SHORT COMMUNICATION

Genetic variability of multifunctional HC-Pro gene of *Lettuce mosaic virus* in northern region of India

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ABSTRACT: *Lettuce mosaic virus* (LMV) is an economically important pathogen causing mosaic disease in spinach (*Spinacia oleracea*). Isolates of LMV originating from different geographical regions in northern India were collected during the survey of 2013-2014. *Lettuce mosaic virus* (LMV) belongs to *Potyvirus* family (genus *Potyvirus*) and is mainly transmitted by aphids. This study covered the genetic variability of Helper component proteinase (HC-Pro) gene of LMV isolates. HC-Pro is multifunctional activity gene, which helps in transmission of virus. The amplified PCR products of ~1.3 kilo basepairs (kb) were obtained from five different isolates, sequenced and deposited in NCBI GenBank. Two isolates R4 and HR1 from Rajasthan (KJ558375, KM501033), isolate R5 from Haryana (KP635365), isolate R6 from Uttar Pradesh (KP635366) and isolate R4 from Delhi (KP635367). The phylogenetic analysis of LMV HC-Pro sequences is showed divergence with other isolates. These isolates showed two different clusters *vis-a-vis* diversity amongst each other.

Keywords: Diversity, HC-Pro, Lettuce mosaic virus, *Potyvirus*, spinach

Spinach (*Spinacia oleracea*) is a common edible vegetable crop grown in the temperate region throughout the world. Being a high nutritional value, it is a rich source (> 20% of the Daily Value) of vitamin A, vitamin C, vitamin K, magnesium, manganese, folate and iron. According to UC pest management guidelines several plant viruses are reported from spinach (<http://www.ipm.ucdavis.edu/PMG/r732100611.html>). These viral diseases may cause growth stunting and yellowing of plants and reduction in the yield. Several society reports (<http://www.apsnet.org/publications/commonnames/Pages/Spinach.aspx>) indicated that spinach is infected by as many as 10 viruses belonging to the genus namely; *Hybrigeminivirus*, *Luteovirus*, *Umbravirus*, *Fabavirus*, *Cucumovirus*, *Potyvirus*, *Tobamovirus* and *Tospovirus* are reported from worldwide (Fotopoulos *et al.*, 2011).

LMV belongs to the genus *Potyvirus* and reveals characteristic symptoms of dwarfing, poor heading, mottling, and vein clearing (Lim *et al.*, 2014). Besides the non-persistent transmission by aphid/vector (*Myzus persicae* and *Macrosiphum euphorbiae*) it is also transmitted by exchange of contaminated seeds and it depends on cultivars as well as age of seedling at the time of inoculation (Jones *et al.*, 2003). It is possible to differentiate between vector of LMV that land but do not replicate in the crops (transient vectors) (e.g. *Myzus persicae* Sulzer), transient nonvectors [e.g. *Hyalopterus pruni* (Geoffroy)], colonizing vectors [e.g. *Macrosiphum euphorbiae* (Thomas)] and colonizing non-vector species (e.g. *Nasonovia ribisnigri* Mosley) (Moreno *et al.*, 2007).

Helper component proteinase (HC-Pro) is a multifunctional protein and it plays an important role in transmission of *Potyvirus* (Verma *et al.*, 2014; Mishra *et al.*, 2014). HC-Pro is generally divided into three functional domains: domain I (N-terminal domain) whose main function is to mediate aphid transmission, has likely been coevolving with the other two domains, a central region, and a cysteine protease domain (CPD) in the C-terminal region. N terminal region is required for aphid transmission and efficient genome amplification, the central region for long distance movement in plants (Bihong *et al.*, 2011). PCR based detection method is primary approach for identification the HC-Pro gene of LMV.

Leaf of spinach showing characteristic symptoms of virus infection including dwarfing, mottling, mosaic and vein clearing (Fig. 1) were collected during field

Fig. 1. Spinach leaves showing potyvirus infection (curling in leaves)

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investigation of different regions of northern India (Rajasthan, Haryana, Delhi and Uttar Pradesh) in 2013-14. All samples of were kept frozen (-20°C) prior to testing.

Total RNA was extracted from each collected infected leaf using TRIzol[®] Genei[™] Merk. The leaf samples were homogenized or disrupted in the TRIzol[®], subsequently, chloroform addition was followed by centrifugation to obtain three distinct layers of lysate. Furthermore, the RNA was precipitated from aqueous phase with isopropanol. The total RNA was reverse transcriptase to first strand of c-DNA by c-DNA synthesis kit (MERK, Genei, INDIA) using Oligo (dT) as primer (followed the manufacturer's instructions). This first strand of c-DNA was used as template to amplify HC-Pro. For the amplification of HC-Pro gene RT-PCR reaction was performed in a total volume of 20 µl containing 2 µl of first strand cDNA, 2µl of 10x PCR buffer, 1 µl of each forward and reverse primer (HPF 5'ATGCATNACACCRNGAAYGATATTCTTG3' and H P R 5' T C A T G C G Y A A A T T I C A C C A G C T G G R G G G T C A C G 3') (100 ng/µl), 1 µl of dNTPs (2.5 mM each), 1.35 unit of *Taq* polymerase and nuclease free water to a final volume. For the amplification of HC-Pro gene degenerate primer was used, which are designed after the multiple sequence alignment analysis with 30 different sequences of LMV HC-Pro from NCBI databank.

