

Introduction

Apple chlorotic leaf spot virus (ACLSV) is the type member of family *Flexiviridae* of the genus *Trichovirus* (Martelli et al., 1994) and is known to infect most fruit tree species including apple, pear, peach, plum, cherry and apricot (Nemeth, 1986; Desvignes and Boye, 1989). The economic importance of ACLSV is largely due to its worldwide distribution and its capacity to induce severe graft incompatibilities in some *Prunus* combinations, causing major problems in nurseries. Although most strains are latent in fruit trees, others can be responsible for russetting, topworking disease and lethal decline of

* Corresponding author: culubas@mku.edu.tr

apple on some rootstock varieties (Desvignes and Boye, 1989). The virus causes serious disease in stone fruits including plum bark split, plum pseudopox or false plum pox, peach dark green sunken mottle, severe leaf and fruit deformation known as butteratura in peach and viruela in apricot, and graft incompatibility in some apricot combinations (Nemeth, 1986; Desvignes and Boye, 1989; Sutic et al., 1999). Although a natural spread in the field has been detected, the natural mode of spread is unknown (Brunt et al., 1996).

The virions of ACLSV are usually flexuous and have filamentous particles of 720 x 12 nm (Brunt et al., 1996). The ACLSV genome consists of a single strand polyadenylated RNA molecule of 7555 kb nucleotides excluding the poly-A tail, with the open reading frames (ORFs 1, 2 and 3) encoding protein with molecular masses of 216.5, 50.4 and 21.4 kDa, respectively (German et al., 1990). The 216.5 kDa ORF encodes a protein suspected to be involved in viral replication and the 50.4 kDa protein is suggested to be the movement protein, while the 21.4 kDa product is the viral coat protein (German et al., 1992).

In Turkey, ACLSV was detected in fruit trees using GF-305 peach seedlings for biological indexing in Yalova (Yürektürk, 1984). It was also detected using ELISA in Yalova and İzmir (Dunez, 1986), in Malatya, Elazığ and Iğdır provinces (Elibüyük, 1998; Sipahioğlu et al., 1999), and in the East Mediterranean (Çağlayan Yıldızgördü and Çalı, 1994; Çağlayan and Gazel, 1998). Cherry nurseries in Eğirdir and Yalova were tested using ELISA for viral diseases in the EPPO certification table, and infection by ACLSV was detected at levels of 23.2% and 45.1%, respectively (Fidan and Özdemir, 1998).

Since trees are infected latently, the detection of the virus in nurseries is a matter of great importance. There are currently a number of methods which can be used to detect ACLSV including biological indexing on either GF-305 peach seedlings or *Malus platycarpa*, immunoelectron microscopy and serological indexing by ELISA using polyclonal or monoclonal antibodies. Although quite efficient, these techniques still have several limitations, either in terms of length of time required for indexing or in terms of sensitivity. The major limitations of biological indexing comes from their application length and cost in terms of glasshouse space and labour intensity. In particular ELISA becomes unreliable for the indexing of most host plants during the summer because of the low virus concentration during this period. In some hosts, this assay is considered reliable only during a short window of about 3 months following budbreak in the spring. RT-PCR, as an extremely sensitive detection tool to overcome these obstacles, has been used for testing plants for ACLSV infections (Candresse et al., 1995; Malinowski et al., 1998; Salmon et al., 2002). Using this technique for the diagnosis of plant viruses could be aided by rapid, simple, sensitive and reliable methods for viral nucleic acid determination. The usual approach is to apply the IC-RT-PCR system which has been proved to be more sensitive and suitable for low virus titres (Nemchinov et al., 1995; Moury et al., 2000; Helguera et al., 2001, 2002). This system eliminates the RNA extraction step in RT-PCR by capturing viruses via antisera, and avoids the inhibitor effect.

