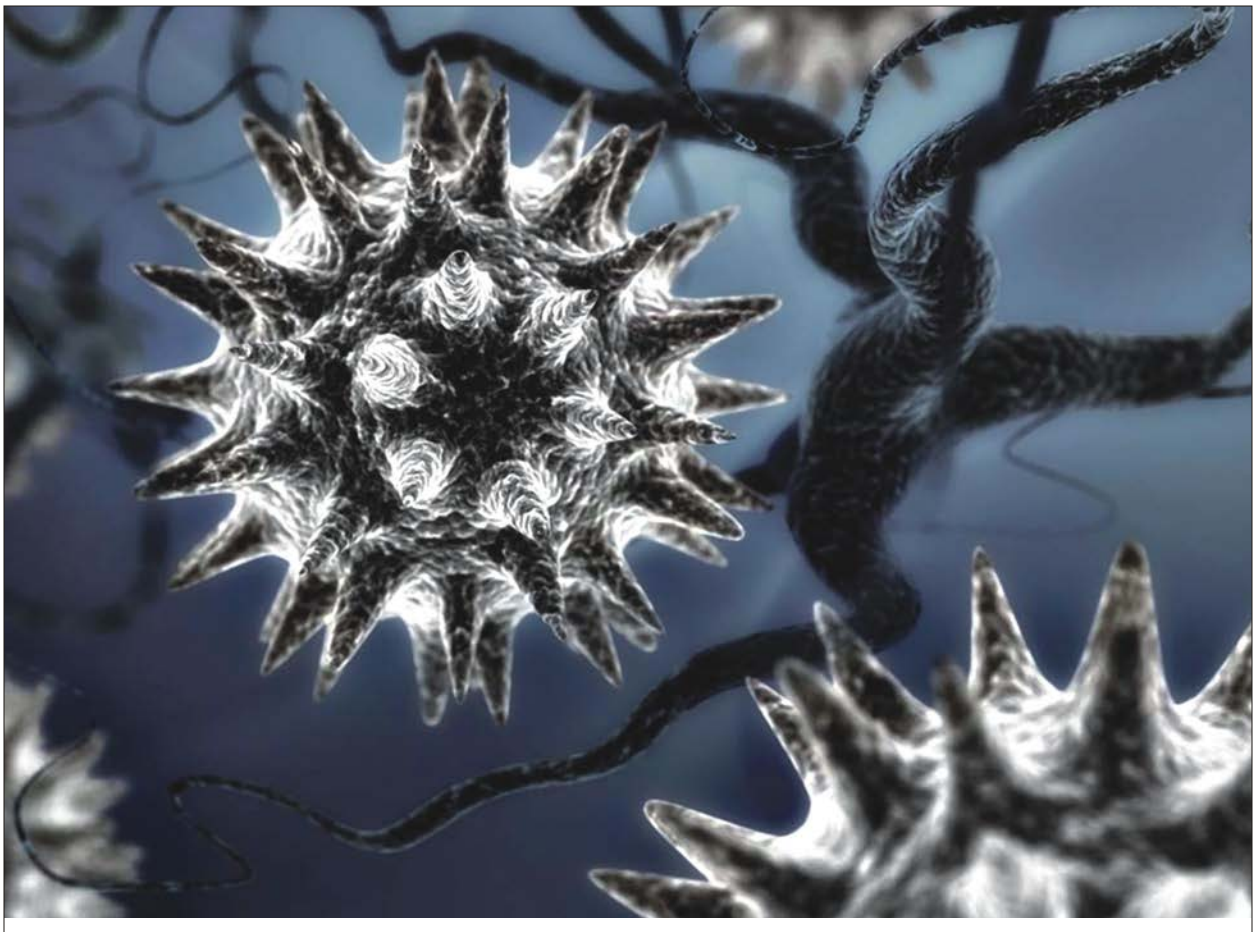

International Journal of Virology

ISSN 1816-4900

Academic Journals Inc.

