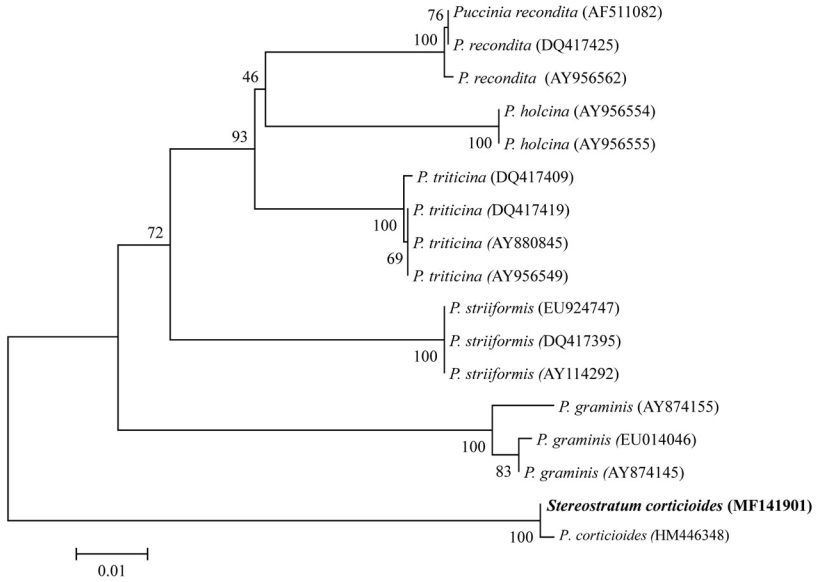


Figure 2. Neighbour-joining tree from ITS sequences showing the relationship between *Stereostratum corticioides* (previously known as *Puccinia corticioides*) of the present study and other closely related *Puccinia* species retrieved from the GenBank. Bootstrap values (1000 replicates) are shown on the branches. Bar = 1 nucleotide substitutions per 100 nucleotides.

According to Thirumalachar, the teliospores produced in very large sized sori are characteristic of this fungus[9].

Earlier studies from India have reported rusts on bamboos[10]. We describe here the occurrence of *Stereostratum corticioides*, an obligate fungal pathogen causing the culm rust disease of a bamboo in Arunachal Pradesh. This rust has been reported from China, Japan and Pakistan[11]. It causes serious culm rust disease on *P. bambusoides* in China[12] and also infects 16 other species of bamboo. It produces urediniospores and teliospores on bamboo; an alternate host is unknown for this rust[11]. In Ziro valley, the maturity of *P. bambusoides* is indicated by the shedding of its leaves and the green stem turning yellow due to the appearance of the rust fungus[13]. Locally known as *Taipona*, the rust usually begins appearing during the month of March. The sori on the culms are scrapped and chewed by the Apatanis. The current report is noteworthy since according to Hyde *et al.*[14], of the more than 1100 fungi associated with bamboo species, relatively fewer are known from India.

1. Kaul, R. N. and Haridasan, K., *J. Econ. Tax. Bot*., 1987, **9**, 378–389.
2. Bharali, S. and Khan, M. L., *Curr. Sci*., 2011, **101**, 855–860.
3. Olson, D. M. and Dinerstein, E., *Conserv. Biol*., 1998, **12**, 502–515.
4. Melkanina, N. P., *Indian For.*, 2008, **134**, 344–350.
5. Cummins, G. B., *The Rust of Cereals, Grasses and Bamboos*, Springer-Verlag, New York, 1971.
6. Chen, X., Line, R. F. and Leung, H., *Genetics*, 1993, **83**, 1489–1497.
7. White, T. J., Bruns, T., Lee, S. and Taylor, J., In *PCR Protocols*: *A Guide to Methods and Application* (eds Innis, M. A. *et al.*), Academic Press, San Diego, 1990, pp. 315–322.
8. Tamura, K., Stecher, G., Peterson, D., Filipski, A. and Kumar, S., *Mol. Biol. Evol*., 2013, **30**, 2725–2729.
9. Thirumalachar, M. J., *Mycologia*, 1947, **39**, 231–248.
10. Mundukur, B. B and Kheswalla, K. F., *Mycologia*, 1943, **35**, 201–206.
11. Mohanan, C., *Diseases of Bamboos in Asia: An Illustrated Manual. International Network for Bamboo and Rattan*, International Development Research Centre, New Delhi, 1997, pp. 79–80.
12. Kuai, S. Y., *J. Forest Sci. Technol*., 1996, **4**, 64–71.
13. Tangjang, S. and Nair, P. K. R., *Int. J. Environ. Agric. Res*., 2016, **2**, 25–34.
14. Hyde, K. D., Zhou, D. Q. and Dalisay, T., *Fungal Divers.*, 2002, 9, 1–14.