Accurate disease diagnosis combined with sensitive, rapid and early detection of plant viruses is critical for the effective management of most crop systems. By this point the aims of this paper, were to survey ACLSV in important stone fruit growing areas in Turkey except for East Anatolia and to conduct RT-PCR and IC-RT-PCR for Turkish ACLSV isolates and thus prove the reliability of the assay.

Materials and Methods

Plant material

The samples were taken from orchards or rootstock and scion nurseries during May 2001 to May 2002, from fully developed leaves of shoots of cherry, peach, apricot, plum and nectarine showing virus symptoms. Samples were collected from the provinces of Afyon, Amasya, Burdur, Bursa, Çanakkale, Isparta, İzmir and Yalova (Table 1). The provinces were selected on the basis of their extensive stone fruit production determined from statistical data from 1999. Shoots were selected randomly around the trees and placed in plastic sample bags labelled with the location. The samples were stored at 4 °C until DAS-ELISA tests and nucleic acid extractions were performed.

ELISA

All plant materials were subjected to DAS-ELISA using the ACLSV detection kit of Loewe Biochemica (GmbH, Germany). Coating for the IC (immunocapture) step was performed by additional antisera provided by Bioreba (Switzerland) following recommendations of the supplier.

Table 1. Numbers of stone fruit tree samples collected from different provinces in Turkey in 2001-2002.

	Sample Collection Provinces								
Stone Fruit Species	Afyon	Amasya	Burdur	Bursa	Çanakkale	Isparta	İzmir	Yalova	TOTAL
Sweet cherry	49	47	15	1	6	2	76	2	198
Sour cherry	1	0	2	0	0	2	5	8	18
Peach	0	12	9	16	5	4	11	6	63
Nectarine	0	0	3	7	9	0	0	4	23
Apricot	6	3	2	0	0	0	1	3	15
Plum	2	5	2	1	1	8	17	16	52
TOTAL	58	67	33	25	21	16	110	39	369

Immunocapture (IC)

Two protocols with modifications were followed for coating and capturing the virus from the plant extracts. The first protocol (Wetzel et al., 1992) was to coat thin walled PCR tubes with antisera (100 µl) diluted (1:200 Loewe, 1:1000 Bioreba) in carbonate buffer (pH 9.6) at 37 °C for 4 h and then wash them with PBS-tween. Leaf pieces (100 mg) were ground in 1 ml of PBS containing 2% PVP-40. After a quick spin, they were added (100 µl) to precoated tubes and maintained at room temperature overnight. After washing, the tubes were used immediately by filling them with RT-PCR mixture (without RNA), or else stored at –20 °C. The second protocol (Rosner et al., 1998) followed the same procedure but the antisera were diluted in 0.1 M borate buffer (pH 8.5) and plant materials were extracted in TBS (10 mM Tris-HCl, pH 7.4, 8 g l^{-1} of NaCl and 20 g l^{-1} of PVP-40) buffer. The tubes were washed with TBS-tween solution.

RNA extraction

A lithium chloride-based method was used for the isolation of total RNA's (Spiegel et al., 1996): 100 mg of leaf pieces was homogenised using a pestle and mortar with 5 volumes of buffer (200 mM Tris-HCl pH 8.5, 1.5% SDS, 300 mM lithium chloride, 10 mM EDTA, 1% sodium deoxycholate, 1% Igepal). The extract was collected and heated for 15 min to 65 °C, and then 6 M potassium acetate (pH 6.5) was added. After 15 min incubation in ice, the tubes were centrifuged. Nucleic acids were precipitated with isopropanol and collected by centrifugation. The pellet was resuspended after drying in 50 µl of sterile water and extracted RNA's were maintained at -80 °C.

Primers and RT-PCR protocol

The PCR primers designed for ACLSV were A52 and A53 (Candresse et al., 1995). The reverse transcription (RT) primer (A52) has the sequence 5'– CAG ACC CTT ATT GAA GTC GAA-3' (position 7213-7233 nt on ACLSV GenBank no: M58152). The sense primer (A53) has the sequence 5'-GGC AAC CCT GGA ACA GA - 3' (position 6875-6891 nt). The amplified fragment has a size of 358 bp.