www.academicjournals.com

International Journal of Virology 8 (1): 114-120, 2012
ISSN 1816-4900 / DOI: 10.3923/ijv.2012.114.120

Incidence of Fruit Trees Viroid Diseases by Tissue Print Hybridization in Egypt

[1]Kh.A. El-Dougdoug, [2]Rehab A. Dawoud, [2]A.A. Rezk and [3]A.R. Sofy

[1]Virology Lab, Department of Agriculture Microbiology, Faculty of Agriculture, Ain Shams University, 11241 Cairo, Egypt

[2]Virus and Phytoplasma Research Section, Plant Pathology Institute, Agricultural Research Center, Giza, Egypt

[3]Department of Botany and Microbiology, Faculty of Science, Al-Azhar University, 11884, Nasr City, Cairo, Egypt

Corresponding Author: A.R. Sofy, Department of Botany and Microbiology, Faculty of Science, Al-Azhar University, 11884, Nasr City, Cairo, Egypt

ABSTRACT

Direct tissue printing on membranes has been applied on a large scale for an initial detection of CEVd, HSVd and PLMVd in fruit trees in Egypt. CEVd was detected mainly in sweet orange trees and occasionally in grapevine and mango. The principal characteristics of the disease on sweet orange trees. It was incidence with 15.4, 4.5 and 1.5%, respectively. HSVd was detected mainly in sweet orange trees and occasionally in apple, apricot, mandarin, grapevine, mango, peach, pear and plum trees with 25.2, 2.2, 7.2, 10.5, 12.4, 15.7, 65.6, 40.5 and 5.7%, respectively. The principal characteristics of the disease on sweet orange trees. PLMVd was detected mainly in peach and occasionally in apple, apricot, grapevine, mango, pear and plum with 45.0, 5.4, 2.5, 0.5, 13.5, 23.4 and 3.5% incidence. The principal characteristics of the disease on peach trees. The three viroids; CEVd, HSVd and PLMVd were detected frequently in sweet orange and peach occasionally in grapevine, pear, mango, plum and apricot in Egypt.

Key words: Detection, hybridization, viroids, CEVd, HSVd, PLMVd

INTRODUCTION

Viroids are the smallest plant pathogens cause serious diseases in economical herbaceous crops (cucumber, hop, potato, tomato), fruit trees (apple, apricot, avocado, coconut, citrus, garapevine, peach, plum) and ornamentals (*Chrysanthemum*) (El-Dougdoug *et al*., 1993, 1997; Randles, 2003; Marei *et al*., 2005; Sofy *et al*., 2010). The viroids are known to infect fruit trees CEVd, HSVd and PLMVd (Pallas *et al*., 2003; Torres *et al*., 2004).

Several efficient methods for detecting viroids infecting fruit trees have been reported previously (Ambros *et al*., 1995; Di Serio *et al*., 2001, 2002; Ragozzino *et al*., 2004). These technologies, based on RT-PCR or on molecular hybridization of labeled probes with plant extracts, need technical expertise for nucleic acid preparations which is time consuming and relatively expensive.

In contrast, tissue printing hybridization, an alternative detection method based on molecular hybridization, does not require nucleic acid preparation because nucleic acids are applied to the membrane by directly imprinting the fresh plant tissues to be tested.

In addition, for large scale indexing and in scheme certification programs, sample manipulation must be reduced to a minimum. This can be achieved by using the tissue printing technique which only requires the direct application of fresh cut sections (stem, petiole leaf or fruit) to nitrocellulose membranes (Mas and Pallas, 1995; Muhlbach *et al*., 2003; El-Dougdoug *et al*., 2010).

Available data on the incidence and biodiversity of fruit trees viroid diseases in Egypt are limited. So, starting a research incidence to fill this gap, a tissue- printing hydridization method to detect Citrus Exocortis Viroid (CEVd), Hop Stunt Viroid (HSVd) and Peach Latent Mosaic Viroid (PLMVd) has been developed and validated.

