

Review Article

<https://doi.org/10.20546/ijcmas.2018.704.281>

Ilarviruses and the Importance of Certified Elite Planting Material in Apple Production System - An Overview

Shelly Kapoor¹, Abhilasha Sharma², Bunty Shylla³ and Anil Handa^{2*}

¹Department of Biotechnology, ²Department of Plant Pathology, Dr. Y S Parmar University of Horticulture and Forestry, Nauni- 173230, Solan, Himachal Pradesh, India

³Horticulture Research & Training Station and KVK, Kandaghat, Himachal Pradesh, India

**Corresponding author*

ABSTRACT

A wide range of graft transmissible pathogens (GTPs) of viral, viroid and phytoplasma etiology affect apple trees resulting in diseases with adverse effects in orchards around the globe. The significance of diseases caused by these GTPs on tree health, fruit shape and quality has resulted in the imposition of legislation based quarantine measures at both domestic and international front. Losses resulting from viruses in apple often remain unnoticed as the impact of these pathogens on productivity is evident over a period of time. Out of all the viruses infecting apple, ilarviruses infecting apple are of utmost importance because of their worldwide occurrence and latent nature in a number of hosts. Since effective management strategies largely depend on the correct identification of diseases and their etiological agents, diagnosis and detection are the most important aspects of managing viruses in an economically viable apple production system. Early detection of viruses in the propagative material is a pre-requisite for checking their effective spread and to guarantee a sustainable fruit production system. Many quarantine programs are in place to reduce inter-continental spread of viruses during international exchange of germplasm. All these phytosanitary measures are overseen by governments based on agreements produced by international organizations. Additionally, certification schemes applied to fruit trees allow the production of planting material of known variety and plant health status for local growers by controlling the propagation of pathogen-tested mother plants. They ensure to obtain propagative material not only free of quarantine organisms under the national legislation but also of important non-quarantine pathogens. Research carried out on the description of ilarviruses (the most important viruses associated with apple industry) with respect to their geographical distribution, taxonomic position, virion properties, host range, symptomatology, transmission, detection and certification schemes for the production of virus indexed elite planting material of apple has been reviewed and discussed in the preceding paragraphs.

Keywords

Graft transmissible pathogens (GTPs),
Apple

Article Info

Accepted:
20 March 2018
Available Online:
10 April 2018

Introduction

Temperate fruits include pome (apple, quince, and pear) and stone fruits (apricot, peach, plum, almond, and cherry) and are members of the family Rosaceae. These fruits are valued for their attractiveness, taste, nutritional quality and as a source of earning foreign exchange. Cultivation of temperate fruits is the mainstay of the economy of the hill farmers of India, though their national productivity is far below the international level.

Among all temperate horticulture crops, apple (*Malus × domestica* Borkh.) is the most important fruit crop. China is the largest apple producing country in the world whereas India ranks third in apple productivity with an area of 3,05,000 ha and production of 22,65,000 MT (Anonymous 2017). In India, apple is mainly cultivated in North Western Himalayan region which include states of Jammu and Kashmir, Himachal Pradesh and Uttarakhand in addition to the North Eastern hilly states of the country. Jammu and Kashmir is the leading apple growing state covering an area of 1,62,971 ha with production and productivity of 17,26,834 MT and 10.1 MT/ha, respectively followed by Himachal Pradesh that covers an area of 1,07,700 ha with production and productivity of 7,38,700MT and 6.9 MT/ha, respectively (Anonymous 2017). Sixty six percent of the apple production in India is contributed by Jammu and Kashmir followed by Himachal Pradesh and Uttarakhand accounting for 29.6 and 3.1 percent, respectively. Any losses occurring to crop would affect the economy and livelihood of many growers for whom apple is the only cash crop.

Various factors such as unfavourable climatic conditions, unsuitable varieties, inadequate pollinizers, lack of pollinators, inadequate nutrition, poor soil conditions, poor canopy

management, old orchards, and low planting density, besides pathological and entomological problems are described to be responsible for low productivity. Climate is the most significant environmental variable affecting the production of apple as it needs low temperatures to break dormancy. The chilling requirement varies from species to species. It is an established fact that changes in climatic condition have a major impact on the qualitative and quantitative aspects of apple fruit production as presence of pathogens is influenced by changes in prevailing climatic conditions which in turn increase the risk of introduction of exotic diseases. It is particularly important in case of systemic pathogens like viruses, viroids and phytoplasma infecting apple which are of worldwide distribution.

Apple crop is attacked by a wide range of fungal, bacterial and viral pathogens and amongst the pathological problems, viral diseases need more attention because virus infection is systemic in nature which passes to the successive generations through the propagating material thus causing decline in health of trees. Unlike bacterial and fungal pathogens, viruses cannot be controlled or eliminated by chemical means. Viral pathogens, particularly members of the genus *Ilarvirus*, cause latent infection which generally remain unnoticed and gradually result in the loss of plant vigour, reduction in quality of produce as well as yield of the crop. In perennial crops, damage is more profound in comparison to annuals (Nemeth 1986; Cambeli *et al.*, 2003; Cieslinska and Rutkowski 2008).

Ilarviruses in apple fruit trees

Losses resulting from viruses in apple often remain unnoticed as the impact of these pathogens on productivity is evident over a period of time. Out of all the viruses infecting

apple, ilarviruses infecting apple are latent (produce no visible symptoms) in nature in a number of hosts and their infection often results in retarded plants growth, fewer small sized fruits with a reduced shelf life or other impacts that often go unnoticed (Hadidi and Barba, 2011). These viruses cause a wide range of symptoms, ranging from symptomless (latent) to a general decline in vigour and productivity. Leaf symptoms include distortion or twisting, mottling, rolling, necrotic spots, shot holes, and unusual color patterns. Fruits may show reductions in size and quality. Economically, ilarviruses induce financial losses and have enormous economic and social impacts to all components of apple production chain. Ilarviruses induce significant losses in orchards reducing the sustainability of many commercial orchards in different regions of the world. The major economically important ilarviruses identified on apple are apple mosaic virus and prunus necrotic ringspot virus. Besides these two ilarviruses, prune dwarf virus (PDV) has also been reported to be infecting apple trees though the prevalence of PDV is relatively less. A detailed description of these ilarviruses with respect to their geographical distribution, taxonomic position, virion properties, host range, symptomatology, transmission, detection and certification schemes for the production of virus indexed elite healthy planting material of apple has been critically scanned and presented in this review article.

Apple Mosaic Virus (ApMV)

Apple mosaic virus is a member of subgroup III of the *Iilarvirus* genus in the *Bromoviridae* family (Alrefai *et al.*, 1994). This family also includes prunus necrotic ringspot virus (PNRSV) and rose mosaic virus (Rybicki 1995). Mosaic disease of apple is one of the oldest known and most widespread diseases caused by viruses. Apple mosaic virus is

economically important and is a common pathogen in commercial cultivars (Mink 1989 and Stouffer 1989). The virus is known by different names such as apple infectious variegation virus, rose infectious chlorosis virus, rose mosaic virus, european plum line pattern virus.

