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Association of virus complex with capsicum under protected cultivation in Himachal Pradesh and implications in their management

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Abstract

Extensive surveys of capsicum crop grown under polyhouse conditions in Kangra, Mandi, Kullu, Hamirpur and Bilaspur districts of Himachal Pradesh (HP) were carried out during 2011 to 2014. The symptoms on the diseased plants included mosaic, mottling, chlorosis, upward or downward curling and deformation of leaves and fruits along with stunting of plants. The disease samples indexed through DAS ELISA showed the presence of eight viruses namely *Cucumber mosaic virus* (CMV); *Pepper mild mottle virus* (PMMoV); *Tobacco mosaic virus* (TMV); *Tomato spotted wilt virus* (TSWV); *Tomato yellow leaf curl virus* (TYLCV); *Potato virus Y* (PVY); *Pepper mottle virus* (PepMoV); *Pepper veinal mottle virus* (PVMV) in 2013 while only four viruses viz. CMV, TSWV, PVY and PMMoV were found in 2014. The presence of viruses which were found to be prevalent in DAS-ELISA was confirmed with RT-PCR using coat protein (CP) specific primers. The highest incidence of 60 per cent was recorded in Bilaspur district. The presence of three commonly occurring viruses *viz.* CMV, TSWV and PMMoV was confirmed with RT-PCR which yielded the amplification product of size 162 bp, 575 bp and 730 bp, respectively.

Key words: Pepper mild mottle virus**,** capsicum, prevalence, detection.

Capsicum (*Capsicum annuum* L. var. *grossum* Sendt), a member of family *Solanaceae* is highly popular and economic crop grown in India since 15th century, mainly for its fruits. In India, it is being grown on an area of 29.14 thousand ha having 153.35 thousand MT production (2012-13) which contributes for one fourth of the world production of capsicum. Capsicum is extensively cultivated in Andhra Pradesh, Karnataka, Maharashtra, Tamil Nadu, Himachal Pradesh (HP), and hilly areas of Uttar Pradesh (Sreedhara 2013). In HP, area under capsicum is 2072 ha with production and productivity of 34,132 MT and 16.47 MT/ha (2012-13), respectively. Capsicum is an important summer season vegetable and spice crop in HP both in open and protected cultivation and assumes a special significance in the mid hills, as it is grown as an off season crop in summer and rainy seasons, which brings lucrative returns to the farmers. In recent years capsicum has become one of the premier crops under polyhouse condition with the advent of Polyhouse Cultivation Venture of HP Govt which ensure its regular and off-season supply.

Capsicum has been attacked by number of fungi, bacteria and viruses. Besides *Phytophthora* fruit rot, Bacterial wilt and canker, viral diseases are the most common cause of losses to polyhouse grown capsicum as they affect both quantity and quality of the produce and are the most difficult to control. The viruses that infect capsicum are *Alfalfa mosaic virus*, *Andean potato mottle virus*, *Beet curly top virus*, *Chili leaf curl virus*, *Chilli veinal mottle virus*, *Cucumber mosaic virus*, *Pepper mild mottle virus*, *Pepper mottle virus*, *Pepper veinal mottle virus*, *Potato virus Y*, *Tobacco etch virus*, *Tobacco mosaic virus*, *Tomato mosaic virus*, *Tomato spotted wilt virus* (Wetter *et al*. 1984; Sharma *et al.* 1993; Lima *et al.* 2011). In HP, the viruses causing mosaic in capsicum are very common.The popularization of polyhouse venture and use of hybrid seeds has led to the escalated incidence of the viral diseases in the state. Surveys of the state's polyhouses revealed that the major disease symptoms were in the form of mosaic, mottle, chlorosis, upward or down ward curling of the leaves, puckering, deformation and reduction of leaf lamina along with stunting of infected plants in some areas (Sharma and Patiyal 2011).The objective of this study was to assess the prevalence of the virus diseases in polyhouse cultivated capsicum.

Materials and Methods

Surveys and sample collection

To study and monitor the prevalence of the viral diseases in capsicum in the state, surveys were conducted for four consecutive years from 2011 to 2014. Extensive surveys of polyhouses in which capsicum are grown in district Kangra, Mandi, Hamirpur, Kullu and Bilaspur were conducted in each year during this period. Surveys were carried out during every year in same polyhouses of same districts. The per cent incidence was calculated using the formula:

$$\text{Per cent Incidence} = \frac{\text{Number of plants infected}}{\text{Total number of plants observed}} \times 100$$

The plants showing viral like symptoms were observed and samples were collected. The virus cultures were maintained by inoculation on susceptible variety "California Wonder" through sap inoculation using standard leaf rub method after establishing their identity through DAS-ELISA. The virus inoculum was prepared by macerating infected leaves in chilled phosphate buffer (K_2HPO_4, pH7.0) using pestle-mortar. The sap extract was inoculated on healthy seedlings of "California Wonder" at 3-4 leaf stage using celite as an abrasive. The plants were then kept at 25±2°C under green house condition. The inoculated plants were observed for the symptoms appearance. The virus cultures were maintained under polyhouse conditions and leaves from infected plants were stored at -80 ºC for further use.