MATERIALS AND METHODS

Plant materials: The infected fruit trees (apple, apricot, citrus, grapevine, mango, peach, pear and plum) revealed virus and viroid like symptoms were selected from different commercial plantations in Egypt.

Tissue print hybridization: Young leaves petioles were cut from different parts of each tree in the field and immediately printed on the nitrocellulose membranes (CF-11). Twice to obtain duplicate printings of each tree were applied. The membranes were air dried.

Viroid probes: CEVd, HSVd and PLMVd clones, 10 mM DIG-labeling nucleotides 25 pm of viriod (+) primer, 25 pm of viroid (-) primer and 10×PCR buffer with $MgCl_2$, 0.5 Taq DNA polymerase (Roche) and the volume was completed to 25 μL with nuclease free water. the PCR parameters were done and the DNA probes were boiled at 95°C for 5 min before adding in the hybridization buffer.

Digoxigenin hybridization: The digoxigenin labeled DNA probes used in tissue print hybridization assay. Pre-hybridization and hybridization were carried out at 68°C essentially as described by Pallas *et al*. (1998). Binding to anti-digoxigenin Fab fragments conjugated to alkaline phosphatase (Roche) and subsequent chemiluminescent detection using CSPD (Roche) or substrate were used to detect the hybridized probe.

RESULTS

The rapid tissue printing in the field as well as diaoxigenin technique revealed the presence of CEVd, HSVd and PLMVd infecting fruit trees (Fig. 1). These viroids were not detected from healthy tree (Fig. 1). The results obtained in the tissue print assay allowed clear. Discrimination between infected and uninfected trees tested. The unclear signal occasionally observed were not considered as positives.

Citrus Exocortis Viroid (CEVd) was detected in sweet orange, grapevine and mango trees analyzed with 15.4, 4.5 and 1.5%, respectively except in apple, apricot, citrus cv., mandarin, peach, pear and plum trees (Fig. 1, Table 1). The CEVd occurs frequently in citrus (sweet orange) and occasionally in grapevine and mango. The principal characteristics of the disease on sweet orange.

Hop Stunt Viroid (HSVd) was detected in all fruit trees analyzed demonstrating a broad distribution of pathogen in the analyzed fruit trees. The incidence level of HSVd in trees was as follows: apple (2.2%), apricot (7.2%), sweet orange (25.2%), mandarin (10.5%), grapevine (12.4%), mango (15.7%), peach (65.6%), pear (40.5%) and plum (5.7%) (Fig. 2, Table 1). These trees either

Table 1: Incidence levels of CEVd, HSVd and PLMVd in fruit trees analyzed

		Percentage of viroids incidence		
Fruit trees	No. of fruit trees	CEVd	HSVd	PLMVd
Apple	50	0.0	2.2	5.4
Apricot	72	0.0	7.2	2.5
Sweet orange	92	15.4	25.2	0.0
Mandarin	50	0.0	10.5	0.0
Grapevine	75	4.5	12.4	0.5
Mango	50	1.5	15.7	13.5
Peach	100	0.0	65.6	45.0
Pear	25	0.0	40.5	23.4
Plum	25	0.0	5.7	35.0