Geographical distribution and economic importance

Apple mosaic virus (ApMV), an important virus of apple is prevalent in almost all apple growing countries. ApMV was first reported in *Rosa* sp. and *Malus domestica* from the USA by White (1928) and Bradford and Joley (1933). It is a common pathogen in commercial cultivars of apple and is economically important because considerable losses have been reported in apple due to its infection. Singh *et al.*, (1979) from India studied the effect of apple mosaic virus on the growth, yield and quality of apple in 30 year old ApMV infected and healthy apple trees and found that shoot growth, fruit set, fruit weight, yield/tree and fruit ascorbic acid content were reduced by ApMV infection. ApMV was reported to decrease bud take of cvs. Jonathan, Jonared and Golden Delicious, but not that of Idared. Infected trees of all 4 cvs. had fewer laterals and their growth was limited. Symptoms were more pronounced on leaves of Jonared, Jonathan and Idared in the second year after budding than during the first year of growth.

The virus decreased bud take in three apple varieties but not in Idared. Most commercial cultivars are known to be affected with variable severity of symptoms. 'Golden Delicious' and 'Jonathan' are severely affected, whereas 'Winesap' and 'McIntosh' are only mildly affected. Except in severe cases, infected trees can still produce a crop and yield reductions may vary from 0 to 50 percent (Rebandel *et al.*, 1979).

In some cultivars, bud set is severely affected. Apart from that, the presence of ApMV was found to reduce the growth of apple trees (Chamberlain *et al.*, 1971), increase the height of the climacteric and decrease the content of malic acid (Makarski and Agrios 1973).

The virus was also reported to decrease girth of trees (Thomsen, 1975), decrease bud take by 3-20 percent along with stunted growth (Rebandel *et al.*, 1979) and reduction in the quality and quantity of pollen (Lemoine, 1982) in infected apple trees. Forty six percent reduction in yield in 'Golden Delicious' and 9 percent reduction in McIntosh cultivar due to infection with ApMV was reported by Cambeli *et al.*, (2003).

Symptomatology

Infection by ApMV is characterized by chlorotic or, more often, bright yellow discolourations of the leaves in the form of blotching, mottling, vein banding or yellowing, ringspots, line and oak leaf patterns, seldom accompanied by evident deformation of the leaf blades (Fulton 1972, 1980). Pale to bright cream coloured yellow areas develop on apple leaves as they expand during early spring. The mosaic areas may be irregular in outline or may occur in bands along major veins.

These areas often become chrome yellow or white as the season progresses. Fridlund (1989) reported that affected leaves may be interspersed with normal leaves on individual shoot. Leaves which exhibit strong mosaic symptoms in the early season may develop large necrotic areas during period of high temperature or increase sunlight and drop prematurely. In infected plants, symptoms are usually outstanding in spring but tend to fade away as the season progresses and are little or not evident on summer vegetation (Halk *et al.*, 1984; Imed *et al.*, 1997).

Transmission and host range

No insect vector is known to transmit ApMV but the virus is transmitted by pollen, vegetative propagation from infected trees or by mechanical sap inoculation to herbaceous hosts (Nemeth 1986). Dhingra (1972) reported that apple mosaic virus was transmitted by both natural and artificial root grafting between apple seedlings in glasshouse experiments and also by natural root grafting in the nursery. Simple intertwining of the roots of adjacent plants could not transmit the virus from infected to healthy plants.

The virus can infect a number of plants and has a wide host range. Experimentally or naturally, it has infected over 65 species in 19 families (Fulton, 1952, 1965). Under experimental conditions, susceptibility to infection is reported in host species of *Apocynaceae*, *Corylaceae*, *Leguminosae*, *Papilionoideae*, *Rosaceae* and *Solanaceae* families. Species susceptible to experimental virus infection include *Catharanthus roseus*, *Corylus avellana*, *Malus sylvestris*, *Nicotiana tabacum*, *Phaseolus vulgaris*, *Cucumis sativus* (cucumber), *Torenia fournier*, *Vinca rosea* (periwinkle) and *Vigna sinensis* (cowpea). *Malus sylvestris* (apple) cv. Lord Lambourne and Jonathan develop a prominent mosaic and are recommended as woody indicators.

Diagnosis and Detection

Serological detection

ELISA method was successfully used for the detection of 41 isolates of *ilarvirus* in *Prunus* sp. and *Malus* sp. representing the entire symptomatic and serological range of prunus necrotic ringspot, apple mosaic virus and prune dwarf virus (McMorran and Cameron 1983). Barba (1986) used Direct ELISA tests for detecting the viruses in different parts of

two naturally infected almond trees at different times of year. PNRSV was detected by ELISA in all tested plant parts with seasonal variations. Detection of the virus from the leaves was not possible after June. Seeds always gave a positive reaction. Sap transmission tests gave positive reactions for PNRSV only in flowers. ApMV was detected by ELISA both in leaves and seeds. Sap transmission was positive only during the spring from leaves bearing symptoms.

Systemic studies on the distribution of apple mosaic virus in the main apple growing areas of the Czech Republic by employing ELISA to detect ApMV in symptomatic apple plants in almost all the gardens tested were conducted to detect ApMV, ACLSV and ASGV in apple leaf extracts (Polak, 1994). Out of 220 samples collected, 34 (15.45 percent) were infected with ApMV, 63 (28.6 percent) were infected with ACLSV and 52 (23.6 percent) with ASGV. Apple trees which gave positive reactions to ELISA were also tested on herbaceous test plants (Fidan, 1994). Incidence of apple mosaic virus was surveyed in 140 apple orchards in Tarragona and Girona provinces. Mosaic symptoms on leaves were observed during spring. ELISA analysis revealed that most trees of cv. Golden Delicious were infected with the mosaic disease (Rovira and Aramburu, 1998).

Multiplex RT-PCR assays are capable of detecting a range of different virus isolates from various geographic origins throughout the year. Viruses were detected reliably in composite extracts at a ratio of one part total nucleic acid extract from an infected sample mixed with 39 parts of extract from healthy samples (from *Malus*, *Pyrus*, *Prunus* and *Pyronia* sp.). Based on bioassays conducted with *Nicotiana benthamiana*, *N. tabacum* and *Chenopodium quinoa*, the use of the internal control minimizes the risk of obtaining false negative RT-PCR results which is desirable

for routine testing and avoids the need to eliminate contaminating DNA in extracts. The multiplex RT-PCR assays described are reliable, rapid and sensitive methods for the detection of these viruses and may replace techniques need commonly like indexing by woody indicators or ELISA (Menzel *et al.*, 2002).

In serological surveys conducted to determine the distribution of ApMV in Turkey by using DAS-ELISA one hundred sixteen plant samples were collected from 24 orchards and were tested by DAS-ELISA which revealed the presence of ApMV in 6.9 percent of the leaf samples (Ylmaz *et al.*, 2005). The virus was detected in 15.09 percent of the apple plants at two orchards. Polak *et al.*, (2008) detected four pome fruit viruses in germplasm collection in the Czech Republic by using ELISA as well as pentaplex RT-PCR. A total of sixty-eight accessions covering native and foreign cultivars were tested for the presence of the main pome fruit viruses: apple stem pitting virus (ASPV), apple stem grooving virus (ASGV), apple chlorotic leaf spot virus (ACLSV) and apple mosaic virus (ApMV). Regardless of the cultivars or the origin, different combinations of mixed infections of viruses were found in infected samples. All positive samples detected by ELISA were confirmed by pentaplex RT-PCR; however, RT-PCR revealed more infected trees than by ELISA. ACLSV and ASPV were the most prevailing viruses in apple and pear, the two viruses were detected in almost all mixed infections. Twenty-eight out of 29 apple accessions representing fifteen cultivars were carrying mixed infection. The most prevailing viruses of apple were ACLSV (96.5 percent), ASPV (89.7 percent) and ASGV (34.5 percent) whereas infection of ApMV was detected only in one apple tree.