The RT and PCR were performed following a single noninterrupted thermal cycling program (Spiegel et al., 1996). The total volume of reaction was 25 µl for RT-PCR and 50 µl for IC-RT-PCR and each reaction contained the RNA template (about 1 µg), virus specific primers (0.8 µM each), 400 µM dNTPs, 1.5 mM $MgCl_2$, 10x reaction buffer (final concentration of 10 mM Tris-HCl pH 8.8, 50 mM KCl and 0.08% Igepal), 1 U of Taq DNA polymerase (Promega, Madison, USA), 0.8 U of M-MLV reverse transcriptase (MBI Fermentas, GmBH Germany) and 0.8 U RNase inhibitor (MBI Fermentas, GmBH, Germany). The amplification protocol was as follows: 1 h at 42 °C for reverse transcription, 35 cycles 30 s at 94 °C, 45 s at 55 °C, 1 min at 72 °C and finally 10 min at 72 °C. PCR products were analysed using electrophoresis in 1% agarose gel stained with ethidium bromide (EtBr) and viewed under UV.

Results and Discussion

Sample collection areas were selected from the most extensive stone fruit growing provinces and scion and/or rootstock stations in Turkey, except for East Anatolia. Thus, 369 samples from stone fruits including sweet and sour cherry, peach, nectarine, apricot and plum trees

were collected from Afyon (58 samples), Amasya (67 samples), Burdur (33 samples), Bursa (25 samples), Çanakkale (21 samples), Isparta (16 samples), İzmir (110 samples) and Yalova (39 samples). All samples were subjected to DAS-ELISA and RT-PCR assays. Thirteen out of the 369 fruit trees tested using DAS-ELISA were found to be naturally infected with ACLSV. RT-PCR analysis of the samples revealed that 51 plants were infected by ACLSV. This number detected using RT-PCR was much higher than that detected using DAS-ELISA. Virus concentration fluctuates during individual growing seasons and between seasons, and becomes undetectable by ELISA (Torrence and Dolby, 1984; Scott et al., 1989). It was reported that testing cherry leaves from infected trees in April or at the beginning of May using ELISA gave negative results, whereas the same trees tested in June or July reacted positively in ELISA (Cieslinska et al., 1995). This suggests that the reason for ELISA detecting low numbers of ACLSV infected trees may be low virus concentration at the sampling time or uneven distribution throughout the buds and limbs of infected trees. Other hand, there have been several RT-PCR is superior to DAS-ELISA. The threshold of plum pox virus (PPV) detection was 5000-fold lower per assay compared with the ELISA test (Wetzel et al., 1991). The detection limit of purified *Prunus* necrotic ring spot virus was 4 ng ml^{-1} using DAS-ELISA, whereas RT-PCR assay revealed greater sensitivity by detecting a limit of 1.28 pg ml^{-1} (Sanchez-Navarro et al., 1998). PCR was used successfully for the detection of various plant viral RNA's extracted from bark samples collected from dormant peach trees in winter (Spiegel et al., 1996), and from ligneous tissue of fruit trees (Korschinek et al., 1991). These reports suggested that PCR could be used at all times of the year and that all parts of the plants can be tested for viral diseases.

Most of the sweet cherry, peach and apricot trees detected to be ACLSV infected were generally symptomless and collected randomly. These results were confirmed by reports on the natural infections of stone fruit trees by ACLSV as a latent virus (Nemeth, 1986; Sutic et al., 1999). On the other hand, wavy lines and light coloured rings on leaves caused by ACLSV were observed on some naturally infected peaches like those induced by PPV.

