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STATUS OF APPLE MOSAIC VIRUS IN KASHMIR VALLEY

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ABSTRACT

Leaves of apple cv. 'Red Delicious' and 'Golden Delicious' were randomly collected from commercial apple growing areas of four districts of Jammu and Kashmir State viz., Shopian, Pulwama, Bandipora and Kupwara for the detection of *Apple mosaic virus* (ApMV) by RT-PCR technique using coat protein gene-specific primers. ApMV was present in all the locations. RT-PCR amplified approximately 800 bp amplicons from 30 out of 276 leaf samples thereby confirmed the infection of ApMV in Kashmir. District Bandipora recorded highest disease incidence of 13.3% followed by Shopian (11.3%), Kupwara (10.2%) and Pulwama (8.6%). Nineteen of the 161 cv. 'Red Delicious' samples and 11 of the 115 cv. 'Golden Delicious' samples were found RT-PCR positive.

Keywords: Apple mosaic virus, ApMV incidence, Kashmir, RT-PCR detection

Apple (*Malus* x *domestica*) is an important fruit crop considered to be the backbone of the economy of Jammu & Kashmir State. Of the various diseases infecting apple, viruses have gained importance in recent years. Amongst viruses, Apple mosaic virus (ApMV), Apple chlorotic leaf spot virus (Mink, 1989), Apple stem grooving virus and Apple stem pitting virus are economically important viruses of apples. ApMV, belonging to genus *Ilarvirus*, family *Bromoviridae*, is common pathogen in commercial apple cultivars (Mink, 1989). In India, ApMv was first reported based on symptoms from Uttarakhand (Bhargava and Bist, 1957). Since then, it has been reported from all commercial growing areas of Himachal Pradesh (Thokchom *et al.*, 2009). Trees infected with ApMV showed 30-50% decrease in fruit yield with around 50% reduction in growth and 20% reduction in trunk diameter (Sutic *et al.*, 1999). Extensive studies on detection of viruses infecting apple using various techniques like biological indexing and ELISA have been carried out across globe (Rwahnih *et al.*, 2004; Ulubas and Ertunc, 2005; Thokchom *et al.*, 2009; Rana *et al.*, 2010). Although quite efficient, these techniques have limitations of either being time consuming for indexing or have less sensitivity and accuracy. In particular, ELISA becomes unreliable for indexing of most host plants during summer because of low virus concentrations in tree parts. This assay is considered reliable only for about 3 months soon after bud-break in spring. RT-PCR, as an extremely sensitive detection tool to overcome these obstacles, has been used for testing virus infections in plants. Realizing that an accurate disease diagnosis combined with sensitive, rapid and early detection of plant viruses is critical for their management, survey of some important apple fruit growing areas of Kashmir was conducted for detection of ApMV using RT-PCR technique.

Surveys were undertaken in four districts of Kashmir namely Shopain, Pulwama, Bandipora and Kupwara during April-June in the years 2009 and 2010 for detection of ApMV in apple plantations. Two hundred seventy six leaf samples with or without typical mosaic symptoms were collected randomly from most predominant apple cultivars 'Red Delicious' and 'Golden Delicious'. The samples

were stored at -80°C until nucleic acid extractions performed. In order to elucidate and confirm infection of virus, total RNA was extracted from leaf tissue using TRI reagent (Life Technologies India) as per the manufacturer's instructions. The primer pairs PAPMCP-3 and PAPCP-3(1) designed for ApMV coat protein (CP) gene by Thokchom *et al.* (2009) were used. Reverse transcription reaction was carried out in a 25 µl reaction mixture using 5 µl RNA (2 µg), 100 pmol PAPMCP-3 primer, 0.5 mM dNTP, 20 units of RNase inhibitor (Fermentas Life Sciences), 1 x RT buffer and 50 units of M-MuLV Reverse Transcriptase (Fermentas Life Sciences). The reaction mixture was incubated at 37°C for 75 min. followed by 5 min. at 70°C. PCR amplification was carried out in a T-Gradient thermocycler (Biometra, Gmbh, Germany) in a 25 µl reaction mixture containing 5 µl cDNA, 5 pmols of PAPCP-3 and PAPCP-3(1) primer, 0.2 mM dNTP and 1 units of Taq DNA polymerase (Fermentas Life Sciences). The DNA was amplified using 30 cycles of denaturation at 94°C for 30 sec., annealing at 59°C for 1 min., elongation at 72°C for 1 min., and a final elongation at 72°C for 7 min. The amplified PCR product was subject to electrophoresis in a 1% agarose gel and then stained with ethidium bromide.