In a study conducted in Turkey by Birisik *et al.*, (2008) on the incidence of apple (*Malus*

domestica) viruses, a total of 108 orchards and 10 varietal collections were visited in the districts of Adana, Antalya, K. Maras and Osmaniye. Some 413 samples of leaves and/or dormant cuttings were obtained from apple trees. Sanitary testing was conducted by ELISA, biological indexing, and RT-PCR. All samples were tested by ELISA for the presence of apple chlorotic leaf spot virus (ACLSV), apple stem grooving virus (ASGV) and apple mosaic virus (ApMV). The overall virus infection rate as revealed by ELISA was 18.8 percent. The prevailing viruses were ACLSV (10.6 percent), ASGV (5.0 percent) and ApMV (3.1 percent). Biological indexing was conducted with the indicators *Malus pumila* cultivars Virginia Crab and Radiant and R 12740 7A against apple viruses in Turkey. Biological indexing revealed higher rates of ACLSV (46.8 percent), ASGV (60.8 percent) and apple stem pitting virus (ASPV; 54.5 percent) infection. RT-PCR tests also confirmed the presence of ASPV, ASGV and ACLSV detected previously by ELISA and biological indexing. This preliminary survey reveals high rates of virus infection in apple in eastern Turkey. Concentrations of ApMV varies in different plant parts as indicated by Svoboda and Polak (2010) who used leaves, flower petals, dormant buds and phloem tissues for the detection of ApMV and reported the highest relative virus concentration in young leaves in April before flowering.

Molecular detection

The complete nucleotide sequence of ApMV has been characterized from several parts of the world (Alrefai *et al.*, 1994; Shiel *et al.*, 1995; Shiel and Berger 2000; Petrzik and Lenz 2002). Saade *et al.*, (2000) developed nonisotopic molecular hybridization and multiplex reverse-transcription polymerase chain reaction (RT-PCR) methodologies that could detect ApMV, PNRSV and ADV in 5

stone fruits simultaneously. For RT-PCR, a degenerate antisense primer was designed which was used in conjunction with three virus-specific sense primers. The amplification efficiencies for the detection of the three viruses in the multiplex RT-PCR reaction were identical to those obtained in the single RT-PCR reactions for individual viruses.

A sensitive and reliable multiplex RT-PCR-ELISA technique for the detection of apple chlorotic leaf spot virus, apple stem pitting virus, apple mosaic virus and apple stem grooving virus was developed by material Menzel *et al.*, (2003). Roussel *et al.*, (2004) developed RT-PCR protocols suitable for a routine diagnosis of latent and ilarviruses in fruit tree certification. This technique was simplified by using crude plant extracts instead of total RNA preparations and by the analyses of pooled samples. Paunovic and Jevremovic (2008) detected pome fruit viruses (ACLSV, ASPV, ASGV and ApMV) in twenty pome fruit cultivars (11 apple, 6 pear and 3 quince cultivars) through the use of different methods. The reliability of virus detection was tested by biological indexing under field and the results were compared with those of laboratory DAS-ELISA tests and RT-PCR method for ASPV and ASGV. The viruses, either individually or in mixed infection, were detected in 7 of the cultivars. ApMV was not detected in any of the cultivars by biological and laboratory testing. With regard to the detection of ACLSV, the results were not dependent on the detection technique and the use of DAS-ELISA for routine testing was justified. The results also suggested that ASPV and ASGV may be positively and more rapidly detected by RT-PCR. Both viruses were detected by DAS-ELISA tests in flower petals of all infected apple cultivars. Since petals were not always available during certification process, routine usage of DAS-ELISA was rendered unreliable in detection of

these viruses. Various other workers (Seigner, 2000; Petrzik and Lenz, 2002; Hou *et al.*, 2004; Petrzik, 2005; Caglayan *et al.*, 2006; Polak *et al.*, 2006; Yardmc *et al.*, 2008; Thokchom *et al.*, 2009) also reported the use of RT-PCR for successful detection of different temperate fruit viruses.

Prunus Necrotic Ring Spot Virus (PNRSV)

Prunus necrotic ring spot virus (PNRSV) was first reported in peach from USA by Cochran and Hutchins (1941) and the name PNRSV was given by Allen (1941). It is a member of the subgroup 3 of the genus *Illarvirus* in the family *Bromoviridae* (Bujarski *et al.*, 2012). The virus is known by various names like European plum line pattern virus, hop B virus, red currant necrotic ring spot virus, rose vein bending virus, rose yellow vein mosaic virus and sour cherry necrotic ring spot virus (Fulton 1985). The virus is of worldwide occurrence and is prevalent in many countries viz., Jordan (Salem *et al.*, 2003); India (Kulshrestha *et al.*, 2005); Egypt (Salam *et al.*, 2007); Croatia and Czech Republic (Sucha and Svobodova, 2010); Albania, Bosnia, Herzegovina, Montenegro, Serbia, Turkey and Ukraine (EPPO PQR, 2012) and Brazil (Fajardo *et al.*, 2015). Other synonyms of the virus are European plum line pattern virus, hop B virus, red currant necrotic ring spot virus, rose vein bending virus, rose yellow vein mosaic virus and sour cherry necrotic ring spot virus.

Geographical distribution and economic importance

Prunus necrotic ring spot virus (PNRSV) is an economically important virus and is a common pathogen in commercial peach cultivars (Brunt *et al.*, 1996). A comprehensive description of PNRSV was made by Fulton (1970) and a review of the virus was prepared by Hammond (2011).

Turkey is one of the most important stone fruit suppliers as it produces around 1.3 million tonnes of stone fruits annually (Gumus *et al.*, 2007). The studies reported the occurrence and distribution of all the stone fruit viruses and viroids in commercial plantings of *Prunus* species in Western Anatolia of Turkey and the studies concluded that PNRSV along with other viruses like PDV, PPV, ApMV, ACLSV and PLMVd were causing major losses to the growers of *Prunus* species. A total of 1732 specimens of stone fruits were tested and it was found that overall infection level with these graft transmissible agents was 30 percent with the PDV as the predominant one followed by PPV and PNRSV. PNRSV was reported to be the most common virus detected in peach and apricot trees grown throughout Algeria (Aouane, 2003).

It is a common pathogen in commercial cultivars of peach and is economically important because considerable losses have been reported in peach due to its infection. Scott (2018) studied the effect of prunus necrotic ring spot virus on growth, yield and quality of peach and found a reduction in tree growth between 12 to 70 percent and yield loss of 5 to 70 percent with fruits having lower soluble sugar content. PNRSV has also been reported to cause significant crop losses depending on the host (15 percent yield loss in sweet cherry and up to 100 percent in peach) and can reduce bud-take in nurseries, decrease growth of fruit from 10 to 30 percent and fruit yield reduction from 20 to 60 percent with delayed fruit maturity (Pallas *et al.*, 2012).

Symptomatology

Regardless of the host, infection by PNRSV remains symptomless as it is a latent virus. The virus is however, characterized by brown lines, rings and leaf curling in most of the hosts (Fulton 1970; Brunt *et al.*, 1996 and Hammond 2011).

