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Case Report

A Viroid Resembling Hop Stunt Viroid in Infected Apple Trees with Apple Scar Skin Disease in the Northeast of Iran

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Abstract

Background and Aims: Apple scar skin disease (ASSD) is one of the destructive diseases of pome fruits which is caused by ASSVd.

Case presentation: We report molecular detection of ASSVd in symptomatic apples cv. Red Delicious with simultaneous presence of a new variant of HSVd from Khorasan Razavi province.

Conclusion: This is the first report on the association of HSVd with ASSVd in apple trees showing merely symptoms of apple dapple on the fruit.

Keywords: Apple, Viroid, Hop stunt, Khorasan

Introduction

Viroidsas single-stranded circular RNA molecules are the smallest infectious agents (1). Their genome is small and cannot code for any protein. Apple scar skin (ASSVd) and Hop stunt viroid (HSVd) belong to Pospiviroidae family whose members possess a central conserved region and an asymmetrical rolling loop type of replication and have no enzyme activity (2). ASSVd and HSVdare the viroids reported to affect pome fruit trees. ASSVd belongs to the genus Apscaviroid and has been first reported to infect Malus, Pyrus and Cydonia spp. (3). Later ASSVd was isolated from the foliage of an infected apple, pear, peach and apricot trees and its RNA was sequenced (4, 5). The Symptoms inflicted by ASSVd are restricted to fruits and include color dappling, cracking, scarring and distortion depending upon the cultivar, render the fruit unmarketable (6). Apple is one of the most important commercial fruit crop and is grown all over the world. In northeastern and northwestern Iran climatic conditions are well suited for commercial production of apple. Symptoms of ASSVd infection have been observed in the past few years in several cultivars of apple in Chenaran and Ghuchan areas in Khorasan–Razavi province in northeastern Iran (7). The Iranian isolates of ASSVd have been shown to be 329 to 334 nucleotide in length (7).

HSVd belongs to the genus Hostuviroid and has the broadest host-range known for any viroid. HSVd was first described as the causal agent of the stunt disease of hop in Japan but since then it has been found in several plant species including some fruit trees like citrus, pear, peach, plum, apricot, almond, cherry, pomegranate and grapevine (8, 9). Some of these plants have shown specific abnormalities or appeared symptomless. The diseases known as cachexia of citrus (10-12) and dapple fruit of plums and peaches (13) have been associated with sequence variants of HSVd.

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HSVd variants has been detected in citrus, grapevine, mulberry and fig trees in Iran (14-16). The sequence of the isolate from fig has been deposited in GenBank but the report has not been published yet. In surveys, made in 2013-2014 symptoms of dapple were observed in some trees of Red Delicious apple in Neyshabour, in Khorasan Razavi (36º30′ N and 58º43′ E). Affected fruits of Red Delicious apple which showed colour dappling, cracking, scarring and distortion Fig. 1, were collected and brought to the laboratory.

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The fruits were peeled off finely and RNA was extracted from the fruit peel tissues using TRIzol (Invitroge, Carlsbade, CA) according to the manufacturer's protocol. The integrity of the viroid molecule was confirmed by electrophoresis in agarose. Return polyacrylamide gel electrophoresis (R-PAGE) and Reverse transcription (RT) polymerase chain reaction (PCR) was performed as described by Singh, 1991 (17) and Astruc et al. 1996 (8), respectively. Specific primers used for amplification of HSVd and ASSVd are shown Table 1.

To optimize resolution of viroid bands the concentration of polyacrylamide gel was adjusted to 5%. Also the sensitivity of detection was enhanced by staining the gel with silver (18) Fig 2 .

PCR products were run on 2% agarose gel, stained with ethidium bromide (1µg/ml), visualized on a UV transilluminator (Fig. 3). As the banding profiles in polyacrylamide gel for both viroids were similar in different samples the bands were randomly chosen, excised from the gel, eluted and sent for sequencing.

Fig. 1. Dapple symptoms caused by apple scar skin viroid on apple cv. Red Delicious from northeast of Iran.

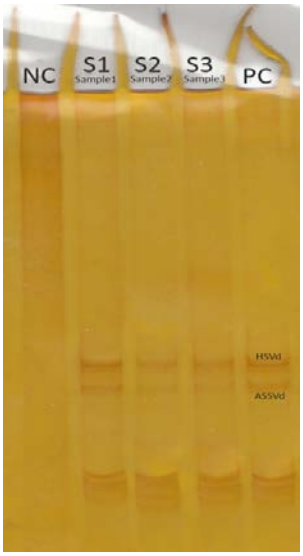

Fig. 2. Return polyacrylamide gel electrophoresis (R-PAGE) profiles of RNA extracted from apple fruits with symptoms of apple dapple disease (viroid). Lanes NC and PC are the negative and positive controls, respectively.