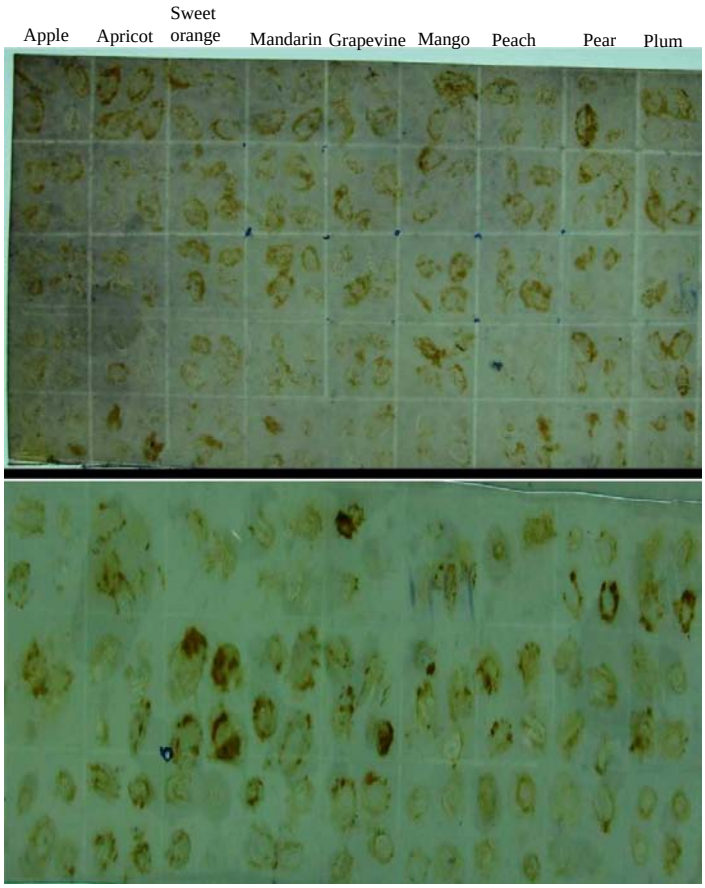

Fig. 1: Tissue print hybridization of naturally infected fruit trees (apple, apricot, sweet orange, mandarin, grapevine, mango, peach, pear and plum) on nitrocellulose membrane using Dig-labeled RNA-DNA probe showing signals of CEVd

showed specific disorders or symptomless. Sweet orange trees were visually inspected for characteristics as a line of reddish brown, gum-impregnated tissue can be seen around the

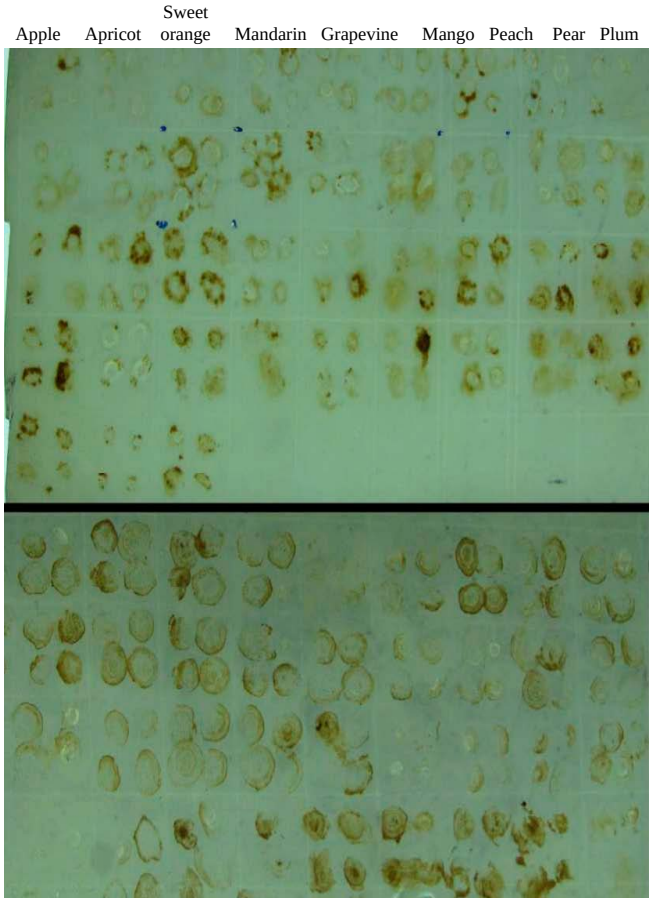

Fig. 2: Tissue print hypridization of naturally infected fruit trees (apple, apricot, sweet orange, mandarin, grapevine, mango, peach, pear and plum) on nitrocellulose membrane using Dig-labeled RNA-DNA probe showing signals of HSVd

circumference and especial near the bud union. The discoloration and gummy may extend about 60 cm or more in the bark of the trunk.