Plate.1 ApMV symptoms on apple cv. Golden Delicious



Plate.2 Severe mosaic symptoms on apple leaves



Plate.3 PNRSV induced ringspots and chlorotic spots on apple leaves



Plate.4 Diffused chlorotic spots and rings from PNRSV infection in apple



Almaraz *et al.*, (2014) observed leaf damage in the form of yellow mottle, chlorotic rings, linear pattern, mosaic, bright yellow discolourations of the leaves in the form of blotching, mottling, vein banding or yellowing, ringspots, line and oak leaf patterns in commercial peach orchards in the Estado de Mexico. The virus is seldom accompanied by evident deformation of the leaf blades (Fulton 1980). Twenty one percent of PNRSV infected sweet cherry trees showing chlorotic ringspots that evolved to dark brown necrotic areas in both secondary veins and interveinal regions of the leaf were reported by Sanchez *et al.*, (2004). Smith *et al.*, (2009) also observed chlorotic and necrotic spots on the leaves of sweet cherry trees infected by PNRSV but the centres of these necrotic spots often disappeared, affording a shothole effect. The presence of PNRSV was also reported by (Scott 2018) in many woody hosts and observed that the infection initially causes shock later developing chronic symptoms. Symptoms can be classified as chlorosis, necrosis, leaf deformity, stunting and shot holes. Chlorosis symptoms include patterns of rings, lines, bands, spots, mottles and mosaic occurring only during the initial acute stage.

Transmission and host range

The virus is not transmissible through insect vector but is transmitted by pollen, vegetative propagation from infected trees or by mechanical sap inoculation to herbaceous hosts (Brunt *et al.*, 1996; Fulton, 1970; Hammond, 2011) and by seeds and pollen in several natural hosts, including *Prunus* sp., hops and roses and in some experimental hosts like *Cucurbita maxima* (Card *et al.*, 2007, Hammond, 2011). Yuan *et al.*, (1990) studied the transmission of PNRSV via *Cricodemella xenoplax* handpicked from the root zone of infected peach trees. The studies concluded that *Cricodemella xenoplax* failed

to transmit the virus to cucumber or peach seedlings as seedlings rootstocks remained symptomless and ELISA also showed negative results for them.

Thrips were used to test the transmission of PNRSV to cucumber and peach seedlings using thrips as vector by applying the infected plum pollen onto cucumber and peach seedlings (Greber *et al.*, 1991). It was found that Fifty six percent of virus was transmissible on to both seedlings with *Thrips tabaci* and sixty six percent with a mixture of five thrips species when pollen were taken from highly infected flower buds whereas only 7 percent transmission rate was reported when pollen was taken from flowers with less infectivity. Hence, high rate of infection in pollen is also very important for the transmission of virus to the plants.

Diagnosis and detection

Serological detection

Although many variants of agar gel immunodiffusion tests were commonly used for the detection of serological relationships among ilarviruses (Casper, 1973; Mink *et al.*, 1987; Crosslin and Mink, 1992), sometimes these failed to detect these viruses in infected plants due to very low concentrations in infected woody plants or the presence of inhibitors (Thomas, 1980). Despite all these problems, serological reactions among ilarviruses could be easily confirmed by DAS-ELISA. A preliminary survey was conducted in Tunisia to identify stone fruit viruses as diseases were occurring in orchards and mother block stands. Two ilarviruses viz., prunus necrotic ring spot virus (PNRSV) and prune dwarf virus (PDV) were detected by DAS-ELISA (Boulila and Marrakchi, 2001). Apple orchards were surveyed in various parts of Himachal Pradesh and samples from infected trees were collected on the basis of

necrotic lesions on leaves (Chandel *et al.*, 2008). DAS-ELISA was performed using antisera for PNRSV and 88-97 percent of virus infection was reported in samples taken from Kullu and Kalpa regions. Similar type of sero-surveys conducted by Kapoor and Handa (2017 b) in peach, almond, cherry, plum, nectarine and apricot resulted the presence of PNRSV in all the hosts tested except for apricot and plum. Scott *et al.*, (2001) conducted field trails to check the effects of PNRSV and PDV on peach and conclusively proved it to be Peach stunt disease. The integrity of the viral treatments was assessed using ELISA. Salem *et al.*, (2003) tested the level of PNRSV infection in almond, peach and plum cultivars over the course of entire year by testing different plant parts of naturally infected trees using the double antibody sandwich-enzyme linked immunosorbent assay (DAS-ELISA). The data revealed spring to be the best time of year for PNRSV detection in flowers, active growing buds and young leaves. Similar results were reported by Kapoor and Handa (2017 a) on the peach cv. July Elberta. Apples grown in Himachal Pradesh were found to be susceptible to many viruses and viroids (Rana *et al.*, 2011). Symptomatic apple cultivars and rootstocks were selected and analysed using DAS-ELISA. The presence of five viruses viz., ApMV, ASGV, ASPV, ACLSV and PNRSV were observed on the basis of ELISA tests. Sanchez-Perez *et al.*, (2017) investigated the status of sour and Duke cherry genetic resources in the Iberian Peninsula for the presence of PNRSV and used DAS-ELISA as the detection method and reported the highest infection rate of forty six percent in the leaf samples of both types of cherries.

Molecular detection

Coat protein gene primers in reverse transcriptase polymerase chain reaction (RT-

PCR) were used by Chandel *et al.*, (2008) for further detection of PNRSV after ELISA. Almaraz *et al.*, (2008) surveyed three locations of commercial peach orchards of Mexico on the basis of symptoms after performing DAS-ELISA. The infected sap was further inoculated onto *Chenopodium quinoa*, *C.amaranticolor*, *Nicotiana tabacum*, *N. glutinosa* and *Datura stramonium* and RT-PCR was performed using these indicator plants. Expected size amplicon of approximate 450 bp were generated from all these parts thereby clearly indicating the presence of PNRSV in the test samples. Partial characterisation of PNRSV in apple was conducted by Abdel-Salam and Mokbel (2014) in Egypt. The trees were marked on the basis of symptoms like chlorotic and necrotic ring spot along with shotholes on the leaves from the apple orchards. RT-PCR using degenerate primer pair of CP for *Ilarvirus* was successfully used for the detection and amplification of PNRSV.

Molecular detection studies by Hu *et al.*, (2016) in apple orchards to assess the incidence of apple chlorotic leaf spot virus (ACLSV), apple stem pitting virus (ASPV), apple stem grooving virus (ASGV) and apple mosaic virus (ApMV) revealed that shoot and leaf samples were drawn from 216 trees and total RNA was extracted using RT-PCR with primer pair ILAR1/ ILAR 2. An amplified fragment of 204 bp corresponding to the partial Coat Protein (cp) gene of ApMV AND PNRSV from 39 samples was obtained. It was further cloned into the pMD18-T easy vector and sequenced. BLAST results showed that all the sequences had the highest identities to CP gene of PNRSV with 78 percent identity to one isolate (L38823) from United States (Hammond and Crosslin, 1995). To confirm the PNRSV infection in apple, a 1487 bp fragment from RNA3 was amplified using primer pair PNRSV-RNA3-F1 and PNRSV-RNA3-R1 which was further cloned and

sequenced. The sequence (KU144878) from one infected tree (FSO6) showed identities of 86.3 to 99.1 percent to previously reported PNRSV genome sequences.