Serological assays through DAS-ELISA

The plants were indexed for the presence of different viruses through DAS-ELISA kits (Bioreba, Switzerland) using polyclonal antibodies (Clark and Adams 1977) by following standard procedure and protocols provided by the manufacturer. The entire naturally infected as well as samples from the artificial inoculated plants were screened for the presence of the viruses. The samples were tested for important capsicum viruses viz. *Pepper mild mottle virus* (PMMoV), *Pepper veinal mottle virus* (PVMV), *Tobacco mosaic virus* (TMV), *Tomato spotted wilt virus* (TSWV), *Cucumber mosaic virus* (CMV), *Tomato yellow leaf curl virus* (TYLCV), *Potato virus Y* (PVY) and *Pepper mottle virus* (PepMoV).

Molecular detection using coat protein specific primers

To confirm the identity of the virus observed in DAS-ELISA assay, samples giving positive results were amplified by polymerase chain reaction (PCR) using coat protein (CP) gene specific primers designed using Primer3 software program available online (www.frado.wi.mit) (TSWVF: 5'-AGTTCTGCAAGTTTTGTCTGTTTT-3' , TSWVR: 5'-CATCACATTAAAACCCTAAGAAACG-3'; CMVF: 5'-GGCTGCAGTGGTCTCCTT-3', CMVR: 5'-GAGTCGAGTCATGGACAAATC-3'; PMMoVF: 5'-CCAATGGCTGACAGATTACG-3', PMMoVR: 5'-CAACGACAACCCTTCGATTT-3') (Rialch *et al.* 2015). The total RNA extracted from the plants with positive reaction in DAS-ELISA was subjected to cDNA synthesis using the MMLV reverse transcriptase (USB). The synthesized cDNA was then subjected to RT-PCR. RT-PCR amplification was carried out in 25 µl reaction volume using 2.5 µl of 10x Taq buffer, 1 µl of 25 mM $MgCl_2$, 2.5 µl of 2mM dNTPs mix, 1 µl of 10mM forward and reverse primers, 2.0 µl of cDNA, 0.2 µl of 5U/ µl Taq polymerase (Merck Genei) and final volume was adjusted with nuclease free water. Amplification was performed in GeneAmp PCR system 9700 (Applied Biosystems) with initial denaturation of 94°C for 4 min followed by 35 cycles of 94°C for 15 s, annealing at 48°C for 40s for PMMoV, 50°C for 40 sec for CMV and TSWV and extension at 72°C for 1 min with a final extension of 5 min at 72°C.

Results and Discussion

Surveys in the districts mentioned in previous section revealed the presence of viruses in almost all the districts. Diseased plants in various polyhouses exhibited symptoms like mosaic, mottling, chlorosis, upward or downward curling and deformation of leaves along with stunting of plants. The fruits produced by diseased plants were deformed and had lumpy appearance with mosaic pattern on their surface. Highest incidence of mosaic diseases was observed in Bilaspur district (Berthin) with about 60 per cent average incidence followed by Kullu and then Kangra with 24 and 20 per cent average incidence, respectively (Fig. 1). In HP, total area under protected cultivation is 223.18 ha in which the major contributing districts are Bilaspur (38.06 ha) followed by Kangra (36.02 ha) and Mandi (28.53 ha) (Spehia 2015). However, very less incidence was recorded in Mandi and Hamirpur districts.

DAS-ELISA using commercial Kits (BIOREBA & SeDIAG) was employed to identify the associated viruses in various diseased samples collected from different districts of HP. Eight viruses viz. CMV, TMV, PVY, TSWV, PepMoV, PYMV, TYLCV and PMMoV were intercepted in the samples collected from 5 districts during this period (Fig. 2). CMV followed by PMMoV and TSWV were the most abundant virus in occurrence while PVY and TYLCV were the least prevalent. The variation was there in number of viruses intercepted during different years. This variation was due to replacement of the hybrid seed used by the farmers due to non-availability of the previous year hybrid and secondly the source of the seed. This fact is being supported by one of our study in which the seed of same hybrid found infected with PMMoV was free of the virus when the same hybrid seed was procured from other areas during the next year (personal communication, unpublished)

The three viruses viz. CMV, PMMoV and TSWV found prevalent in the serological tests were further assayed through RT-PCR based detection using CP gene specific primers. RT-PCR of the coat protein gene of representative isolates found positive for PMMoV, CMV and TSWV in DAS ELISA yielded ampilcons of 730, 162 & 575 bp, respectively (Fig. 3) whereas no such amplification was observed in healthy leaf samples. Thus, confirmed the presence of these three viruses in the state.