Peach Latent Mosaic Viroid (PLMVd) was detected in apple, apricot, grapevine, mango, peach, pear and plum trees analyzed except in sweet orange and mandarin demonstrating a broad distribution of his pathogen in the analyzed fruit trees. The incidence level of PLMVd in fruit trees was as follows: apple (5.4%), apricot (2.5%), grapevine (0.5%), mango (13.5%), peach (45.0%), pear (23.4%) and plum (3.5%) (Fig. 3, Table 1). The principal characteristics of the viroid on fruit trees includes: delayed flowering 4 to 6 days with similar effects in vegetation and fruit maturity. On infected trees, fruits are deformed, dull in color, discolored or pigmented. Vegetative buds turn necrotic, drops off and produce weak growth. Yield declines in quantity and quality. Occasionally leaves exhibit discoloration in the form of either diffuse mosaic or yellowish mottles: blurred chlorotic blotches. The leaves are generally not deformed. Other plant species either showed specific disorders or symptomless.

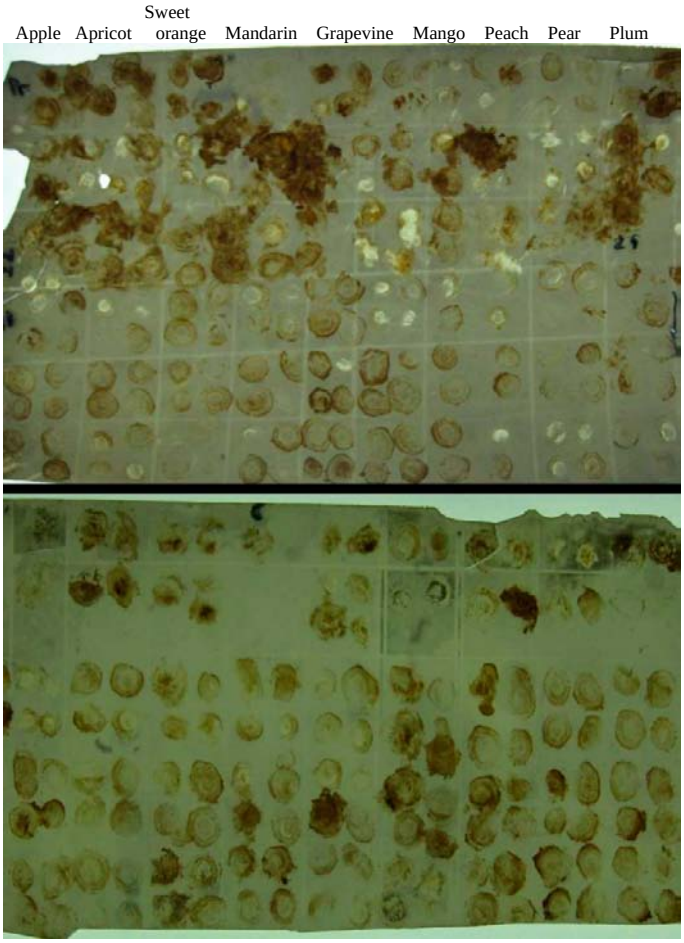

Fig. 3: Tissue print hypridization of naturally infected fruit trees (apple, apricot, sweet orange, mandarin, grapevine, mango, peach, pear and plum) on nitrocellulose membrane using Dig-labeled RNA-DNA probe showing signals of PLMVd