Among the fruit tree species tested in this research, nectarine was found to have the highest infection rate (34.78%) followed by apricot, peach, sour cherry, plum and sweet cherry, respectively (Figure 1a). The samples collected from Bursa exhibited the highest ACLSV infection 64% (Figure 1b) and the incidence of the virus in nectarines and peaches was much higher than that in the other provinces. Bursa is the most productive stone fruit growing area after Malatya, especially in peach and nectarine (SIS, 2000). The ACLSV in Bursa is therefore a major threat to peach and nectarine growing. The samples provided from Yalova were determined as showing the second highest ACLSV incidence. All plant materials from this province were collected from the Atatürk Central Horticultural Research Institute, in Yalova, which collects and maintains the cultivars and works on new variety adaptations. Our results confirmed the investigation by Fidan and Özdemir (1998) into cherry rootstock/scion nurseries at the same institute. These researchers determined that ACLSV was the most prevalent virus among the tested stone fruit viruses. The virus contamination in this institute is a serious concern in terms of providing rootstock and scion materials for new stone fruit plantations.

IC-RT-PCR was applied to ACLSV infected sweet cherries and peaches. Viral particles immunocaptured

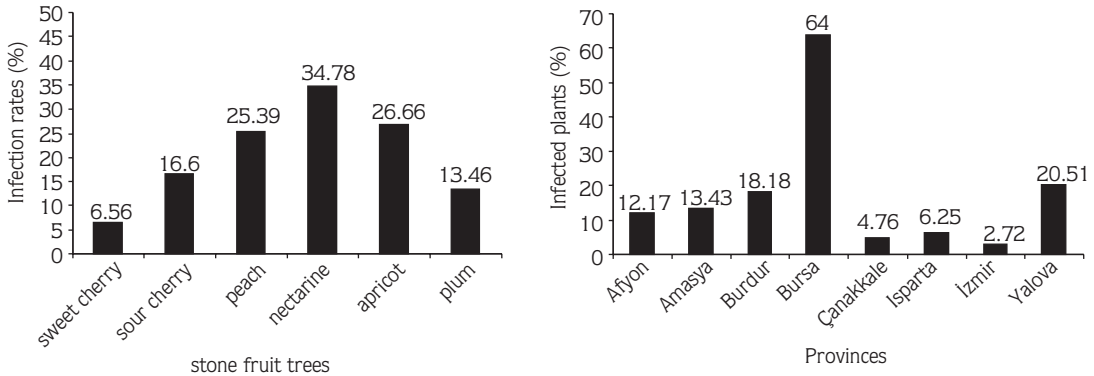

Figure 1. Infection rates of ACLSV in stone fruit species (a) and provinces (b).

from infected plant material sap were efficiently amplified by PCR. Amplified products showed clear bands of the expected size (Figure 2a). All selected isolates were amplified, thus confirming the polyvalence of the assay. No amplification was observed in uninfected tissue. These results are comparable with those obtained by Nemchinov et al. (1995) and further validate the method's use under various conditions. No differences were determined between Bioreba and Loewe antisera or between the 2 protocols followed for immunocapture. Both antisera and IC-RT-PCR are recommended for detection of ACLSV in stone fruit trees as a result of our investigation.

Inhibitors as a limiting factor in PCR amplification may be present in certain plant tissues (Vunsh et al., 1991). Diluting plant sap for IC is one means of overcoming this difficulty (Rosner et al., 1998). Samples from serial dilutions of sap prepared from an ACLSV infected sweet cherry were used in IC and RT-PCR. Greater than 1:10 sap dilutions was reduced the PCR amplifications (Figure 2b), probably due to the lower amounts of virus RNA template available. It was recorded that PCR products amplified from PNRSV infected plant sap were increased by dilution up to 1:250 (Rosner et al., 1998).