ACKNOWLEDGEMENTS. S.T. and T.S.S. thank the Department of Biotechnology, New Delhi for funding the NE-Twinning project (BT/431/NE/TBP/2013) and Swami Shukadevananda, Chairman, VINSTROM, for facilities.

Received 30 May 2017; revised accepted 9 July 2018

S. TANGJANG[1]
M. SUDHAKARA REDDY[2]
T. S. SURYANARAYANAN[3,*]
TAPI TAKA[1]

[1]*Department of Botany, Rajiv Gandhi University, Rono Hills 791 112, India*
[2]*Department of Biotechnology, Thapar University, Patiala 147 004, India*
[3]*Vivekananda Institute of Tropical Mycology, Ramakrishna Mission Vidyapith, Chennai 600 004, India*
**For correspondence.*
e-mail: t_sury2002@yahoo.com

Seed transmissibility of *Pepper mottle virus*: survival of virus

PepMoV was first recognized in Arizona (USA) in 1969 as a new strain of potyvirus that infected peppers. It was first reported from Palm Beach County, Delray Beach, Florida, (USA)[1–3] in *Capsicum annuum*. Recently, the virus has been reported from other pepper growing countries of the world, such as Taiwan, India, Korea, China, Japan, Cuba[4–9]. Indian chilli (*Capsicum annuum*) is infected by potyviruses such as *Potato virus Y* (PVY) and *Pepper veinal mottle virus*[10,11]. Sandhu and Chohan[12] reported PepMoV for the first time in 1979 from Punjab on serology study basis which was later molecularly characterized in 2014 (refs 5, 12). PepMoV is transmitted by insects, i.e. many species of *Aphis* namely *gossypii*, *craccivora* and *Myzus persicae* in a non-persistent manner in the field[13]. The virus belongs to most important family

of plant viruses, i.e. Potyviridae and infects mostly *Capsicum* sp. This virus has flexuous rod-shaped particles which measures around 730 nm × 11 nm and contains a single + sense RNA genome of about ~10 kb in length. PepMoV is transmitted via mechanical inoculation or grafting, but not reported to be transmitted by contact between plants. It has not yet been reported to be transmitted by seed.

In this study, we conducted the experiment to prove the seed transmissibility of PepMoV. This is the first report of seed transmissibility of PepMoV till date. As the mechanism of seed transmissibility can contribute significantly towards the epidemiology of this emerging viral disease, emphasis should be laid on virus-free seed production.

In the field sown chilli crop, some of the cultivars/lines expressed symptoms similar to PepMoV disease. Leaf samples from hot pepper plants showing symptoms of pepper mottle (Figure 1) (dark green vein banding accompanied with fruit deformation as well as stunting of the plant) were collected from the Vegetable Research Farm at Punjab Agricultural University, Ludhiana (Punjab, India). On fruits, symptoms were observed as deformed fruits, reduced fruit size with green streaks on fruits. Typical PepMoV induced symptoms appeared in early March. Leaves were collected from 100 symptomatic and asymptomatic plants of sown cultivars.

From the field tested samples of chilli which were found positive in DAS-ELISA, in 2015, fruits were collected and stored for conducting further experiments. The fruits were collected at ripened stage (Figure 2). Seeds were isolated from fruits using gloves. Sterilized covers were used for storage of seeds. Harvesting of seeds was done on the basis of ELISA reading. Seeds of infected plants from respective cultivars, which showed highest ELISA value, were harvested separately. These seeds were further sown in sterilized soil, in replica of 10 for 10 cultivars. The plants were maintained in insect-proof cages for further study. Fifty seeds from each cultivar were sown under protected conditions, and subsequently around 200 seedlings both symptomatic and asymptomatic were harvested 5 weeks post-emergence. They were further individually assayed via ELISA.

The samples (including leaf, seed, placenta and fruit cover) were tested by DAS-ELISA using a polyclonal antibody to PepMoV, *Potato virus Y* ($PVY^{o/c}$) and *Potato virus Y* (PVY^{N}) (Agdia, Elkhart, USA).