DISCUSSION

The results obtained in the tissue print assays allowed clear discrimination between infected and uninfected fruit trees. The unclear signal occasionally observed were not considered as positives. The tissue printing described rapid method for the viroid detection of fruit trees in the field and was effective and reliable the efficiency of tissue printing method was similar to that of the Polymerase Chain Reaction (PCR). Tissue print hybridization assay gave excellent results and more sensitive the S-PAGE technique (Pallas *et al*., 1998). Any way tissue print hybridization is more advisable because of its possibility to test simultaneously a large number of samples. No reaction occurred with the healthy control, indicating that this tissue printing preparation is able to avoid cross reaction with plant nucleic acids and the carry over deriving from the interference of substances with PCR. In addition, this assay have many advantages such as the small amount of starting plant tissue, the rapidity and the possibility to print petiole on nitrocellulose membranes

in the field as reported by Mas and Pallas (1995), Muhlbach *et al.* (2003) and El-Dougdoug *et al.* (2010). All this characteristics make this method useful for the detection and detection and diagnosis of viroids on fruit trees in particular for preliminary screening of materials candidate for sanitary certification programs. Several reports indicate that viroids can be detected by molecular hybridization of imprinted membranes (Podleckis *et al.*, 1993; Romero-Durban *et al.*, 1995).

Present results clearly demonstrate that tissue printing is a very convenient small amount of starting plant tissue method for analysis of a large number of samples from different geographical areas, faciliting the evaluation of the sanitary status of the stone fruit industry in different countries. This is particularly relevant for countries where no appropriate facilities for viroid diagnosis exist. In addition, all analysis can be conducted in a single facility enhancing the test reproducibility and ensuring that the same evaluation criteria are applied to samples from all region. Interestingly, cuttings originating from Egypt for viroids were collected in winter season with temperature oscillating between 10-24°C, indicating that viroid concentration in the tree is still sufficient for detection under these extreme conditions.

Viroids can be detected by biological, biochemical or molecular methods. The three approaches are time consuming and expensive, with the limiting step for molecular technique being the sample preparation process. In the present study, direct tissue printing on membranes, a procedure that the viroids sample extraction has been applied on large scale for on initial screening of viroids in a different regions.

The results obtained for CEVd are consistent with those previously reported in Egypt (El-Dougdoug *et al.*, 1993) and other countries of the Mediterranean region (Hadidi *et al.*, 2003a) where it has been detected mainly in citrus (sweet orange) trees and occasionally in grapevine (Hadidi *et al.*, 2003b). The PLMVd is consistent with those previously reported in Egypt (Marei *et al.*, 2005) and other countries of the Mediterranean region (Hadidi *et al.*, 2003a), where it has been detected mainly in peach and apple (El-Dougdoug, 1998) occasionally in pear plum and apricot (Hadidi *et al.*, 2003b) the HSVd was detected on all samples with high incidence levels in citrus and pear and apple.

REFERENCES

Ambros, S., J.C. Desvignes, G. Llacer and R. Flores, 1995. Pear blister canker viroid: Sequence variability and causal role in pear blister canker disease. J. Gen., 76: 2625-2629.

Di Serio, F., M. Malfitano, A. Ragozzino, J.C. Desvignes and R. Flores, 2001. Apple dimple fruit viroi: Fulfillment of Koch postulates and symptom characteristics. Plant Dis., 85: 179-182.

Di Serio, F., M. Malfitano, D. Alioto, A. Ragozzino and R. Flores, 2002. Apple dimple fruit viroid: Sequence variability and its specific detection by multiplex fluorescent RT-PCR in the presence of Apple scar skin viroid. J. Plant Pathol., 84: 27-34.

El-Dougdoug, K.H.A., S.H. El-Deeb and A.A. Abou-Zeid, 1993. Anatomical and ultrastructural changes in orange leaves infected with Citrus *exocortis viroid* (CEVd). Annals Agric. Sci. Ain Shams, 38: 101-117

El-Dougdoug, K.H.A., M.A. Awad, A.A. Shalaby and A.A. Abo-Zeid, 1997. Viroids infect mandarin and navel orange in Egypt. Arab Univer. J. Agri. Sci., 5: 209-225.

