Indian J. Plant Genet. Resour. 34(1): 70–74 (2021)
DOI 10.5958/0976-1926.2021.00010.3

RESEARCH ARTICLE

Evaluation of Chilli Genotypes against Pepper Mottle Virus under Artificial Conditions

Shikha Sharma*, Abhishek Sharma and Salesh Jindal
Punjab Agricultural University Ludhiana-141027, Punjab, India

(Received: 13 August, 2020; Revised: 04 January, 2021; Accepted: 08 March, 2021)

One hundred and twenty four genotypes of *Capsicum annuum* were screened for resistance against *Pepper mottle virus* (PepMoV). Artificial screening was done via mechanical inoculation. Phenotypically resistant lines were further subjected against PepMoV antibodies in double antibody sandwich ELISA (DAS-ELISA). The results deciphered that seven genotypes viz., SM-478, IS-263, COO-226, VR-523, PL-412, IS-269, KC-312 exhibited resistant reaction (R), whereas twenty showed moderately resistant reaction. Virus distribution in hot pepper was seen via exposing different plant parts to DAS-ELISA. Maximum absorbance was recorded in leaf tissues. Further the effect of virus on seed yield was also observed in seven genotypes, which included two popular varieties viz., Punjab Tej and Suraj Mukhi and one hybrid CH-1. The results concluded that virus can cause up to 72 per cent seed yield loss in susceptible genotype *i.e* KRS-303.

Key Words: Chilli, Pepper mottle virus, Resistant sources, Seed yield

Introduction

Capsicum annuum (chilli) is an important vegetable and spice crop grown throughout the world. Chilli is a member of family Solanaceae and is native to the tropics and subtropics of America. The crop is attacked by a large number of diseases viz., viral, fungal, bacterial, nematode and phytoplasmal, which leads to great economic loss (Muthukumar and Bhaskaran, 2007). Natural occurrence of many viruses *e.g Pepper leaf curl virus, Pepper veinal mottle virus, Pepper vein banding virus* and *Pepper mottle virus* have been reported by different workers infecting chilli pepper worldwide (Green and Kim, 1991; Kaur *et al.,* 2014). *Pepper mottle virus* (PepMoV) is a potyvirus and belongs to the largest family of plant viruses (Shukla *et al.,* 1994). The virus was first recorded in USA during 1969 (Nelson and Wheeler, 1972) as a new strain of Potyvirus that infected peppers. By 1975 it became evident that PepMoV was contributing to crop losses in pepper growing areas of the United States. The virus infects many species of *Solanaceae* family, including several species of *Capsicum* (*C. annuum*, *C. frutescens*), *Datura* spp., *Lycopersicon esculentum, Physalis floriana*, tobacco (*Nicotiana* spp.) and nightshade (*Solanum* sp.) (Kaur *et al.,* 2014; Sharma *et al.*, 2019). The virus was reported to be of seed borne nature in India in 2018 (Sharma *et al.,* 2018). The management of viral diseases is majorly dependent upon resistant varieties cultivation. At present, there is clear evidence for four independent loci in *Capsicum,* each with alleles that confer resistance to viral isolates that belong to one or more of the viruses, PVY, PepMoV and *Tobacco etch virus* (TEV). Systemic movement of viruses inside the infected plant is well known. In resistant varieties, general location or types of tissues associated with the blockage have been identified, though most appeared to involve an inability of the virus to enter the phloem or exit the phloem or combinations thereof. Resistant varieties are available in both hot and sweet peppers. However, available commercial resistant varieties may not be effective against all isolates of PepMoV found. Given the seed borne nature of virus (Sharma *et al.,* 2018), the effect of virus on the seed yield in most popular varieties or hybrids was taken up. Furthermore, as the blockage of virus inside the plant is reported by previous workers, so tracing PepMoV in different parts of chilli plant was also investigated. Available germplasm was screened via artificial inoculation to hunt for resistant sources which can be further used in resistance breeding programme.

Material and Methods

Localization of PepMoV in different plant parts

Pepper seedlings of *cv.* SL-466 showing symptoms

*Email: shikhasharma-coapp@pau.edu

of PepMoV were tagged in the nursery. Seedlings exhibited symptoms such as mottling, vein banding and mosaic. To localize the virus in the whole plant parts the twigs and leaves of the plant were collected from the nursery stage. Later on the red ripe fruits were also collected from the tagged plant. The sap was extracted from above mentioned plant parts and was subjected to ELISA against PVY and PepMoV. Healthy plant parts were used for comparison.