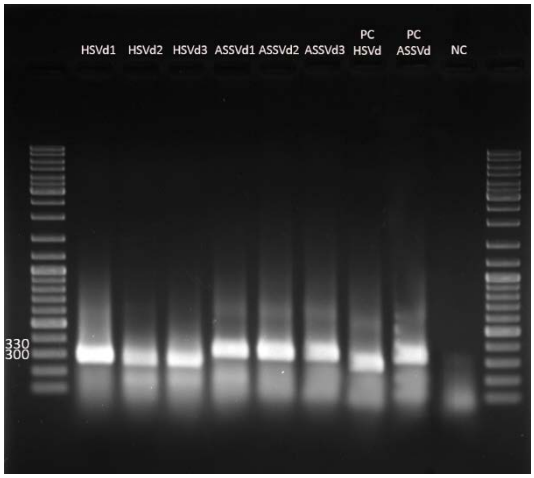

Fig. 3. Amplified RT-PCR products of RNA extracted from apple fruits with symptoms of apple dapple disease (viroid) on 2% agarose gel; the gel was stained with ethidium bromide and observed on an UV-transilluminator.

ASSVd and HSVd RNA bands were well resolved on R-PAGE, with HSVd-RNA moving faster than that of ASSVd (Fig. 2).
The PCR products obtained with The ASSVd primer set #1 yielded faint bands on the gel, whereas the PCR product obtained with primer set #2 was distinctly sharper. The expected fragments of ca 300 bp typical of HSVd and 330bp of ASSVd respectively, were detected in several samples (Fig.3).

Discussion

The sequence of the isolate of ASSVd characterized in the present study constituted of 334 nucleotide (KM213398) and appeared to be identical with the nucleotide sequence of an Iranian ASSVd isolate reported previously (7).
The HSVd variant found in the present study (KM213398) differed in one nucleotide (G insertion between the nucleotide sequence positions 154 to 155) with HSVd strain reported from pome fruits in Greece (EU925591). Vegetative propagation, particularly grafting, which is the common practice in propagation of budlings, constitutes the principal contributing factor in maintenance, perpetuation and spread of viruses and viroids including HSVd and ASSVd (19, 20).
To obtain viroid-free propagation stocks and for their maintenance, detection assays of high sensitivity are to be employed. Bioassay involving transmission to and observation of differentiating symptoms on the indicator plants are laborious and time-consuming methods of low to acceptable sensitivity, are abandoned in most cases, especially wherever molecular methods, mainly the ones employing nucleic acids, including PCR-based detection method have become available. In cases like the present study in which infections with more than one transmissible agent is involved and specially when one or more of the infectious entities produce no or not yet descriptive symptoms, one should employ more than one

Table 1: Primers used in PT-PCR for amplification and detection of Apple scar skin viroid (ASSVd) and Hop stunt viroid (HSVd).

Viroid	Primer	Number of bases	Sequence	Reference
ASSVd No. 1	sense	17	5´-CTCGTCGTCGACGAAGG-3´	Za et al., (2008)
	antisense	22	5´-CAGCACCACAGGAACCTCACGG-3´	Zhoa et al., (2008)
ASSVd No. 2	sense	23	5´-CCGGTGAGAAAGGAGCTGCCAGCA-3´	Wang et al., (2012)
	antisense	15	5´-CCTTCGTCGACGACGA-3´	Wang et al., (2012)
HSVd	sense	18	5´-GGCTCCTTTCTCAGGTAAG - 3´	This work
	antisense	24	5'-CCGGGGCAACTCTTCTCAGAATCCA-3'	This work

detection method, preferably include one sensitive nucleic acid-based method. To the best of our knowledge, this report presents the first molecular evidence of multiple infection of apple with HSVd and ASSVd. The impact of this combination on the type and severity of symptoms, whether additive, synergistic or else, awaits to be determined.

Compliance with Ethical Standards:

- We hereby confess that there are no conflict of interest, whatsoever, among us on publication of the manuscript, including the order of authors, affiliations and the content.
- This research is not involving Human Participants and/or Animals.