During the survey major symptoms exhibited by infected trees were typical mosaic pattern on leaves on a few branches or twigs and a few trees showed vein clearing. Two hundred seventy six random leaf samples of apple cv. 'Red Delicious' and 'Golden Delicious', collected from 8 locations of Shopian, Pulwama, Bandipora and Kupwara districts of Kashmir valley and subjected to RT-RCR detection of ApMV, revealed the prevalence of virus in all the locations with varying incidence (Table 1). Bandipora recorded highest disease incidence of 13.3% followed by Shopian (11.3%), Kupwara (10.2%) and Pulwama (8.6%). Out of 276 samples, 30 showed positive RT-PCR results thus exhibiting an overall disease incidence of 10.86%. Among locations, Kakapora in district Pulwama recorded lowest ApMV incidence of 8.2 % followed by Muran in the same district (8.6%) and Imam Sahib in district Shopian. Based on RT-PCR, Aloosa in Bandipora and Syedpora in Shopian recorded highest ApMV incidence of 15.5 and 14.6%, respectively. Among the apple cultivars, 13.04% 'Red Delicious' samples (21 out of 161) were RT-PCR positive compared to 10.45% (11 out of 115) in 'Golden Delicious' samples across location. In present investigation, molecular detection of ApMV was carried out using RT-PCR by employing primer pairs specific to coat protein gene of ApMV. The RT-PCR amplified approximately 800 bp amplicon from 32 out of 276 leaf samples (Fig. 1) thus confirmed the ApMV infection in apple in Kashmir.

Table 1: RT-PCR positive samples and disease incidence of ApMV in different leaf samples collected from various locations in Kashmir valley

Districts	Locations	Apple cultivars				Mean disease incidence (%)
		Red Delicious		Golden Delicious		
		ApMV +ve samples	Disease incidence (%)	ApMV +ve samples	Disease incidence (%)	
Shopian	Imam Sahib	2 (20)*	10.00	1 (13)	7.69	8.85
	Syedpora	2 (12)	16.67	1 (08)	12.50	11.59
Pulwama	Muran	1 (15)	6.67	2 (19)	10.52	8.60
	Kakapora	2 (23)	8.70	1 (13)	7.69	8.20
Bandipora	Aloosa	3 (18)	16.66	2 (14)	14.28	15.47
	Asham	3 (26)	11.53	2 (17)	11.67	11.65
Kupwara	Chakpuran	6 (25)	16.00	1 (21)	4.76	10.38
	Ashipora	2 (22)	9.09	1 (10)	10.00	9.55
Total		21 (161)		11 (115)		

*Total number of samples tested

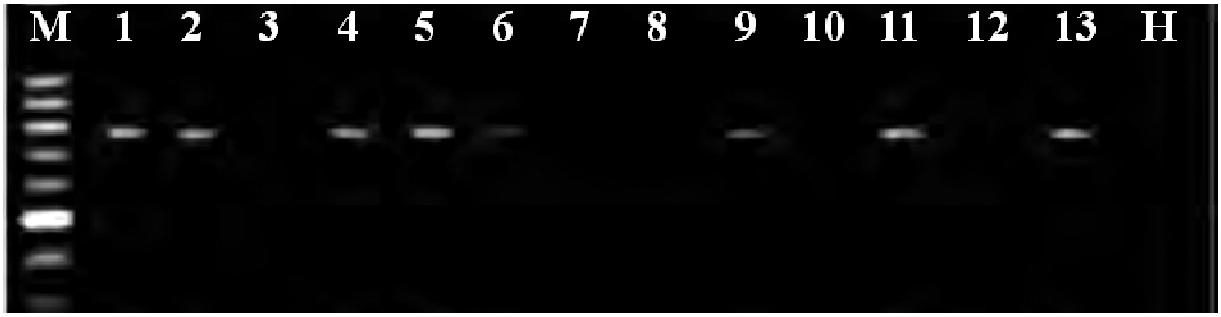

Fig. 1: RT-PCR amplification from ApMV infected and non-infected leaf samples: Lane 1 to 13 show the amplification of approximately 800 bp fragment, M is 100 bp marker and H is healthy sample.

