New Disease Reports

# First report of *Apple dimple fruit viroid* in apple trees in Iran

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***Received:*** *18 Oct 2016.* ***Published:*** *12 Jan 2017.* ***Keywords:*** *Malus domestica*

During inspections in July 2016, ten sets of three stem cuttings were collected from different parts of apple trees (*Malus domestica*) cv. Red Delicious showing dappling, crinkling and irregular yellow spots on their fruits. The samples were collected from two orchards in the area of Maragheh, northwest Iran and viroid-like symptoms were observed in approximately 5% of the trees in the surveyed orchards.

Total RNA was extracted from leaf and bark of shoots using an RNeasy Plant Mini Kit (Qiagen, Germany) and preparations were subjected to RT-PCR with the apscaviroid-specific PCR primers, PBCV100C and PBCV194H which amplify the central conserved region of viroids in this genus (He *et al*., 2010). Synthesis of cDNA was done using a RevertAid First Strand cDNA Synthesis Kit (ThermoScientific, USA). PCR thermocycling involved 35 cycles of denaturation at 94°C for 30 s, annealing at 58°C for 45 s and extension at 72°C for 1 min, followed by a final extension at 72°C for 10 min. PCR amplicons were analysed by electrophoresis on a 1.2% agarose gel and viewed with an UV transilluminator after staining by ethidium bromide. A 215 bp fragment was amplified from six of the ten samples, while there was no amplification in negative controls. The amplicons from two positive samples (designated as IRN1 and IRN2) were directly sequenced in both directions and the sequences were assembled using the ContigExpress programme included in the Vector NTI package (Invitrogen, USA). The BLAST results showed that both IRN1 and IRN2 isolates have 96% identity with the ADFVd-10 isolate of *Apple dimple fruit viroid* (ADFVd) from Italy (GenBank Accession No. EF088666).

In order to amplify the full length of the viroid, a specific primer pair, AD5/AD6 (Di Serio *et al*., 2002) was used. Bands of the expected size (306 bp) were amplified from extracted RNA using the above-mentioned conditions and directly sequenced in both directions. The IRN1 and IRN2 sequences were deposited in GenBank (KX909575 and KX909576, respectively). BLAST analysis of sequences from this study with those of full length ADFVd sequences (e.g. EF088660, EF088661 and EF088666) showed identities ranging from 79-99%. To our knowledge, this is the first report of ADFVd in apple trees in Iran.

## References

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Figure 1

**To cite this report**: Roumi V, Gazel M, Caglayan K, 2017. First report of *Apple dimple fruit viroid* in apple trees in Iran. *New Disease Reports* **35**, 3. http://dx.doi.org/10.5197/j.2044-0588.2017.035.003

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