IC-RT-PCR obviates the need for the time consuming RNA purification step, and thus, simplifies RT-PCR assay. Technically, RT-PCR amplification of ACLSV from crude sap is a much simpler procedure than IC-RT-PCR and it can be easily applied in large scale testing. However, it should be noted that IC-RT-PCR testing was proved to be a more sensitive procedure (Wetzel et al., 1992; Rosner et al., 1998; Helguera et al., 2001), and therefore this should be applied in cases of lower virus content.

Conclusions

In this paper we demonstrate the potential of RT-PCR for the detection of ACLSV and the prevalence of the virus in Turkey. The highest potential in developing sensitive detection for routine purposes can be expected by the application of already existing PCR methods. RT-PCR and IC-RT-PCR provide possible alternatives to ELISA, which

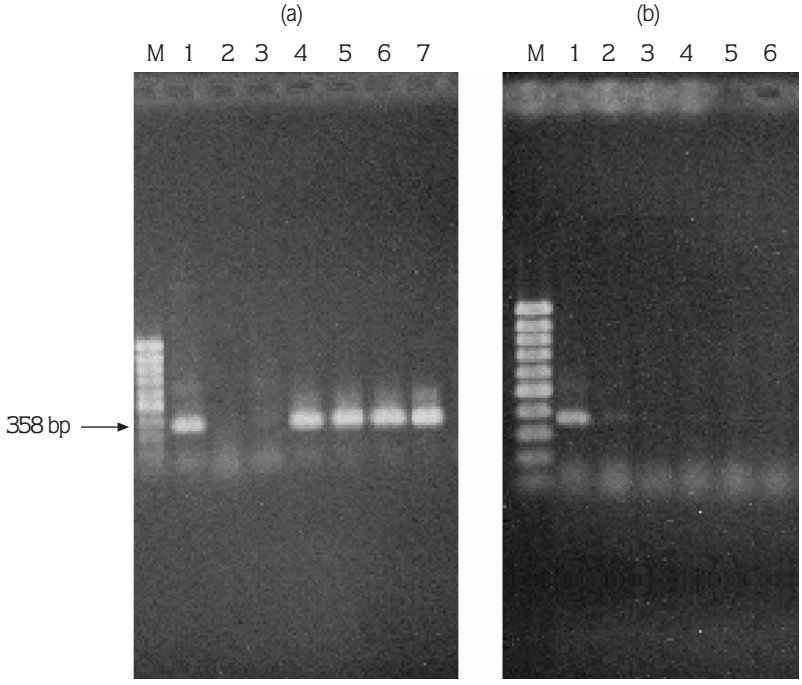

Figure 2. a) IC-RT-PCR tests of some ACLSV isolates. M DNA ladder, low range (MBI Fermentas), 1-ACLSV plasmid DNA as a positive control, 2-negative control without RNA, 3-healthy plant control, 4,5- sweet cherries, 6,7-peaches. b) IC-RT-PCR with diluted sap of ACLSV infected leaf sample. 1-1:10, 2-1:1000, 3-1:2000, 4-1:3000, 5-1:4000, 6- negative control.

often fails because of low virus titre and inhibitory effects of compounds in the sap of woody plants. The amount of ACLSV infected plants detected using RT-PCR is almost 4-fold more than that detected using ELISA, meaning that PCR needs to be used as a detection tool in accurate testing of nurseries. In addition, testing of imported dormant budwood, commonly used for international movement, and other woody plant materials in certification schemes need not be delayed for months these techniques are used. Additionally, these tools provide an efficient and rapid tool for large scale early screening of plant material, especially in virus sanitation programmes.

The absence of any symptoms in most of the ACLSV infected plant samples exemplifies the need to test more samples to determine the infection rate of the virus in Turkey. The high infection rate in nectarine among the tested samples reveals that ACLSV may originate from abroad. This possibility increases the importance of healthy budwood importation.

References

Brunt, H.A., K. Crabtree, M.J. Dallawitz, A.J. Gibbs and L. Watson. 1996. Viruses of plants. CAB International, Cambridge, UK.