The PCR products were further cloned into pGEM-T vector (Promega, USA), using standard molecular biology procedures. Positive clones were identified by colony PCR as well as restriction digestion of plasmid recombinant. Positive clones (5 recombinants) were sent for custom sequencing.

The HC-Pro sequences were initially analyzed using the BLAST program (<http://www.ncbi.nlm.nih.gov/BLAST>). The sequence identity matrix between amino acid sequences and nucleotide sequences was calculated using the BioEdit software version 5.09.04. Amino acid sequences were aligned using Clustal W

(Verma *et al.*, 2016; Yadav and Khan, 2015) and Neighbor-joining tree was generated using MEGA v. 7.0 program (Kumar *et al.*, 2016).

To examine genetic variability of the LMV population in north region of India, field surveys were performed in 2013-2014. Therefore, we have sampled spinach from the fields. All LMV infected samples were identified by RT-PCR. After the PCR amplification we have found ~1.3 kb band in all five infected samples. After PCR amplification, eluted amplicons were cloned in pGEM-T vector separately and transformed in *E.coli* DH5 α . Near about twenty clones were identified by colony PCR as well as by restriction digestion with *EcoRI*. After the confirmation all clones (five clones for each) were sequenced (Xcelris Pvt Ltd, Ahmedabad, Gujarat, India) and submitted to the NCBI GenBank [Accession No: KJ558375 (isolate R4 from Rajasthan), KM501033 (isolate HR1 from Rajasthan), KP635365 (isolate R5 from Haryana), KP635366 (isolate R6 from Lucknow, Uttar Pradesh) and KP635367 (isolate R4 from Delhi)].

BLAST analysis LMV Indian isolates HR1, R5 and R7 showed 90%, 78% and 77% identity respectively with LMV strain E from France. R4 and R6 isolates showed 75 % and 76% sequence identity with LMV isolate Tn515-ev Tunisia and LMV isolate muju respectively. Sequence identity matrix analysis of the 5 Indian HC-Pro isolates revealed 60-82% nucleotide sequence identity themselves and other LMV isolates compared in this study. Comparative analysis of amino acid sequences showed 70-99% sequence identity themselves and other LMV isolates (Table 1). The phylogenetic analysis of LMV HC-Pro sequence showed the ancestry and evolutionary relationship with other isolates LMV Brazil isolates and LMV France strain E (Fig. 2).

The sequence identity matrix showed the clear diversity among Indian isolates (under this study) at nucleotide and amino acid level. Similar types of genetic variability study have been described for the HC-Pro genes of sugarcane streak mosaic virus (SCSMV). Phylogenetic analysis of LMV isolates from north India

Fig. 2. Cladogram of LMV HC-Pro by Neighbour-joining (NJ) method. (*) mark indicates this study sequences

Table 1. Percent nucleotide (Above the diagonal line) and amino acid (below the diagonal line) between HC-Pro genes of Lettuce mosaic virus (LMV) isolates from India (this study)

LMV isolates	HR1	R4	R5	R6	R7
HR1	100	82.1	76.8	76.0	61.1
R4	99.3	100	76.6	80.7	59.7
R5	99.0	99.7	100	75.8	61.2
R6	99.0	99.7	99.5	100	60.1
R7	70.0	69.3	69.3	69.0	100

(under this study) showed in two separate clusters in which isolates HR1 and R7 from Rajasthan and Delhi respectively in one cluster and rest of three isolates R4, R5 and R6 from Rajasthan, Haryana, Lucknow (Uttar Pradesh) respectively in another cluster. HC-Pro of LMV Indian isolate HR1 and R7 showed phylogenetic relationship related with LMV France (strain E) and another hand side LMV isolate R4 shows clustering with LMV Brazil isolate AF 199 2013 France isolates.

Several LMV isolates have been characterized from different geographical regions and analysed on the basis of capsid protein (CP) genes (Lim *et al.*, 2014). There are some reports for HC-Pro genetic diversity of different species of potyvirus (Jaroszewska *et al.*, 2013) but in our knowledge, for LMV, this is the first evidence of phylogenetic analysis and diversity of HC-Pro in India. The study presented shows the diversity of Lettuce mosaic virus in Northern India. Our results will certainly help to develop strategies for developing resistance varieties against LMV and it might be useful in future efforts to control the disease; particularly should there be further epidemics.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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