The present study has shown the presence of atleast eight viruses on capsicum cultivated under polyhouse conditions in HP with wide prevalence of CMV, TSWV and PMMoV detected using DAS ELISA and RT-PCR based indexing. CMV, which is a geographically prevalent virus may cause upto 60 per cent losses is known to be transmitted by 26 species of aphid in non-persistent manner under natural conditions infecting more than 770 plant species belonging to 85 families (Green and Kim 1991). TSWV

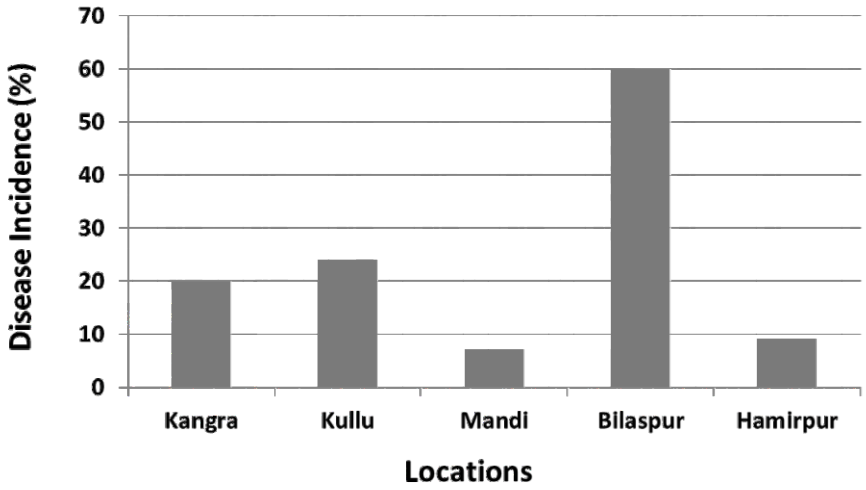

Fig. 1. Average incidence of mosaic diseases on capsicum grown under polyhouse conditions in Himachal Pradesh during 2011-2014

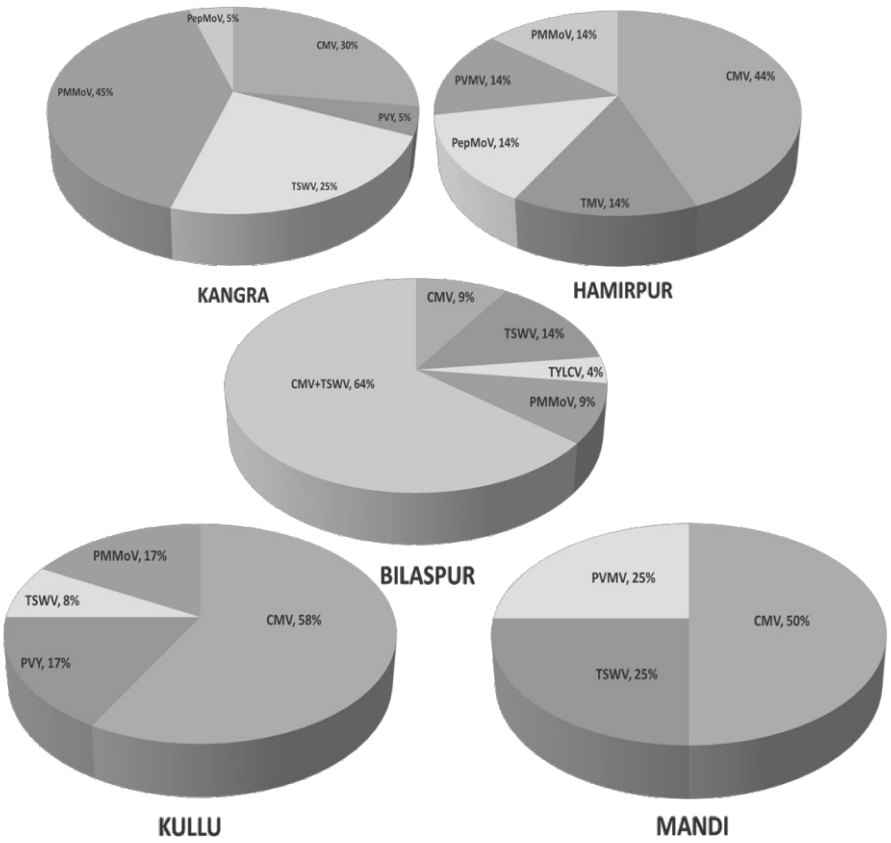

Fig. 2. Distribution patterns of capsicum viruses grown under polyhouse conditions in Himachal Pradesh