Çağlayan Yildizgördü, K. and S. Çalı. 1994. Occurrence and detection of virus and virus like diseases of plum and apricot trees in the East Mediterranean. In: Proc. of 9th Cong. of the Mediter. Phytopath. Union. Sept. 18-24, Kuşadası-Aydın Türkiye. 551-553.

Çağlayan, K., and M.H. Gazel. 1998. Virus and virus-like diseases of stone fruits in the Eastern Mediterranean area of Turkey (Eds : A. Hadidi). Proc. 17th Int. Symp. on Fruit Tree Virus Diseases, Acta Hort. 472: 527-529.

Candresse, T., M. Lanneau, F. Revers, N. Grasseau, G. Macquaire, S. German, T. Malinowski and J. Dunez. 1995. An immuno-capture PCR assay adapted to the detection and the analysis of the molecular variability of the apple chlorotic leaf spot virus. Acta Hort. 386: 136-147.

Cieslinska, M., T. Malinowski and B.J. Zawadzka. 1995. Studies on several strains of apple chlorotic leaf spot virus (ACLSV) isolated from different fruit tree species. Acta Hort. 386: 63-71.

Desvignes, J.C. and R. Boye. 1989. Different diseases caused by chlorotic leaf spot virus on the fruit trees. Acta Hort. 235: 31-38.

Dunez, J. 1986. Preliminary observations on virus and virus like diseases of stone fruit trees in Mediterranean and Near East countries. FAO Plant Protection Bulletin, 34: 43-48.

Elibüyük, İ.Ö. 1998. Malatya ilinde yetiştirilen sert çekirdekli meyve ağaçlarındaki virusların tanılanması üzerinde araştırmalar. Doktora tezi, Ankara Üniversitesi, Fen Bilimleri Enstitüsü, pp 120, Ankara.

Fidan, Ü. and S. Özdemir. 1998. Kiraz ağaçlarında görülen virüslerin DAS-ELISA ile belirlenmesi. Zirai Mücadele Araştırma Enstitüsü, Bornova/İzmir. <www.tagem.gov.tr/yeni%20web/projeler/uygulamaya%20aktarilan/projeler00/bsad00/11.HTM>

German S., T. Candresse, M. Lanneau and J. Dunez. 1992. Genomic organisation of apple chlorotic leaf spot closterovirus (ACLSV). Acta Hort. 309: 31-8.

German, S., T. Candresse, M. Lanneau, J.C. Huet, J.C. Pernollet and J. Dunez. 1990. Nucleotide sequence and genomic organisation of apple chlorotic leaf spot virus. Virology 179: 104-112.

Helguera, P.R., D.M. Docampo, F. Nome and D.A. Ducasse. 2002. Enhanced detection of Prune dwarf virus in peach leaves by immunocapture-reverse transcription-polymerase chain reaction with nested polymerase chain reaction (IC-RT-PCR nested PCR). J. Phytopathology 150: 94-96.

Helguera, P.R., T. Taborda, D.M. Docampo and D.A. Ducasse. 2001. Immuno capture reverse transcription-polymerase chain reaction combined with nested PCR greatly increases the detection of prunus necrotic ringspot virus in the peach. J. Virol. Meth. 95: 93-100.

Korschineck, I., G. Himmler, R. Sagl, H. Steinkleellner and H.W.D. Katinger. 1991. A PCR membrane spot assay for the detection of plum pox virus RNA in bark of infected trees. J. Virol. Methods 31: 139-146.

Malinowski, T., B. Komorowska and T. Candresse. 1998. Characterisation of SX/2 an apple chlorotic leaf spot virus isolates showing unusual coat protein properties. Acta Hort. 472: 43-50.

Martelli, G.P., T. Candresse and S. Namba. 1994. Trichovirus, a new genus of plant viruses. Arch. Virology 134: 451-455.