Accurate detection of viral infection in early stages of orchard establishment is indispensable, in view of viral dissemination through rootstock and bud wood and lack of effective disease eradicative and chemical control measures. In present study efforts were made to assess the status of ApMV in commercial apple growing areas of Kashmir by employing RT-PCR as a sensitive and reliable detection tool. The results revealed the presence of virus in apple orchards in all the locations surveyed with varying incidence. The major symptoms exhibited by virus infected trees were typical mosaic pattern leaves showing vein clearing. The molecular detection of causative agent, using coat protein specific primers, amplified its coat protein indicating that the disease is of viral etiology and not of malnutrition. RT-PCR amplification using coat protein specific primers gave positive results, thereby confirming the infection of ApMV in Kashmir valley. RT-PCR is a highly efficient detection tool used by many researchers across the globe for detection of viruses infecting temperate fruits (Moury *et al.,* 2000; Salmon *et al.,* 2002; Helguera *et al.,* 2002). Thokchom *et al*. (2009) for the first time reported ApMV in Himachal Pradesh using RT-PCR. The present results confirm the prevalence of ApMV in apple orchards in Kashmir. Since the virus is known to spread by graft transmission and may or may not express symptoms in host, efforts are required to identify ApMV-free apple plants of elite genotypes and rootstocks in all the areas of Kashmir by thorough surveys and RT-PCR detection, and to serve as mother plants for establishment of virus free apple orchards. The situation becomes still grim in view of the fact that almost all pome fruits are infected by the virus with or without producing symptoms (Thokchom *et al.,* 2009). This emphasizes more research for the identification of ApMV and other viruses infecting apple and other pome fruits in of Kashmir.

REFERENCES

Bhargava, K.S. and Bist, N.S. 1957. Three virus diseases of hill fruits in Kumaon. *Current Science,* **26:** 324–325.

Helguera, P.R., Docampo, D.M., Nome, F. and Ducasse, D.A. 2002. Enhanced detection of *Prune dwarf virus* in peach leaves by immunocapture-reverse transcription-polymerase chain reaction with nested polymerase chain reaction (IC-RT-PCR nested PCR). *Journal of Phytopathology*, **150:** 94-96.

Mink, G. I. 1989. Apple chlorotic leafspot. pp. 8–19; 34-39 In: *Virus and Virus-like Diseases of Pome Fruits and Simulating Non-infectious Disorders* (eds. P.R. Fridlund). Pullman, USA/ Cooperative Extension College of Agriculture and Home Economics, Washington State University, Washington, USA.

Moury, B., Cardin, L., Onesto, J.P., Candresse, T. and Poupet. A. 2000. Enzyme-linked immunosorbent assay testing of shoots grown *in vitro* and the use of immunocapture-reverse transcription polymerase chain reaction improve the detection of *prunus necrotic ringspot virus* in rose. *Phytopathology,* **90:** 522-528.

Rana, T., Chandel, V., Kumar, Y., Ram, R., Hallan, V. and Zaidi, A.A. 2010 Molecular variability analyses of *Apple chlorotic leaf spot virus* capsid protein. *Journal of Bioscience,* **35:** 605–615.

Rwahnih, M.A., Turturo, C., Minafra, A., Saldarelli, P., Myrta, A., Pallás, V. and Savino, V. 2004. Molecular variability of *apple chlorotic leaf spot virus* in different hosts and geographical regions. *Journal of Plant Pathology*, **86:** 117-122.

Salmon, M.A., Vendrame, M., Kummert, J. and Lepoivre, P. 2002. Detection of *apple chlorotic leaf spot virus* using 5' nuclease assay with a fluorescent minor groove binder-DNA probe. *Journal of Virological Methods,* **104:** 99-106.

Sutic, D.D., Ford, R.E., and Tosic, M.T. 1999. Virus diseases of fruit trees. pp. 345–347. In: *Handbook of Plant Virus Diseases*. CRC Press, Boca Raton, Florida, USA.

Thokchom, T., Rana, T., Hallan, V., Raja Ram and Zaidi, A.A. 2009. Molecular characterization of the Indian strain of *Apple mosaic virus* isolated from apple (*Malus domestica*). *Phytoparasitica*, **37:** 375-379.

Ulubas, C. and Ertunc, F. 2005. *Apple chlorotic leaf spot virus* (ACLSV) status in Turkey and sensitive detection using advanced techniques. *Turkish Journal of Agricultural Forestry*, **29:** 251-257.