El-Dougdoug, K.A., 1998. Occurrence of Peach latent masaic viriod (PLMVd) in Apple fruit (*Malus domestica*). Ann. Agric. Sci., Ain Shams Univ., Cairo. Egypt, 43: 21-30.

El-Dougdoug, K.A., M.E. Osman, S.A. Hayam, A. Rehab, Dawoud and Reham M. Elbaz, 2010. Biological and molecular detection of HSVd-infecting peach and pear trees in Egypt. Austr. J. Basic Appl. Sci., 4: 19-26.

Hadidi, A., H.H. Mazyad, M.A. Madkour and M. Bar-Joseph, 2003a. Viroids in the Middle East. In: Viroids, Hadidi, A., R. Flores, J.W. Randles and J.S. Semancik (Eds.)., CS/RO., Collingwood, Australia, pp: 275-278.

Hadidi, A., L. Giunchedi, H. Osaki, A.M. Shamloul and P. Gentit *et al*., 2003b. Peach Latent Mosaic Viroid in Temperate Fruit Hosts. In: Viroids, Hadidi, A., R. Flores, J.W. Randles and J.S. Semancik (Eds.)., CS/RO., Collingwood, Australia.

Marei, E.M., M.A. Amer, K.A. El-Dougdoug, B.A. Othman and E.K. Allam, 2005. Molecular detection of Peach latent mosaic viroid Egyptian isolate. Egyptian J., 2: 315-329.

Mas, P. and V. Pallas, 1995. Non-isotopic tissue-printing hybridization: A new technique to study long- distance plant virus movement. J. Virol. Methods, 52: 317-326.

Muhlbach, H.P, U. Weber, G. Gomez, V. Pallas, N. Duran-Vila and A. Hadidi, 2003. Molecular Hybridization. In: Viroids, Hadidi, A., R. Flores, J.W. Randles and J.S. Semancik (Eds.)., CS/RO., Collingwood, Australia, pp: 103-114.

Pallas, V., G. Gomez and N. Duran-Vila, 2003. Viroids in Europe. In: Viroids, Hadidi, A., R. Flores, J.W. Randles and J.S. Semanick (Eds.)., CS/RO., Collingwood, Australia, pp: 268-274.

Pallas, V., J.A. Sanchez-Navarro, P. Mas, M.C. Canizares, F. Aparicio and J.F. Marcos, 1998. Molecular diagnostic techniques and their potential role in stone fruit certification schemes. Options Mediterr., 19: 191-208.

Podleckis, E.V., R.W. Hammond, S. Hurtt and A. Hadidi, 1993. Chemiluminescent detection of potato and pome fruit viroids by digoxigenin labeled dot blot and tissue blot hybridization. J. Virol. Methods, 43: 147-158.

Ragozzino, E., F. Faggioli and M. Barba, 2004. Development of a one tube- one step RT-PCR protocol for the detection of seven viroids in four genera: Apscaviroid, hostuviroid, pelamoviroid and pospiviroid. J. Virol. Methods, 121: 25-29.

Randles, J.W. 2003. Economic Impact of Viroid Diseases. In: Viroids, Hadidi, A. R. Flores, J.W. Randles and J.S. Semancik (Eds.)., CS/RO., Collingwood, Australia, pp: 3-14.

Romero-Durban, J., M. Cambra and N. Duran-Vila, 1995. A simple imprint-hybridization method for detection of viroids. J. Virol. Methods, 55: 37-47.

Sofy, A.R., A.M. Soliman, A.A. Mousa, S.A. Ghazal and K.H.A. El-Dougdoug, 2010. First record of *Citrus viroid* II (Cvd-II) associated with gummy bark disease in sweet orange (*Citrus sinensis*) in Egypt. New Dis. Reports, 21: 24-24.

Torres, H., G. Gomez, V. Pallas, B. Stamo and A. Shalaby *et al*., 2004. Detection by tissue printing of stone fruit viroids, from Europe, the Mediterranean and North and South America. Acta Hortic., 657: 379-383.