Evaluation of Ppepper Germplasm against PepMoV

To find the sources of resistance against PepMoV, a total of 125 genotypes of *Capsicum annuum* were screened artificially using mechanical inoculation method. A genotype, SL-475 was used as susceptible check.

Maintenance of Inoculum

The pepper plants showing virus infection under field conditions were subjected to ELISA and the plants positive for *Pepper mottle virus* were used as inoculum source. The inoculum was multiplied by sap inoculating young healthy seedlings of susceptible genotype SL-475and was further maintained under insect proof conditions.

Artificial Screening by Mechanical Inoculation

The seedlings of 125 genotypes were raised in pro-trays using cocopeat, vermiculite and perlite in the ratio of 3:1:1. Nursery of genotypes was sown under insect proof conditions following proper cultural practices. Ten plants of each genotype were sap inoculated at 2-3 true leaf stage. The inoculum was prepared by grinding infected young leaves in a grinder with Phosphate buffer (0.01M). Sap inoculations were done in the evening hours. Before inoculating plants at 2-3 true leaf stage, celite-545 powder was dusted on to the upper side of leaves to cause injury for easy entry of virus inside the host. With the help of a cotton swab the sap was rubbed on the upper surface of leaves in same direction. After inoculation leaves were washed with double distilled water to avoid any injury by carborandum powder and to remove excess inoculum. The inoculated plants were kept in insect proof cage and observed regularly for symptom appearance from inoculation up to six weeks. Inoculation was repeated at an interval of three days twice. The disease incidence and severity was recorded on visual basis. The severity of plants was categorized as highly resistant, resistant, moderately resistant, moderately susceptible, susceptible and highly susceptible as scale suggested by Mughal and Khan (2011).

Confirmation of Resistance

After six weeks of visual observations, all the inoculated lines (both symptomatic and asymptomatic) were subjected to DAS-ELISA against antisera of PepMoV (Agdia).

Modified 0-5 severity scale for *Pepper mottle virus* screening (Mughal and Khan, 2001).

Symptoms	Severity grade	Disease reaction
No visible symptoms	0	HR
Mild mottling on the upper leaves	1	R
Banding of vein on few leaves, Mosaic initiation on all leaves	2	MR
Distinct mosaic and vein-banding symptoms on all leaves	3	MS
Severe mosaic and vein-banding along with narrowing of leaves, and misshapen leaf lamina	4	S
Severe mosaic and vein-banding, misshapen leaves and fruits, defoliation, small number of fruits	5	HS

Estimation of seed yield loss by PepMoV

Ten red ripe fruits from seropositive (Mild to High) plants of eight genotypes *viz.* CH-1, SL-475, IS-269, Punjab Tej, Suraj mukhi, FLP-483 and KRS-304were collected. The genotypes which were highly susceptible as well as which showed resistant response in DAS-ELISA were included to conclude the Experiment. Two widely adapted varieties viz., Punjab Tej and Suraj Mukhi and one popular hybrid (CH-1) were also included to see the effect of virus on seed yield. The seeds were extracted and counted. To calculate the per cent seed yield loss, seeds of ten healthy fruits were also taken into account. Per cent seed yield loss was calculated using formula:

$$\text{Per cent seed yield loss} = \frac{\text{HSY} - \text{ISY}}{\text{HSY}} \times 100$$

Results and Discussion

Localization of PepMoV in Different Plant Parts

The leaves and stems samples of pepper seedlings of *cv.* SL-475 and a healthy plant of same cultivar were exposed to DAS-ELISA against antisera of PepMoV. The absorbance value of leaves and stem of infected plants was 3.58 and 4 times higher than the healthy leaves respectively. Whereas the absorbance value of fruit and seeds in ELISA was 3.63 and 2.6 times greater