Prune Dwarf Virus (PDV)

Prune dwarf virus (PDV) was first described by Thomas and Hildebrand (1936) on *Prunus domestica* showing stunting and leaf malformation symptoms. In plum, PDV causes stunting and leaf malformation and shortened internodes. In Italian prune, it decreases the length of shoots, their diameter, number of leaves, and the photosynthetic total area (Hadidi and Barba, 2011). In cherry, PDV may cause leaf chlorotic spots, rings and diffuse mottling, and possibly stem pitting and flat limb. Fruits can be malformed and their production is reduced. In some apricot cultivars, PDV has been reported to induce gummosis on the trunk. In most peach cultivars, PDV induces mild stunting while leaves become dark green and more erect than those of noninfected trees, but infection by severe isolates can cause important yield reduction and poor quality of fruits. Peaches infected with both PDV and PNRSV (peach stunt disease, PSD), display bark splitting, increased sucker production and yield is reduced by up to 60 percent. The virus causes economic losses on stone fruit trees, especially in sour and sweet cherry, almond, and peach (Nolasco *et al.*, 1991; Rampitsch *et al.*, 1995; Uyemoto and Scott, 1992). PDV frequently occurs in mixed infections with other ilarviruses.

PDV is a multicomponent virus with five types of particles differing in size. Unenveloped virions vary from quasi-isometric, about 19–20 nm in diameter, to bacilliform with length up to 73 nm (Caglayan *et al.*, 2006). Several strains of PDV have been described. The virus is transmitted by grafting (buds, scions), pollen

and seed. Pollen transmission depends on many factors such as fruit tree species and circumstances affecting pollination. Pollen transmission in sweet and sour cherry shows the highest transmission rates (George and Davidson 1964; Gilmer 1965). Seed transmission occurs in sweet cherry, sour cherry, mahaleb, and myrobalan, but infection rates vary with the species (Caglayan *et al.*, 2006). PDV infection causes yield reduction and is responsible for significant losses in almond (Nolasco *et al.*, 1991), peach, and sweet cherry (Rampitsch *et al.*, 1995; Uyemoto and Scott, 1992).

Certification schemes and programmes

Plant material produced in accordance with the certification scheme is derived from nuclear stock plants that have been tested and found free from viruses and produced under conditions minimizing infection by other graft transmissible pathogens infecting apple trees. Certified plant material for export should in any case satisfy the phytosanitary regulations of importing countries, especially with respect to any of the pathogens covered under the category of quarantine pests.

There are well established certification programs and plant protection organizations in developed countries operate within the framework of these certification programmes. Some of these certification schemes are in operation since the 1960s and have been delivering high quality virus indexed propagation material preventing the introduction of viruses in the fields. NCPN-FT (formerly NRSP5 or IR-2), NCPP, and FPS in the USA, CTIFL and SOC in France, EMLA and SASA in the UK, Naktuinbouw in the Netherlands, and CVIPS in Spain are only some examples to show the investment various countries have made to promote their respective agricultural economy (Boye and Desvignes, 1984; Cutting and Montgomery,

1973; Ebbels, 1979; Navarro *et al.*, 2002; NCPN-FT 2012; Reed and Foster, 2011; Rowhani *et al.*, 2005).

By the end of the 1990s, Regional Plant Protection Organizations (RPPOs) such as the European and Mediterranean Plant Protection Organization (EPPO) and the North American Plant Protection Organization (NAPPO) began drafting technical guidelines for their member countries concerning the production of certified plant propagation material. According to EPPO, a certification scheme is a “System for the production of vegetatively propagated plants for planting, intended for further propagation or for sale, obtained from nuclear stock after several propagation stages under conditions ensuring that stated health standards are met. The filiations of the material are recorded throughout the scheme.” In line with this, NAPPO defines that a “virus-certified stock refers to plants for planting and propagation produced under an official virus testing and certification programme” (NAPPO 2013). Consequently, certification schemes are essentially quality control systems for propagating and planting material which will be officially certified by the officially delegated authorities by the issuing of a certificate or label. Through certification, propagating material is assured to be free not only from quarantine organisms but from important indigenous nonquarantine pathogens as well, in compliance with each country’s requirements as dictated by the local and international markets.

It is pertinent to note that not all pathogens or viruses can be excluded by a certification scheme. In the past, two types of categories, virus free and virus tested, have been used. The first corresponds to individual plants tested for all virus and virus-like pathogens known to infect the host in a specific region, while the second one focuses on the most important pathogens. Although the first term

is older and more popular to nurserymen, the preferred term nowadays is pathogen tested and covers only organisms particularly mentioned in the published scheme (usually viruses, viroids, and phytoplasmas). All recent EPPO certification schemes refer only to the latter category (EPPO, 2006).

Furthermore, different approaches for the production of healthy planting material of a certain cultivated plant, certification or classification, may apply. In a typical EPPO certification scheme, the certified material is descended by not more than a fixed number of steps from individual plants each of which is tested and found free from pests, and is then maintained and propagated under rigorous conditions excluding recontamination. In a classification scheme, the classified material is descended by one or more steps from material which, as a population, meets certain health standards and is maintained and propagated under conditions minimizing recontamination. Which of the approaches is appropriate for a given cultivated plant depends on considerations of cost and resources, health status required, practical possibilities for testing, rate of recontamination, and value of the final material.

Normally, the operation of certification schemes is run by official governmental authorities or officially recognized private organizations, although there are differences between countries and continents. Private companies and nurseries’ associations are not precluded from participating in an official scheme (e.g., MIVA in Italy and AVASA in Spain) (Pina *et al.*, 2012; Savino, 1992) or from running their own schemes (e.g., companies producing ornamentals) (Waterworth, 1998).

The origin of each plant should be known so that any problems of health or trueness to type

may be traced throughout the certification scheme. The use of propagation material in nurseries to produce certified plants should be checked by an official or officially authorized organization that controls the health, origin and amount of such material on the basis of field inspections and of the records and documents presented by the nursery. The nursery plant protection programme and the check inspections should also take account of other important pests that can affect quality, so that the certified plants delivered to the fruit grower are substantially free from these pests. Certified planting material for export should in any case satisfy the phytosanitary regulations of importing countries. Certified plants leaving the scheme should carry an official certificate (which may be a label) indicating the certifying authority, the plant producer and the certification status of the plants.