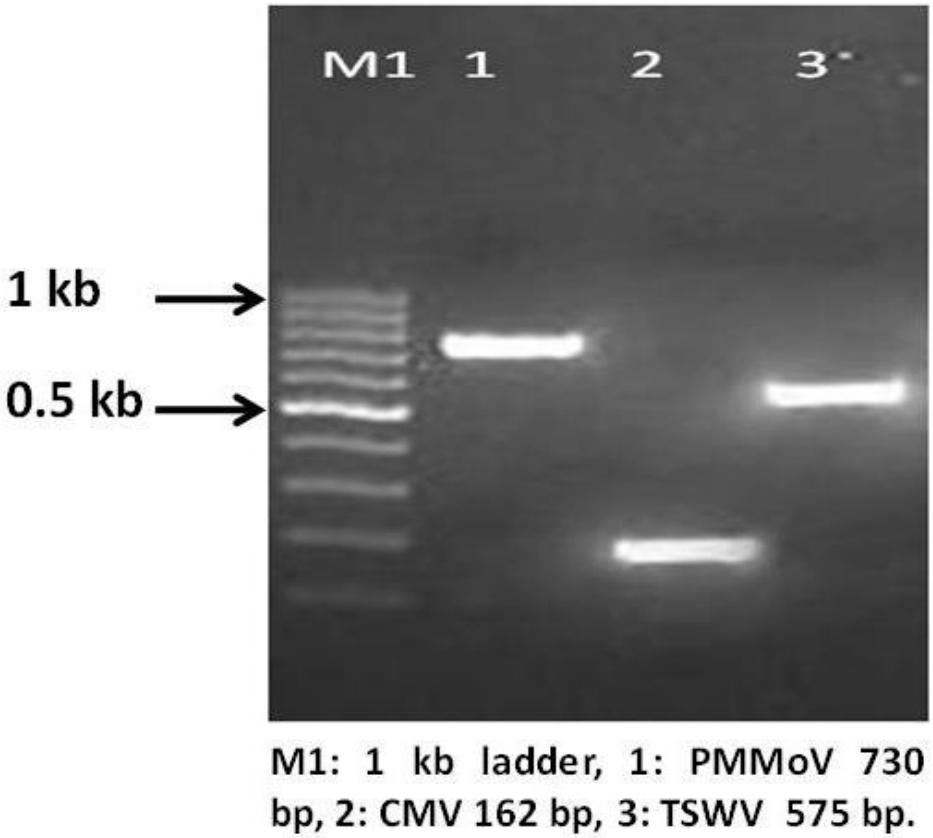

Fig. 3. Molecular detection of PMMoV, CMV and TSWV through RT¯PCR using CP specific primers

transmitted by thrips has a very wide host range and infect about 400 species of Monocotyledonous and Dicotyledonous plants mainly in the tropical and sub-tropical environments. Involvement of vectors in the spread of these two viruses poses serious threat for their spread under protected cultivation as both the vectors experiences a very congenial environment under polyhouse conditions otherwise suitable for plant growth. Hence control of vectors and eradication of weed hosts around the polyhouses can help in avoiding the attack of these viruses. Another important virus PMMoV intercepted in the polyhouse grown capsicum during the past 4-5 years has threatened the off-season cultivation of this crop (Sharma and Patiyal 2011). PMMoV, member of *Virigaviridae* family and genus *Tobamovirus* is a highly contagious, seed and soil borne virus known to attack capsicum around the world (Wetter *et al*. 1984; Sharma *et al*. 1993; Mnari-Hattab and Ezzaier 2006; Lima *et al*. 2011; Caglar *et al*. 2013). The studies on this virus have shown its invasion in India through the imported hybrid seeds as it was first detected from the seed lots procured from the local vendors (Rialch *et al*. 2015). The high seed transmission and survival of this virus in soil and crop debris alarms its seriousness to threat the capsicum cultivation in the state. Seed transmission up to 75 per cent and soil transmission has also been reported by many workers (Martinez-Ochoa *et al*. 2003; Toyoda *et al*. 2004). The virus has not been reported to be transmitted by any insect species. In polyhouse cultivation the plants are trained on two or four branches and pinching of the buds is a common practice which can result in rapid spread of the virus through the infected sap which contaminate the cutting knives and pinching hands as the virus is highly contagious and can tolerate high temperatures prevalent in the polyhouses. Thus, the management of this virus becomes a challenge once established in the polyhouse. This study envisages the necessity of proper quarantine and certification procedures to check the spread of this virus through seed. In addition precautions are needed to check its spread during pruning and training of the plants. However to achieve its effective management through various exclusionary means and cultural operations, proper indexing of the seed and the nursery is most important pre-requisite to prevent the losses caused by such dreaded viruses.

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