Total RNA was extracted from seeds, leaf, placenta and fruit cover of the chilli that had tested positive by ELISA. RNA was extracted using FastRNA ProGreen kit (MP Biomedicals, USA) according to the manufacturer's protocol (Figure 3). M-MuLV Reverse Transcriptase (Promega, USA) was used for reverse transcription of RNA. First strand cDNA was synthesized separately using RevertAid first strand cDNA synthesis kit. Total 20 µl reaction mixture containing 5 µl of total RNA (700 to 1000 ng), 1 µl reverse primer NIb3R (for amplification with Nuclear Inclusion b gene based primer pair) (20 pM), 4 µl reaction buffer (5×), 1 µl ribolock RNase inhibitor (20 U/µl), 2 µl of dNTP mix (1 mM), 1 µl M-MuLV reverse transcriptase (200 U/µl) and 6 µl nuclease-free water was used. The reaction mixture was incubated in a thermal cycler at 42°C for 45 min followed by heating at 70°C for 10 min according to manufacturer's protocol. PCR was performed separately in a 25 µl reaction volume containing 5 µl of cDNA, 5 µl reaction buffer (5×), 1.5 mM $MgCl_2$, 0.2 mM dNTP mix, NIb2F/NIb3R (degenerate primer) given by Zheng *et al.*[14], 20 pmol/ml each and 1U DNA polymerase. The PCR cycling profile consisted of initial denaturation at 94°C for 3 min, followed by 30 cycles consisting of denaturation at 94°C for 1 min, primer annealing at 52°C for 1 min and extension at 72°C for 1 min and a final extension at 72°C for 10 min. The PCR products were analysed on 1% agarose gel with 100 bp DNA marker (Promega, USA).

Chilli is one of the most valuable vegetable crops globally[15], cultivated in many countries. In production India ranks first, followed by China and Pakistan. However, PepMoV is a recent threat to chilli production, but there is a need to work out the yield loss potential of the virus.

The common symptoms associated with the virus disease were mottling, green stripes on fruits and fruit deformation as well as dark green vein banding on infected chilli cultivars. Similarly, mottling, crinkling and dark green vein banding has been observed to be caused by PepMoV from pepper growing countries of the world, such as Taiwan, India, Korea, China, Japan and Cuba[4–9]. This continental distribution of virus suggests that transmission of virus is not mediated through any other possible way but by seed, as the trade of germplasm among

Figure 1. Symptomatic chilli plant infected by PepMoV.

Figure 2. Fruits harvested from naturally PepMoV infected plant.

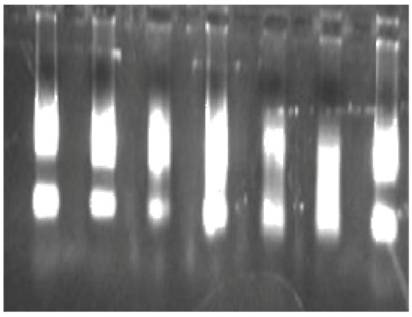
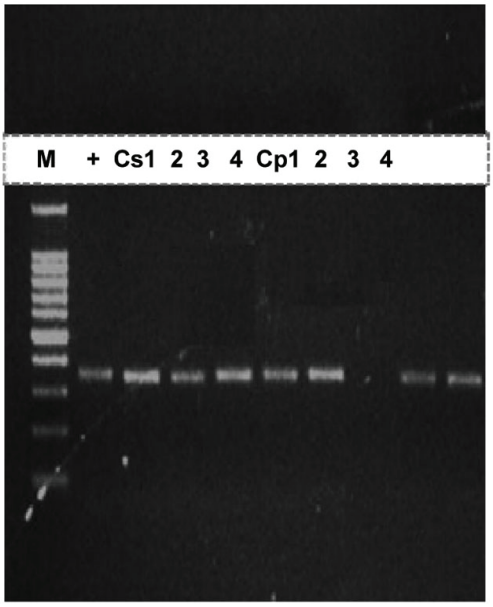


Figure 3. RNA isolated from seed and placenta of chilli samples.



NIB	NIb2F – GTITGYGTIGAYGAYTTYAAYAA NIb3R – TCIACIACIGTIGAIGGYTGNCC	NIb gene	Zheng *et al.*[14]

Figure 4. PCR results with Nib gene based primers. 100 bp base pair marker (M), positive control (+) with PepMoV-infected chilli seed (Cs1-4) and placenta (Cp1-4).

the scientific community is recurring. ELISA results confirm presence of PepMoV in all infected samples, which were responsible for most of the observed symptoms.