References

- Almaraz TD, Sanchez-Navarro J and Pallas V. 2014. Detection of *Prunus* necrotic ringspot virus in peach (*Prunus persica* L.) in Mexico and molecular characterization of its RNA component-3. *Agrociencia* 48: 583-598.
- Allen WR. 1941. *Prunus* necrotic ringspot virus in Peach. *Phytopathology* 12: 325-333.
- Abdel-Salam AM and Mokbel SA. 2014. Partial characterization of *Prunus* necrotic ringspot virus on apple in Egypt. *Egyptian Journal of Virology* 11: 280-287.
- Almaraz Torre DL, Montoya-Piña JV, Rangel AS, Camarena-Gutiérrez G and Salazar-Segura M. 2008. First Report of *Prunus* necrotic ringspot virus in Peach in Mexico. *Plant Disease* 92: 482-482.
- Alrefai RH, Shiel PJ, Domier LL, D'Arcy CJ, Berger PH and Korban SS. 1994. The nucleotide sequence of apple mosaic virus coat protein has no similarity with the other *Bromoviridae* coat protein genes. *Journal of General Virology* 75: 2847-2850.
- Aouane B. 2003. Preliminary studies on stone fruit tree viruses in Algeria. *Peach* 12: 56-286.
- Anonymous. 2017. <https://www.faostat.fao.org> (8:00 PM, 10th February, 2018)
- Barba M. 1986. Detection of apple mosaic and *Prunus* necrotic ringspot viruses in almond by ELISA. *Archives of phytopathology and plant protection* 22: 279-282.
- Birisik N, Myrta A, Hassan M and Baloglu S. 2008. A preliminary account on apple viruses in Mediterranean Region of Turkey. *Acta Horticulturae* 781: 125-130.
- Boulila M and Marrakchi M. 2001. Sequence Amplification (RT-PCR) and Restriction Fragment Polymorphism (RFLP) Analysis of Some Isolates of *Prunus* necrotic ringspot *ilarvirus*. *EPPO Bulletin* 31: 173-178.
- Boye´ R and Desvignes JC. 1984. Bilan des quinze anne´es de se´lection conservatrice du mate´rielve´ge´tale fruitier. *Fruits* 39: 637-645.
- Bradford FC and Joley L. 1933. Infectious variegation in the apple. *Journal of Agricultural Research* 46: 901-908.
- Brunt A A, Crabtree K, Dallwitz MJ, Gibbs AJ, Watson L and Zurcher EJ. 1996. Apple mosaic *Ilarvirus*. *Plant Viruses Online: Descriptions and Lists from the VIDE Database*.
- Bujarski J, Figlerowicz M, Gallitelli D, Roossinck MJ and Scott SW. 2012. Family *Bromoviridae*. In: *Virus Taxonomy: Classification and Nomenclature of Viruses*.
- Caglayan K, Serce CU, Gazel M and Jelkmann W. 2006. Detection of four apple viruses by ELISA and RT-PCR assays in Turkey. *Turkish Journal of Agriculture and Forestry* 30: 241-246.
- Cambeli T, Folwell R J, Wandschneider P, Eastwell K C. and Howell W. 2003. Economic implications of a virus prevention program in deciduous tree

- fruits in the US. *Crop Protection* 22: 1149-1156.
- Card SD, Pearson MN, and Clover GRG. 2007. Plant pathogens transmitted by pollen. *Australasian Plant Pathology* 36:455-461.
- Casper R. 1973. Serological properties of Prunus necrotic ringspot virus and Apple mosaic virus isolates from rose. *Phytopathology* 63: 238-240.
- Chamberlain EE, Atkinson JD, Hunter JA and Wood GA. 1971. Effect of apple mosaic virus on growth and cropping of "Freyberg" apples trees. *New Zealand Journal of Agricultural Research* 14: 936-943.
- Chandel V, Rana T, Handa A, Thakur PD, Hallen V and Zaidi AA. 2008. Incidence of Prunus necrotic ring spot virus on *Malus domestica* in India. *Journal of Phytopathology* 156: 382-384.
- Cichal PE and Sala-Rejczak K. 2011. Biological and molecular characterization of Prunus necrotic ringspot virus isolates from three rose cultivars. *Acta physiologiae plantarum* 33: 2349-2354.
- Cieslinska M and Rutkowki KP. 2008. Effect of apple chlorotic leaf spot virus on yield and quality of fruits from 'Golden Delicious' and 'Sampion' cultural apple trees. *Acta Horticultiurae* 781: 119-124.
- Cochran LC and Hutchins LM. 1941. A severe ring spot virosis of peach. *Phytopathology* 31: 860.
- Crosslin JM and Mink GI. 1992. Biophysical differences among Prunus necrotic ringspot ilarviruses. *Phytopathology* 82: 200-206.
- Cutting CV and Montgomery HBS. 1973. More and better fruit with EMLA. East Malling/Long Ashton: *Report of the East Malling/Long Ashton Research Station*.
- Dhingra KL. 1972. Transmission of apple mosaic by natural root grafting. *Indian Journal of Horticulture* 29: 348-350.
- Ebbels DL. 1979. A historical review of certification schemes for vegetatively-propagated crops in England and Wales. *ADAS Quarterly Review*: 32: 21-58.
- EPPO. 2006. EPPO Standards PM 4/17 (2) Schemes for the production of healthy plants for planting. *Pathogen-tested olive trees and rootstocks. Bulletin OEPP/EPPO* Bulletin 36: 77-83.
- EPPO PQR. 2012. EPPO Technical Document No. 1061. Study on the Risk of imports of plants for planting. EPPO, Paris.
- Fajardo TVM, Nascimento MB, Eiras M, Nickel O and Pio-Ribeiro G. 2015. Molecular characterization of Prunus necrotic ringspot virus isolated from rose in Brazil. *Ciência Rural* 45: 2197-2200.
- Fidan U. 1994. Indexing of apple trees for apple mosaic virus, apple chlorotic leaf spot virus and apple stem grooving virus by ELISA. *Journal of Turkish Phytopathology* 23: 127-132.
- Fridlund PR. 1989. Virus and virus like diseases of pome fruits and non-infectious disorders. WSU Press, USA. 330p.
- Fuchs E, Gruntzig M and Alkai B. 1988. Serological detection of mechanically transmissible viruses of pome and stone fruits. *Newsletters for plant protection in the GDR* 42: 208-211.
- Fulton RW. 1965. A comparison of two viruses associated with plum line pattern and apple mosaic. *Zastita Bilja* 16: 427-430.
- Fulton R. 1952. Mechanical transmission and properties of Rose mosaic virus. *Phytopathology* 42: 413-416.
- Fulton RW. 1970. Prune dwarf virus. *CMI/AAB Description of Plant Viruses* 3: 23-25.
- Fulton RW. 1972. Apple mosaic virus. *CMI/AAB Descriptions of Plant Viruses*, No. 83. Wellesbourne, UK: *Association of Applied Biology*. pp. 4-7.
- Fulton RW. 1980. Iilarviruses In: *Handbook of Plant Virus Infections and Comparative Diagnosis*. (ed.): Kurstak E. North Holland, Amsterdam. Elsevier. pp. 377-421.
- Fulton RW. 1985. PNRSV *Iilarvirus*. In: Brunt A A, Grabtree K, Dollwitz S, Gibbs A J, Watson L. and Zurches E J (eds). *Plant viruses*. pp. 21-27.
- George JA and Davidson TR. 1964. Further evidence of pollen transmission of