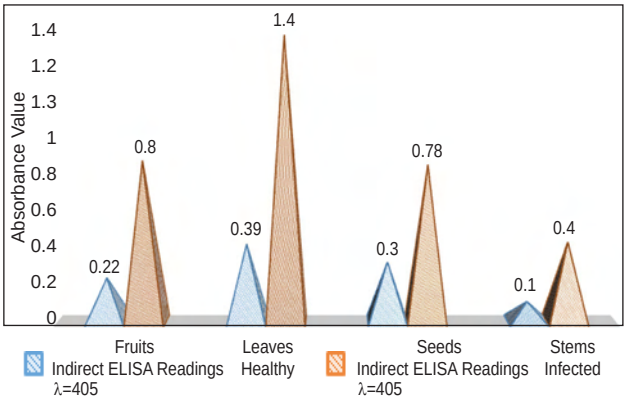

Fig. 1. Prevalence of PepMoV in different organs of pepper plants using indirect ELISA

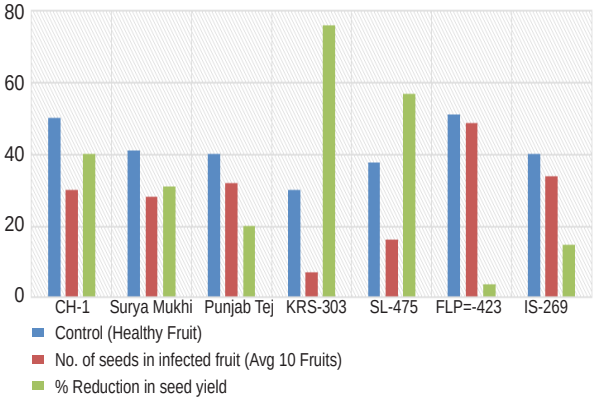

Fig. 2. Per cent reduction in seed yield due to PepMoV in Chilli

than the comparative corresponding parts of healthy plant of same genotype. It is evident from the results that virus was present systemically in the infected plant. However maximum viral load was in leaves, followed by fruits and seeds. Kogovsek *et al.* (2011) also found that the PVY inoculum was highest in infected leaves and stem.

Evaluation of Chilli Genotypes against *Pepper Mottle Virus* under Artificial Conditions

To find the resistance sources among the available genotypes, artificial or natural screening methods are adopted. In present study, artificial screening was performed to evaluate 125 genotypes of pepper against Pepper mottle virus disease at PAU, Ludhiana (Fig. 3). The plants were regularly observed for symptom appearance up to five weeks after inoculations and the genotypes were evaluated based on modified 0-5 scale (Mughal and Khan, 2001).Out of total 124 genotypes evaluated, seven genotypes viz., SM-478, IS-263, COO-226, VR-523, PL-412, IS-269, KC-312 were found to be resistant. Among rest of genotypes twenty showed moderately resistant reaction. Whereas twelve were moderately susceptible, fifty eight were susceptible and twenty eight were highly susceptible against PepMoV under artificial screening conditions (Table 1). The morphological responses showed by these genotypes after sap inoculation with the crude sap from *Pepper mottle virus* infected plant was also correlated and cross verified with the serological assay (DAS ELISA) as per Moury *et al.* (2005).

Capsicum annuum L. cv. Avelar was shown to have a monogenic, recessively inherited factor for resistance to Pepper mottle potyvirus (PepMoV) (Zitter and Cook, 1973; Guerini and Murphy, 1999). Resistance against

Scale for interpreting DAS-ELISA results*

OD at 405 nm	Response	Symbol
Same as Negative control	Negative	-
2-5 times higher than negative	Mild positive	+
5-10 times higher than negative	Positive	++
10-15 times higher than negative	Strong positive	+++

Chilli veinal mottle virus and *Pepper veinal mottle virus* was identified at AVRDC in two accessions of *C. annuum*, Perennial HDV and PSP-11, which were of Indian origin (Anonymous, 1990, 1991). However in India, information on resistant sources against PepMoV is very limited. Kaur *et al* (2018) screened 140 genotypes of hot pepper and found eight resistant sources against PepMoV. Unlike other insect pests and disease, for viral disease use of chemicals as control measure is not found to be very effective. Hence, for management of virus disease the best approach is use of resistant sources. These sources could serve as donors for promising resistance genes in breeding programmes to transfer the resistance into popular varieties or they may be used directly. So finding resistance sources will be the most economical, adaptable and sustainable approach for viral disease management.