Moury, B., L. Cardin, J.-P. Onesto, T. Candresse and A. Poupet. 2000. Enzyme-linked immunosorbent assay testing of shoots grown in vitro and the use of immunocapture-reverse transcription-polymerase chain reaction improve the detection of prunus necrotic ringspot virus in rose. Phytopathology 90: 522-528.

Nemchinov, L., A. Hadidi, J.A. Foster, T. Candresse and T. Verderevskaya. 1995. Sensitive detection of apple chlorotic leaf spot virus from infected apple or peach tissue using RT-PCR, IC-RT-PCR, or multiplex IC-RT-PCR. Acta Hort. 386: 51-62.

Nemeth, M. 1986. Virus, Mycoplasma and Rickettsia Diseases of Fruit Trees. Martinus Nijhoff Publishers, The Netherlands and Akademini Kiado, Hungary.

Rosner, A., Y. Shiboleth, S. Spiegel, L. Krisbai and M. Kölber, 1998. Evaluating the use of immuno-capture and sap-dilution PCR for the detection of prunus necrotic ringspot virus. Acta Hort. 472: 227-233.

Salmon, M.A., M. Vendrame, J. Kummert and P. Lepoivre, 2002. Detection of apple chlorotic leaf spot virus using 5' nuclease assay with a fluorescent minor groove binder-DNA probe. J. Virol. Methods 104: 99-106.

Sanchez-Navarro, J.A., F. Aparicio, A. Rowhani and V. Pallas. 1998. Comparative analysis of ELISA, nonradioactive molecular hybridization and PCR for the detection of prunus necrotic ringspot virus in herbaceous and Prunus hosts. Plant Pathol., 47: 780-786.

Scott, S.W., O.W. Barnett and P. Burrows. 1989. Prunus necrotic ringspot virus selected peach orchards in South Carolina. Plant Dis., 73: 913-916.

Sipahioğlu, H.M., A. Myrta, N. Abou-Ghanem, B. Di Terlizzi and V. Savino. 1999. Sanitary status of stone fruit trees in East Anatolia (Turkey) with particular reference to apricot. Bull. OEPP/EPPO 29(4): 439-442.

Spiegel, S., S.W. Scott, V. Bowman-Vance, Y. Tam, N.N. Galiakparov and A. Rosner. 1996. Improved detection of prunus necrotic ringspot virus by polymerase chain reaction. Europ. J. Plant Pathol. 102: 681-685.

State Institute of Statistics. 2000. Tarımsal yapı (üretim, fiyat, değer). T.C. Başbakanlık Devlet İstatistik Enstitüsü, Ankara.

Sutic, D.D., R.E. Ford, and M.T. Tosic, 1999. Handbook of Plant Virus Diseases. CRC Press. London, New York, Washington.

Torrance, L. and C.A. Dolby. 1984. Sampling conditions for reliable routine detection by enzyme-linked immunosorbent assay of three ilarviruses in fruit trees. Ann. Appl. Biol. 104: 267-276.

Vunsh, R., A. Rosner and A. Stein. 1991. Detection of bean yellow mosaic virus in gladioli corms by the polymerase chain reaction. Ann. Appl. Biol. 119: 289-294.

Wetzel, T., T. Candresse, G. Maquaire, M. Ravelonandro and J. Dunez. 1992. A highly sensitive immunocapture polymerase chain reaction method for plum pox potyvirus detection. J. Vir. Methods 39: 27-37.

Wetzel, T., T. Candresse, M. Ravelonandro and J. Dunez. 1991. A polymerase chain reaction adapted to plum pox potyvirus detection. J. Virol. Methods, 33: 355-365.

Yürektürk, M. 1984. Marmara Bölgesi'nde sert çekirdekli meyvelerde görülen Sharka virüs hastalığı üzerine çalışmalar. Atatürk Bahçe Kültürleri Araştırma Enstitüsi, p 37.