Research Article

Using Reverse Transcription-Polymerase Chain Reaction (RT-PCR) for Determination of Apple mosaic ilarvirus (ApMV) in Hazelnut (Corylus avellana L.) Cultivars

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Abstract

Apple mosaic ilarvirus (ApMV) is a very detrimental pathogen that the production of apple and hazelnut. The virus is spreaded with vegetative plant materials and it is gradually distributing in both pome fruits, hazelnut and oil rose plantations in Turkey. In this study, Symptomatically ApMV infected and uninfected hazelnut leaves obtained from Hazelnut Research Station, Giresun – Turkey and Total RNA of hazelnut cultivars ("Fosa", "Mincane", "Palaz" and "Tombul") was extracted according to lithium chloride based protocol and we use one tube RT-PCR technique for molecular determination of ApMV in some symptomatically infected Turkish hazelnut cultivars. The results showed that RT-PCR can be used efficiently for the determination of ApMV.

ABBREVIATIONS

ApMV: Apple mosaic ilarvirus; DAS-ELISA: Double Antibody Sandwich Enzyme-Linked ImmunoSorbent Assay; RT-PCR: Reverse Transcription-Polymerase Chain Reaction

INTRODUCTION

Plant viruses cause major losses to several agricultural and horticultural crops around the world. Unlike other plant pathogens, there are no direct methods available yet to control viruses and, consequently, the current measures rely on indirect tactics to manage the viral diseases. Hence, methods for detection and identification of viruses, both in plants and vectors, play a critical role in virus disease management. Diagnostic techniques for viruses fall into two broad categories: biological properties related to the interaction of the virus with its host and/or vector (e.g., symptomatology and transmission tests) and intrinsic properties of the virus itself (coat protein and nucleic acid; [1]).

Recent advances in biotechnology and molecular biology have played a significant role in development of rapid, specific and sensitive assays for detection of plant viruses. Nucleic acid based methods have increasingly been used in recent years to develop diagnostic assays for plant pathogens. These methods have the potential to be very sensitive and highly specific and are based on the unique nucleic acid sequence of the pathogen [2,3].

Inexpensive and effective nucleic acid extraction methods have already been described, including total RNA, double stranded RNA (dsRNA) and DNA extractions from plant material [4,5].

The most economically damaging Ilarvirus affecting hazelnut on a worldwide scale is related to Apple mosaic ilarvirus (ApMV). ApMV is present worldwide and preferably exist on woody hosts such as apple, apricot, cherry, almond, plum, and peach [6]. The research of hazelnut infestation with ApMV is conducted mostly in the countries where the plant is cultivated commercially: USA, Spain, Italy, and Turkey. Thus, it is important to detect ApMV infection in hazelnut. Although virus infection can be screened symptomatically and DAS-ELISA, the most suitable method for detection of even the low titration of the virus is the usage of reverse transcription polymerase chain reaction [7,8]. Therefore, we aimed to develop an efficient procedure for detection of ApMV in some symptomatically infected Turkish hazelnut cultivars ("Fosa", "Mincane", "Palaz" and "Tombul") via RT-PCR technique in this study.

MATERIALS AND METHODS

Plant Material

Symptomatically ApMV infected and uninfected leaves and young stems of Turkish hazelnut cultivars ("Fosa", "Mincane", "Palaz" and "Tombul") obtained from Hazelnut Research Station,
Giresun – Turkey in April to May. Ten to sixteen samples for each cultivar (symptomatically infected or uninfected) were analysed for virus determination. Symptomatically infected hazelnut leaves develop pale yellow to bright cream colored areas as they expand during early spring. Mosaic areas can be irregular or occur in bands along major veins. Growth may be retarded and yield reduced in sensitive cultivars.

RNA Isolation

Total RNA of hazelnut cultivars was extracted according to lithium chloride based protocol [9]. Each sample (0.6 g fresh leaves) was homogenized in a roller press with 5 volumes of buffer (200 mM Tris-HCl, pH: 8.5, containing 1.5 % SDS, 300 mM lithium chloride, 10 mM EDTA, 1% sodium deoxycholate, 1% igepal) and 0.5 % 2-mercaptoethanol. The extract was collected into a 1.5 ml eppendorf tubes, half a volume of 5 M potassium acetate at pH: 6.5 was added and the mixture centrifuged at 1200 g for 15 min. Nucleic acids were precipitated from the supernatant with isopropanol and centrifuged as described above. The pellet was resuspended after drying in 25μl of steril water and treated with DNase I (1U/μl; Fermentas) at 65°C for 15 min.

RT-PCR

Two oligonucleotide primers were used in this study;

Sense: 5’-ATC CGA GTG AAC AGT CTA TCC TCT AA-3’

Antisense: 3’-GTA ACT CAC TCG TTA TCA CGT ACA A-5’ (primer position 1474–1499, 1711–1735; product size 262 bp, accession number U15608; [10])

The reverse transcriptase (RT) and PCR reactions were performed following a single non interrupted thermal cycling programme [11]. Each reaction contained the RNA template (50 ng); primers (20 pmol-μl−1), dNTP (0.4 mM); MgCl₂ (1.5 mM); 2.5 μl reaction buffer (1x). 1 unit of Taq DNA polymerase (Fermentas) and 20 unit RT (Fermentas). The sequence of the primers was indicated below. The total volume of 25 μl was subjected in a thermocycler (AB Applied Biosystems; Veriti 96 well thermalcycler) to the following program: 30 min at 42°C, 3 min 95°C, then 40 cycles of 92°C for 30 sec, 54°C for 30 sec, and 72 for 1 min and finally 5 min at 72°C. PCR products were electrophoresed in a 1.5 % agarose gel and stained with ethidium bromide.

RESULTS AND DISCUSSION

There are only a few reports on apple virus and virus like diseases based on symptomatology, biological indexing and electron microscopy in Turkey [12]. ApMV is a wide spread pathogen in Turkish cultivars and it has been determined before using ELISA technique in hazelnut [13], in apple [14], cherry [15], androsa [16].

Recently [17], analysed Turkish and Ukrainian ApMV isolates and compared them for coat protein composition and same researcher and co-workers reported distribution and molecular detection of ApMV in apple and hazelnut in Turkey [18].

In parallel, this study shows that the potential of RT-PCR for the detection of ApMV and the prevalence of the virus in four Turkish hazelnut cultivars (Figure 1). 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 ApMV infected plants amply reveals the necessity to test more samples to determine the infection rate of the virus in Turkey. ApMV causes obvious symptoms on most apple and hazelnut cultivars and it may not be a big danger in Turkey. Natural vectors of ApMV for spreading have not been described, and obtaining of virus free reproductive and planting material and its usage for the set up of new plantings are key for efficient virus control. Sensitive, reliable and rapid methods for virus detection are most important for the production of virus free plant material. The RT-PCR assay offers a

![Figure 1](image-url)
very effective and reliable detection method for the determination of ApMV rate.

CONCLUSION

Hazelnut (*Corylus avellana* L.), which is an important agricultural product of Turkey. However, hazelnut can be infected by several viruses that can affect its production. The most economically damaging *ilavirivirus* affecting hazelnut on a worldwide scale is related to *Apple mosaic ilavirivirus*. The present results indicate that a sensitive analysis like RT-PCR for the detection of ApMV can be used easily in the certification program. On the other hands, a definite conclusion on the rate of this virus can only be made after detailed scanning of large numbers of hazelnut cultivars. Virus elimination from infected mother plants is a significant matter for the certification system of nursery material for hazelnut growing in Turkey.

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REFERENCES