Reduction in Seed Yield due to Pepper Mottle Virus Infection

As the virus was confirmed to be seed transmitted, the effect of the virus infection on seed yield with an emphasis on the seed formation was studied. Seed is the basic unit for the production of the crop. This virus exhibits symptom onto the fruits such as formation of green stripes with reduction in fruit size as published earlier (Sharma *et al.*, 2018). Popular varieties and hybrid of chilli along with genotypes were selected for

Fig. 3. Artificial screening of genotypes via mechanical inoculation against PepMoV

Table 1. Reaction of genotypes evaluated against PepMoV under artificial screening condition

Disease Reaction	Genotype
HS (28)	PLS-412, PPLS12-1, PP-416, SL-468, SL-475, SL-466, IS-262, VR-523, IS-264, KRS-311, KRS-310, KRS-309, KRS-308, KRS-307, KRS-306, KRS-305, KRS-304, KRS-303, KRS-302, PAU-513, FLS-31, Punjab Tej, Punjab Guchedar, Punjab Sindhuri, AC-105, AC-104, IHR-616, TC-7246
S (58)	PI-419, PL-121, PC-1-1, PPLS-12-1, PL-413, PL-420, PP-421, SL-462, SL-473, SP-479, C-142, FL-201, DL-161, PAU-114, PAU-115, JP-283, MSFL-1-2, MSC-31-4, PAU-212, PAU-215, PAU-216, SR-467, PP-418, PP-421, PAU-217, PP-416, PC-410, FLP-441, PAU-512, FLP-444, JH-271, UP-3, AN-21, PP-158, FLP-301, FLP-445, FLP-482, FLP-485, S-343, PU-423, PC-1, TC-7246, PBC-362, SR-481, SN-921, SN-923, UP-4-1, AC-103, VS-9, PP-414, UJ-503, PL-406, IS-261, AC-102, PC-408, YL-582, PP-402, CH-1,
MS (12)	SHHP-404, IS-266, IS-265, PAU-114, PAU-211, PAU-212, PAU-213, PAU-214, PAU-115, PAU-116, PAU-511, VR-521,
MR (20)	IS-267, IS-268, PAU-211, G-4, GC-222, YB-583, SN-480, PE-422, PE-415, IHR-583, Co-4390, PJ-424, KLC-111, KLC-112, FLP-483, FLP-431, 1-6-4, JL-282, US-501, ML-342,
R (7)	SM-478, IS-263, COO-226, VR-523, PL-412, IS-269, KC-312
HR	NIL

S-Susceptible, HS-Highly Susceptible, MS-Moderately Susceptible, MR-Moderately Resistant, R-Resistant

the experiment. From the naturally infected PepMoV plants, confirmed by DAS-ELISA, fruits were collected and numbers of seeds were counted from these infected fruits of Suraj Mukhi, Punjab Tej, SL-475, CH-1, FLP-483, KRS-303 and IS-269.

It has been found that the *Pepper mottle virus*, lead to the reduction in the number of seed formation, as compared to the healthy plants of the respective genotype (Fig 2). Maximum seed formation was observed to be negatively hampered in case of KRS-303 and

SL-475. It was noticed that in KRS genotype, per cent reduction in seed yield was maximum *i.e* 76 per cent, followed by SL-475 (57%). These two genotypes are found to be highly susceptible to PepMoV infection in artificial screening. Whereas, in the resistant genotypes effect on per cent reduction was minimum in FLP-423 (3.0%) followed by IS-269 (15%) genotype. Where as in popular chilli hybrid, the seed yield was reduced due to PepMoV upto 40 per cent. In other two popular varieties viz., Suraj Mukhi and Punjab Tej, adopted by farmers at large scale the per cent yield reduction was 31 and 20 per cent respectively. Earlier Kumari and Makkouk (1995) also reported the yield loss due to Pea seed borne mosaic potyvirus in different genotypes. They reported that yield losses can be upto 61 per cent due to virus infection. It has also been reported that in case of *Soybean mosaic virus* (SMV) infections that occur after flowering has less than 25% impact on yields or seed quality (Bowers and Goodman, 1979; Ren *et al.,* 1997a; Song *et al.,* 2016). It is evident from these findings that PepMoV can lead to sustainable damage in chilli production if further spread is not contained timely. The study has also found promising resistant sources against the virus infection which can further be utilised in resistance breeding programme against PepMoV. Hot pepper is one of the most important vegetable and spice crop of India. Due to the lack of effective management strategies against plant viruses the most economical approach left is growing of resistant sources. Moreover, if seed yield is affected, it necessities to investigate the underneath factors. The present study was conducted via keeping in view the same objectives. Thus, the promising genotypes identified in the present study can be exploited as source of resistance in breeding programme against this emerging pathogen and it also would became apparent to study the effect of PepMoV infection on seed yield in further developed varieties.