- necrotic ringspot and sour cherry yellows viruses in sour cherry. *Canadian Journal of Plant Science*, 44: 383–384.
- Gilmer RM. 1965. Additional evidence of tree-to-tree transmission of sour cherry yellows virus by pollen. *Phytopathology* 55: 482–483.
- Greber RS, Klose MJ, Milne JR, and Teakle DS. 1991. Transmission of prunus necrotic ringspot virus using plum pollen and thrips. *Annals of Applied Biology* 118: 589-593.
- Gumus M, Paylan IC, Matic S, Myrta A, Sipahioglu HM and Erkan S. 2007. Occurrence and distribution of stone fruit viruses and viroids in commercial plantings of *Prunus* species in western Anatolia, Turkey. *Journal of Plant Pathology* 35: 265-268.
- Halk EL, Hsu HT, Aebig J and Franke J. 1984. Production of monoclonal antibodies against three ilarviruses and alfalfa mosaic virus and their use in serotyping. *Phytopathology* 74: 367-372.
- Hadidi A, and Barba M. 2011. Economic impact of pome and stone fruit viruses and viroids. In A. Hadidi, M. Barba, T. Candresse, and W. Jelkmann (Eds.), *Virus and virus-like diseases of pome and stone fruits* (pp. 1–8). St. Paul, MN: APS Press.
- Hammond RW. 2011. Prunus necrotic ring spot virus. Viroids. In A. Hadidi, M. Barba, T. Candresse, and W. Jelkmann (Eds.), *Virus and virus-like diseases of pome and stone fruits* (pp.207-213). St. Paul, MN: APS Press.
- Hammond RW and Crosslin JM. 1995. The complete nucleotide sequence of RNA 3 of a peach isolate of Prunus necrotic ringspot virus. *Virology* 208: 349-353.
- Hou YL, Yang J I, Dong YF, Zang ZP, Wang SH and Wu P. 2004. Detection techniques for apple mosaic virus diseases by RT-PCR. *China Fruits* 6:5-6.
- Hu GJ, Dong Y F, Zhang Z P, Fan X D, Ren F, Li Z N, and Zhou J. 2016. First Report of Prunus necrotic ringspot virus Infection of Apple in China. *Plant Disease*, 100: 1955.
- Imed A, Boscia D, Boari A, Saldarelli P, Digiario M and Savino V. 1997. A comparison of apple mosaic virus isolates from Prunus trees and production of specific monoclonal antibodies. *EPPO Bulletin* 27: 563-564.
- Kapoor S and Handa A. 2017(a). Prevalence of PNRSV in Peach orchards of Himachal Pradesh and its detection through DAS-ELISA. *Journal of Plant Diseases Sciences* 12: 129-132.
- Kapoor S and Handa A 2017(b). Serological Evidence for the Presence of Prunus Necrotic Ring Spot Virus in Stone Fruits with Particular Reference to Peach. *International Journal of Current Microbiology and Applied Sciences* 6: 4078-4083.
- Kulshrestha S, Verma N, Hallan V, Raikhy G, Singh MK, Ram R and Zaidi AA. 2005. Detection and identification of Prunus necrotic ringspot virus in Pelargonium. *Australasian Plant Pathology* 34: 599-601.
- Lemoine J. 1982. The effect of virus diseases on the behaviour of the ornamental apples. Mokum permanently and Golden Gem. *Revue horticole* 43: 45-47.
- Makarski JSJ and Agrios GN. 1973. Respiration, organic acid and sugar composition of apple fruits collected from apple mosaic virus or russet ring virus-infected trees. *Phytopathology* 63: 1483-1488.
- Mc Morran JP and Cameron HR. 1983. Detection of 41 isolates of necrotic ringspot, apple mosaic, and prune dwarf viruses in *Prunus* and *Malus* by enzyme linked immunosorbent assay. *Plant Disease* 67: 536-538.
- Menzel W, Jelkmann W and Maiss E. 2002. Detection of four apple viruses by multiplex RT-PCR assays with co-amplification of plant mRNA as internal control. *Journal of Virological Methods* 99: 81-92.
- Menzel W, Zahn V and Maiss E. 2003. Multiplex RT-PCR-ELISA compared with bioassay for the detection of four

- apple viruses. *Journal of Virological Methods* 110: 153-157.
- Mink GI, Howell WE, Cole A and Regev S. 1987. Three serotypes of Prunus Necrotic ring spot virus isolated for rugose mosaic-diseased sweet cherry trees in Washington. *Plant Diseases* 71: 91-93.
- Mink GI. 1989. Apple chlorotic leaf spot virus. In: *Virus and virus-like diseases of stone fruits and simulating noninfectious disorders*. (ed.): Pullman WA. College of Agriculture and Home Economics, Washington State University. pp. 8-11.
- NAPPO. 2013. RSPM 5. Glossary of phytosanitary terms. Ottawa, Canada: The Secretariat of the North American Plant Protection Organization.
- Navarro L, Pina JA, Jua´rez J, Ballester-Olmos J F, Arregui JM., Ortega C. 2002. The citrus variety improvement program in Spain in the period 1975–2001. In: *Proceedings of the fifteenth IOCV conference* (pp. 306–316). Riverside, CA: IOCV.
- NCPN-FT. 2012. State level model regulatory standard: *Virus-tested certification program for Prunus, Malus, Pyrus, Chaenomeles, and Cydonia nursery stock production systems*. Prosser, Washington: NCPN-FT.
- Nemeth M. 1986. Virus, mycoplasma and rickettsia diseases of fruit trees. Akademiai Kiado. Budapest, 841p.
- Pallas V, Aparicio F, Herranz MC, Amari K. Sanchez-Pina MA. Myrta A and Sanchez-Navarro JA. 2012. Ilarviruses of Prunus species: A continued concern for Fruit trees. *Phytopathology* 102: 1108-1120.
- Paunović S and Jevremović D. 2008. Comparative results of detection of pome fruit viruses by different methods. *Acta Horticulture* 781: 147-154.
- Petrzik K and Lenz O. 2002. Remarkable variability of apple mosaic virus capsid protein gene after nucleotide position 141. *Archives of virology* 147: 1275-1285.
- Petrzik K. 2005. Capsid protein sequence gene analysis of Apple mosaic virus infecting pears. *European journal of plant pathology* 111: 355-360.
- Pina, JA, Chome´ P, Vives MC and Navarro L. 2012. The citrus nursery tree certification program in Spain. In: *Book of abstracts of XII international citrus congress*, Valencia, Spain. p.250.
- Polak J. 1994. Distribution of apple mosaic virus in apple gardens of the Czech Republic. *Ochrana Rostlin* 30: 85-89.
- Polak J, Hassan M. and Myrta A. 2006. Simultaneous detection and identification of four pome fruit viruses by one-tube pentaplex RT-PCR. *Journal of Virological Methods* 133: 124-129.
- Polak J, Hassan M, and Paprstein F. 2008. Detection and distribution of four pome fruit viruses in germplasm collection in the Czech Republic. *Acta Horticulturae* 781: 113-118.
- Rampitsch C, Eastwell KC, and Hall J. 1995. Setting confidence limits for the detection of Prune dwarf virus in Prunus avium with a monoclonal antibody-based triple antibody-sandwich ELISA. *Annals of Applied Biology* 126: 485–491.
- Rana T, Negi A, Dhir S, Thockchom T, Chandel V, Walia Y, Singh RM, Ram R, Hallan V, and Zaidi AA. 2011. Molecular diagnosis of apple virus and viriod pathogens from India. *Archives of Phytopathology and Plant Protection* 44: 502-512.
- Rebandel Z, Zawadzka BJ and Wierszyllowski J. 1979. Effect of Apple mosaic virus on bud-take and growth of trees in the nursery. *Fruit Science Reports* 6: 9-17.
- Reed PJ, and Foster JA. 2011. Exclusion of pome and stone fruit viruses, viroids and viroids. In A. Hadidi, M. Barba, T. Candresse, and W. Jelkmann (Eds.), *Virus and virus-like diseases of pome and stone fruits* (pp. 381–388). St. Paul, MN: APS Press.
- Rovira M and Aramburu J. 1998. Incidence of Apple mosaic virus (ApMV) disease on hazelnut (*Corylus avellana* L.) in Spain and its effects on yield. *Nucis Newsletter* 7: 18-20.