References

1. Diener TO. The viroid: biological oddity or evolutionary fossil? Advances in virus research. 2001;57:137-84.
2. Li SF, Sano, T. Molecular biology of viroid, Nongye Shengwu Jishu Xuebao. Journal of Agricultural Biotechnology. 2000;8(3):48-53.
3. Koganezawa H, Yanase H, Sakuma T, editors. Viroid-like RNA associated with apple scar skin (or dapple apple) disease. XII International Symposium on Fruit Tree Virus Diseases 130; 1982.
4. Chen W, Tien P, Lin L, Wang G, Liu F. Study of viroid RNA isolated from apple scar skin disease tissues. Chinese Journal of Virology. 1986;2(4):79-84.
5. Wang Y, Zhao Y, Niu J. Molecular identification and sequence analysis of the Apple Scar Skin Viroid (ASSVd) isolated from four kinds of fruit trees in Xinjiang Province, China. Molecular Pathogens. 2012;3(1):12-8.
6. Koganezawa H, Yang X, Zhu SF, Hashimoto J, Hadidi A. Apple scar skin viroid in apple. Hadidi AF, R. Randles, J. W. Semancik, J. S., editor2003. 137-41 p.
7. Yazarlou A, Jafarpour B, Habili N, Randles J. First detection and molecular characterization of new Apple scar skin viroid variants from apple and pear in Iran. Australasian Plant Disease Notes. 2012;7(1):99-102.
8. Astruc N, Marcos JF, Macquaire G, Candresse T, Pallás V. Studies on the diagnosis of hop stunt viroid in fruit trees: identification of new hosts and application of a nucleic acid extraction procedure based on non-organic solvents. European Journal of Plant Pathology. 1996;102(9):837-46.
9. Shikata E, editor Electron-microscopic studies on plant-virus and viroid RNAs. Journal of electron microscopy; 1990: Japanese soc electron micro bus center acad soc Japan 16-9 Honkomagome, 5-Chome Bunkyo-KU, Tokyo 113, Japan.
10. Diener T, Smith D, Hammond R, Albanese G, La Rosa R, Davino M. Citrus B viroid identified as a strain of hop stunt viroid. Plant disease (USA). 1988;72(8):691-3.
11. Semancik J, Roistacher C, Rivera-Bustamante R, Duran-Vila N. Citrus cachexia viroid, a new viroid of citrus: Relationship to viroids of the exocortis disease complex. Journal of General Virology. 1988;69(12):3059-68.
12. Levy L, Hadidi A, editors. Direct nucleotide sequencing of PCR-amplified DNAs of the closely related citrus viroids IIa and IIb (cachexia). Proc Conf Int Organ Citrus Virol, 12th IOCV, Riverside, CA; 1993.
13. Sano T, Hataya T, Terai Y, Shikata E. Hop stunt viroid strains from dapple fruit disease of plum and peach in Japan. Journal of General Virology. 1989;70(6):1311-9.
14. Alavi S, Ahounmanesh A, Rahimian H. Nucleotide Sequence of Hop Stunt Viroid cDNA from Cachexiainfected Citrus Plants in Mazandaran Province. Iranian Journal of Plant Pathology. 2007;43(1):49-64.
15. Zakiaghl M. IK. Identification and partial molecular characterization of grapevine viroids in Fars province. Iranian Journal of Plant Pathology. 2010;46(3):249-62.
16. Amiri Mazhar M, Bagherian SAA, Izadpanah K. Variants of Hop stunt viroid Associated with Mulberry Vein Clearing in Iran. Journal of Phytopathology. 2014;162(4):269-71.
17. Singh R, P. Return-polyacrylamide gel electrophoresis for the detection of viroids. Viroids and Satellites: Molecular Parasites at the Frontier of Life1991. p. 89-118.
18. Igloi G. A silver stain for the detection of nanogram amounts of tRNA following two-dimensional electrophoresis. Analytical biochemistry. 1983;134(1):184-8.
19. El-Dougdoug KA, Osman M, Abdelkader H, Dawoud R, Elbaz R. Elimination of Hop stunt viroid (HSVd) from infected peach and pear plants using cold therapy and chemotherapy. Aust J Basic Appl Sci. 2010;4:54-60.
20. Hadidi A, Barba M, Candresse T, Jelkmann W. Virus and virus-like diseases of pome and stone fruits: St. Paul: The American Phytopathological Society Press; 2011.