Until now PepMoV is not reported to be seed transmitted to the next generation[7]. Infected seeds from field infected fruits of PepMoV were found positive in DAS-ELISA and later showed symptoms in true leaves after 4 weeks of sowing in sterilized soil under insect-proof cage conditions. The fact that the plants were maintained in an insect-proof cage, avoided the possibility of any vector transmission of virus. This indicates that seeds from the field infected fruits carried over the virus to the next generation.

To further establish the role of seed in the carryover of virus to the next generation, RT-PCR was performed. Nuclear inclusion gene-based RT-PCR was conducted on the seed. A desired amplicon of ~350 bp was observed on agarose gel (Figure 4).

Among the thirty highly seropositive samples, amplification with the Nib-based primer was observed in 13. The possible reason could be the low titre of the virus in the seed. Until this level it was confirmed that the virus was seed borne. To localize the virus, RNA was isolated from placenta and fruit cover of chilli. Using upstream primer of NIb region, cDNA was prepared by RT-PCR. Twelve out of 30 samples of the placental part produced the desired amplicon corresponding to 350 bp. But none of the fruit cover samples could be amplified.

Limited studies have been conducted on seed transmission of PepMoV[6,8,9]. There still lies a significant gap with respect to our knowledge about the seed transmission of this virus. Despite widespread presence among the nations including India, not much information has been gathered about the impact of virus on seed transmissibility or the effect on seed quality.

In this study, PepMoV was detected in seeds, placenta and in young seedlings harvested from fruits infected by PepMoV naturally. We also studied and proved that seedlings emerged from PepMoV infected seeds could further act as potential reservoirs of the virus for vector transmission.

Seed-transmissible viruses tend to pose a huge threat for epidemic, as they can act as a primary source of inoculum for further vector-mediated spread over long range of distance. Therefore, it becomes urgent and essential to understand the epidemiology of the virus with respect to seed transmission, as it is important for plant protection against initial infection and its further spread. The nature of this virus spread, to be sporadic over the world and more evenly distributed in the Asian continent, can be attributed to seed transmissibility. Moreover the sporadic spread of the virus that is of common occurrence in bio-nurseries can be resolved via careful study on transmission through seeds.

1. Nelson, M. R. and Wheeler, R. E., *Plant Dis. Reptr*., 1972, **56**, 731.
2. Zitter, T. A., *Plant Dis. Reptr*., 1972, **56**, 586.
3. Zitter, T. A., *Plant Dis. Reptr*., 1972, **56**, 731.
4. Cheng, Y. H., Deng, T. C., Chen, C. C. and Liao, J., *Plant Dis*., 2011, **95**, 617.
5. Kaur, S., Kang, S. S., Sharma, A. and Sharma, S., *New Dis. Rep*., 2014, **30**, 14.
6. Kim, Y. J., Jonson, M. G., Choi, H. S., Ko, S. J. and Kim, K. H., *Virus Res*., 2009, **144**, 83–88.
7. Luo, X. *et al*., *Can. J. Plant Pathol*., 2016, **38**, 506–510.
8. Ogawa, Y., Hagiwara, K., Iwai, H., Izumi, S. and Arai, K., *J. Gen. Plant Pathol*., 2003, **69**, 348–350.
9. Quiñones, M. *et al*., *New Dis. Rep.*, 2011, 24.
10. Jayarajan, R. and Ramakrishna, K., *Madra. Agric. J.*, 1969, **56**, 761–766.
11. Khatri, H. L. and Sekhon, I. S., *Indian J. Mycol. Plant Pathol*., 1984, **4**, 121–125.
12. Sandhu, K. S. and Chohan, J. S., *Indian J. Mycol. Plant Pathol*., 1979, **9**, 177–182.
13. Shukla, D. D., Ward, C. W. and Brunt, A. A., In *The Potyviridae*, Wallingford, UK, CAB International, 1994, pp. 74–110.
14. Zheng, L., Rodoni, B. C., Gibbs, M. J. and Gibbs, A. J., *Plant Pathol.*, 2010, **59**, 211–220.
15. Geetha, R. and Selvarani, K., *Int. J. Adv. Res. Innov. Ideas Eduac.*, 2017, **3**, 205–210.

Received 7 December 2017; revised accepted 10 July 2018

SHIKHA SHARMA[1,*]
SANTOKH SINGH KANG[1]
ABHISHEK SHARMA[2]

[1]*Department of Plant Pathology, and*
[2]*Department of Vegetable Science,*
Punjab Agricultural University,
Ludhiana 141 004, India
**For correspondence.*
e-mail: shikhasharma-coapp@pau.edu