- Roussel S, Kummert J, Dutrecq O, Lepoivre P and Jijakli M H. 2004. Development of molecular tests for the detection of ILAR and latent viruses in fruit trees. *Communications in Agricultural and Applied Biological Sciences* 69: 427-432.
- Rowhani A, Uyemoto JK, Golino DA and Martelli GP. 2005. Pathogen testing and certification of *Vitis* and *Prunus* species. *Annual Review of Phytopathology* 43: 261-278.
- Rybicki EP. 1995. The *Bromoviridae*. In: *Virus taxonomy. VIth report of the International Committee on Taxonomy of Viruses*. (eds.): Murphy FA, Fauquet C M, Bishop D H L, Ghabrial S A, Jarvis S A. pp. 65-68.
- Saade M, Aparicio F, Sanchez Navarro JA, Herranz MC, Myrta A, Terlizzi B and Pallas V. 2000. Simultaneous detection of the three ilarviruses affecting stone fruit trees by nonisotopic molecular hybridization and multiplex reverse-transcription polymerase chain reaction. *Phytopathology* 90: 1330-1336.
- Sanchez RP, Carts RM, Benavides PG and Sanchez MAG. 2004. Main viruses in Sweet Cherry plantation of Central-Western Spain. *Scientia Agricola* 72: 83-86.
- Sanchez RP, Morales-Corts MR. and Sanchez MAG. 2017. Sour and duke Cherry viruses in South-West Europe. *Phytopathologia Mediterranea* 56: 62-69.
- Salem N, Mansour A, Al-Musa A, and Al-Nsour A. 2003. Incidence of *Prunus necrotic ringspot virus* in Jordan. *Phytopathologia Mediterranea* 42: 275-279.
- Salem N, Mansour A and Al Musa A. 2005. Viruses of pome fruit trees in Jordan. *Journal of Plant Pathology*. 87: 123-126.
- Salam AAM, Ibrahim AM, Abdelkader HS, Aly AM and El-Saghir SM. 2008. Characterization of two isolates of *Prunus necrotic ringspot virus* (PNRSV) from peach and apricot in Egypt. *Arab Journal of Biotechnology* 11: 107-112.
- Savino V. 1992. Certification of grapevine in Italy. In: G. P. Martelli (Ed.), *Grapevine viruses and certification in EEC countries: State of the art* (pp. 55-65). Bari, Italy: CIHEAM, Quaderno n. 3.
- Scott SW, Zimmerman MT, Yilmaz S, Zehr EI and Bachman E. 2001. The interaction between *Prunus necrotic ringspot virus* and *Prune dwarf virus* in peach stunt disease. *Acta Horticulturae* 550: 229-236.
- Scott SW. Viruses of peach. www.clemson.edu/extension/peach/commercial/diseases/files/h7.5.pdf (10:10AM, 12th March 2018)
- Seigner L. 2000. Virus detection with the polymerase chain reaction (PCR). *Healthy Plants* 52: 205-211.
- Shiel PJ, Alrefai RH, Domier LL, Korban SS and Berger PH. 1995. The complete nucleotide sequence of apple mosaic virus RNA-3. *Archives of Virology* 147: 1247-1256.
- Shiel PJ and Berger PH. 2000. The complete nucleotide sequence of apple mosaic virus (ApMV) RNA 1 and RNA 2: ApMV is more closely related to alfalfa mosaic virus than to other ilarviruses. *Journal of General Virology* 81: 273-278.
- Svoboda J and Polak J. 2010. Relative concentration of apple mosaic virus coat protein in different parts of apple tree. *Horticultural Science* 37: 22-26.
- Singh BB, Pandey B N, Singh RN and Singh SP. 1979. Effect of apple mosaic virus on the growth yield and quality of apple. *Punjab Horticulture Journal* 19: 80-82.
- Smith I M, Dunez J, Phillips D H, Lelliott R A. and Archer S A eds. 2009. *European handbook of plant diseases*. John Wiley and Sons 583p.
- Stouffer RF. 1989. Apple stem pitting. In: *Virus and Virus-like Diseases of Pome Fruits and Simulating Noninfectious Disorders*. ed: Fridlund P R. College of Agriculture and Home Economics, Washington State University. pp. 138-144.
- Suchá J and Svobodová L. 2010. Incidence of *Prune dwarf virus* and *Prunus necrotic*

- ring spot virus in orchards of sweet and sour cherry in the Czech Republic-Short communication. *Horticultural Science (Prague)* 37: 118-120.
- Thokchom T, Rana T, Hallan V, Ram R and Zaidi AA. 2009. Molecular characterization of the Indian strain of Apple Mosaic virus isolated from apple (*Malus X domestica*) *Phytoparasitica* 37: 375-379.
- Thomas BJ. 1980: The detection by serological methods of viruses infecting the rose. *Annals of Applied Biology* 94: 91-101.
- Thomas HE, and Hildebrand EM. 1936. A virus disease of prune. *Phytopathology* 26: 1145–1148.
- Uyemoto JK, and Scott SW. 1992. Important diseases of Prunus caused by viruses and other graft-transmissible pathogens in California and South Carolina. *Plant Disease* 76: 5–11.
- Thomsen A. 1975. Cross protection with apple mosaic virus. *Journal of Crop* 79: 57-62.
- Waterworth HE. 1998. Certification for plant viruses—An overview. viroids. In A. Hadidi, M. Barba, T. Candresse, and W. Jelkmann (Eds.), *Virus and virus-like diseases of pome and stone fruits* (pp. 325–331). St. Paul, MN: APS Press.
- White RP. 1928. An infectious chlorosis of roses. *Plant Disease Report* 12: 33-34.
- Wong SM and Horst RK. 1988. Comparison of antigen and antibody-coated enzyme-linked immune sorbent assay procedures for the detection of three isolates of purified apple mosaic virus or Prunus necrotic ringspot virus. *Acta Horticulturae* 234: 249-256.
- Yardmc N, Cevik B and Eryigit H. 2008. Dtetction of Apple mosaic virus on apple cultivars growing in South-West Turkey by ELISA and RT-PCR methods. *Acta Horticulture* 781: 561-565.
- Ylmaz ND, Yanar Y, Kadoglu I and Yanar D. 2005. Study on distribution of apple mosaic virus (ApMV) in apple orchards in Tokat Province. *Ondokuz Mays Universitesi Ziraat Fakultesi Dergisi* 20: 12-15.
- Yuan WQ, Barnett OW, Westcott SW and Scott SW. 1990. Tests for transmission of Prunus necrotic ringspot and two nepoviruses by *Criconemella xenoplax*. *Journal of nematology* 22: 489.
- Zheng YY, Hong N, Wang GP and Hu HJ. 2006. Cloning and sequence analysis for the CP gene of Apple stem grooving virus from pears. *Acta Phytopathol. Sinica*. 36: 62-67.

How to cite this article:

Shelly Kapoor, Abhilasha Sharma, Bunty Shylla and Anil Handa. 2018. Ilarviruses and the Importance of Certified Elite Planting Material in Apple Production System - An Overview. *Int.J.Curr.Microbiol.App.Sci*. 7(04): 2444-2462. doi: <https://doi.org/10.20546/ijcmas.2018.704.281>