References

Anonymous (1990) *Progress Report.* Asian Vegetable Research and Development Center, Shanhua, Taiwan.

Anonymous (1991) *Progress Report.* Asian Vegetable Research and Development Center, Shanhua, Taiwan.

Bowers GR and RM Goodman (1979) Soybean mosaic virus: infection of soybean seed parts and seed transmission. *Phytopathol.* **69:** 569-572.

Green SK and JS Kim (1991) Characteristics and control of viruses infecting peppers: a literature review (No. 635.64398/G798). Asian Vegetable Research and Development Center.

Guerini MN and JF Murphy (1999) Resistance of *Capsicum annuum* 'Avelar' to pepper mottle potyvirus and alleviation of this resistance by co-infection with Cucumber mosaic cucumovirus are associated with virus movement. *J Gen Virol.* **80**: 2785-2792.

Kaur S, SS Kang, A Sharma and S Sharma (2014) First report of *Pepper mottle virus* infecting chilli pepper in India. *New Dis Rep.* **30**: 2044-0588.

Kaur S, SS Kang, A Sharma, SK Jindal and MS Dhaliwal (2018) Evaluation of Hot Pepper Germplasm for Multiple Disease Resistance against Root Knot Nematode and Viruses. *Ind J Pl Gen Res.* **31**: 243-50.

Kogovšek P, A Kladnik, J Mlakar, MT Žnidarič, M Dermastia, M Ravnikar and M Pompe-Novak (2011) Distribution of Potato virus Y in potato plant organs, tissues, and cells. *Phytopathol.* **101**: 1292-300.

Kumari SG and KM Makkouk (1995) Variability among twenty lentil genotypes in seed transmission rates and yield loss induced by pea seed-borne mosaic potyvirus infection. *Phytopathol Mediter.* **34**: 129-32.

Moury B, A Palloix, C Caranta and P Gognalons (2005) Serological, molecular, and pathotype diversity of *Pepper veinal mottle virus* and *Chilli veinal mottle virus*. *Phytopathol.* **95**: 227.

Muthukumar A and R Bhaskaran (2007) Tactics to manage disease problems in pepper. *Spice India* **20**: 20-25.

Nelson MR and RE Wheeler (1972) A new virus disease of pepper in Arizona. *Pl Dis Report* **56**: 731.

Ren Q, TW Pfeiffer and SA Ghabrial (1997a) Soybean mosaic virus incidence level and infection time – interaction effects on soybean. *Crop Sci.* **37:** 1706-1711.

Sandhu KS and JS Chohan (1979) Studies on the characterization of the mottle disease of chilli (*Capsicum annuum*) in the Punjab. *Ind J Mycol Pl Pathol.* **9**: 177-182.

Sharma S, A Sharma A and SS Kang (2018) Seed transmissibility of the virus: Survival of virus. *Curr Sci.* **115**: 2014-16.

Sharma S, A Sharma and SI Kaur (2019) Occurrence of pepper mottle virus on tomato in India. *Virus Dis.* **30:** 474-475.

Shukla DD, CW Ward and AA Brunt (1994) *The Potyviridae.* CAB International, Cambridge.

Song YP, C Li, L Zhao, A Karthikeyan, N Li, K Li and HJ Zhi (2016) Disease spread of a popular soybean mosaic virus strain (SC7) in southern China and effects on two susceptible soybean cultivars. *Philli J Agri Sci* **99**: 355-364.

Zitter TA and AA Cook (1973) Inheritance of tolerance to a pepper virus in Florida. *Phytopathol.* **63**: 1211-1212.