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RESEARCH ARTICLE

Himalayan wild cherry (*Prunus cerasoides*) as a new natural host of *Cherry necrotic rusty mottle virus* (CNRMV) and a possible role of insect vectors in their transmission

P. Awasthi[1,2], R. Ram[2], S.G.E. Reddy[3], Gireesh Nadda[3], A.A. Zaidi[1,†] & V. Hallan[2]

1 Academy of Scientific and Innovative Research (AcSIR), New Delhi, India
2 Plant Virology Lab, Floriculture Division, CSIR-Institute of Himalayan Bioresource Technology, Palampur, India
3 Entomology Lab, Hill Area Tea Science Division, CSIR-Institute of Himalayan Bioresource, Technology, Palampur, India

Keywords
Aphids; CNRMV; delphacid; natural host; RNA virus; virus vector.

Correspondence
V. Hallan, Academy of Scientific and Innovative Research (AcSIR), New Delhi, India/Plant Virology Lab, CSIR-Institute of Himalayan Bioresource Technology, Palampur 176061, Himachal Pradesh, India. Email: rnaivi@gmail.com/hallan@ihbt.res.in

†Deceased.

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Abstract

A Himalayan wild cherry (Hwc) tree (*Prunus cerasoides*) growing in the vicinity of an already reported *Cherry necrotic rusty mottle virus* (CNRMV) infected bamboo (*Dendrocalamus hamiltonii*) showed severe virus-like symptoms on the leaves consisting of marginal chlorosis, mosaic, shot holes and necrotic spots. Reverse transcription-polymerase chain reaction (RT-PCR) using virus specific primers confirmed the presence of the virus. In order to assess the prevalence of this virus, 40 symptomatic and asymptomatic Hwc plants along with four different suspected insect vectors were analysed by double antibody sandwich-enzyme linked immunosorbent assay (DAS-ELISA), dot blot hybridisation and RT-PCR. CNRMV was detected in 57% of the wild cherry samples, two aphid species *Astegopteryx bambusae* and *Tinocalloides montanus*, and in a delphacid of *Bambusiphaga* sp. Complete coat protein (CP) gene of CNRMV was sequenced from two randomly selected samples. The isolates characterised from Hwc shared 99% similarity to the already characterised isolates of CNRMV from India, suggesting a conserved nature of the CP gene. This study represents the first report of CNRMV infection in Himalayan wild cherry and its possible insect vectors.

Introduction

Prunus cerasoides D. Don also known as *P. puddum*, Himalayan wild cherry (Hwc), 'phuya', 'panyyan', 'paja', 'pajja', 'paddam', 'padmak', 'padmakh' or 'padmakashta', is a deciduous tree of *Prunus* species in *Rosaceae* family extensively distributed in the forests of the Himalayas (Chatterjee & Pakrashi, 1992; Chauhan, 1999; Chankija, 2006; Singh & Gaur, 2008). In India, sweet cherry (*Prunus avium* L.) and sour cherry (*Prunus cerasus* L.) are grown mainly in the North-Western states, Jammu and Kashmir, Himachal Pradesh and in hilly regions of Uttarakhand. The commercial plantations of sweet cherry are raised mainly on seedling of '*paja*' (*Prunus cerasoides* var. majestic), bird cherry (*Prunus padus*), Mazzard (*P. avium*) and Mahaleb (*P. mahaleb*) (Bajwa *et al.*, 1959; Ghosh, 2005). The economic impact of a virus infection is usually greatest when diseased scion buds are grafted on healthy rootstocks or vice versa to produce the nursery tree.

Hwc has only recently been explored for the presence of major pome and stone fruit viruses and has been found to harbour viruses such as *Apple chlorotic leaf spot virus* (Rana *et al.*, 2008), *Prunus necrotic ring spot virus* (Chandel *et al.*, 2007) and a viroid, *Apple scar skin viroid* (ASSVd) (Walia *et al.*, 2012).

Cherry necrotic rusty mottle virus (CNRMV) is an unassigned species in the family *Betaflexiviridae*. The virus causes symptoms such as brown, angular, necrotic spots, mottling, abnormal leaf fall, yellowing, shot holes and rusty chlorotic areas on the leaves of cherry plants (Wadley & Nyland, 1976), gum blisters, and necrosis of the bark in several cultivars. The disease symptoms (necrotic rusty mottle and rusty mottle) on the leaf of diseased plants can be highly variable and depend upon climatic conditions, virus isolates and plant cultivar. Virus infected trees can exhibit reduced growth, low productivity and early death of trees (Posnette *et al.*, 1968).

The disease caused by CNRMV is easily transmitted by grafting and budding but not mechanically by sap inoculation. There is no evidence of vector, seed or pollen transmission of CNRMV (Rott & Jelkmann, 2012). The

virus has mainly been detected in sweet cherries and reported from many cherry growing regions of the world such as Japan, New Zealand, North America, Europe, Chile, Germany, Korea and India. This virus has also been detected in apricot, peach, plum and sour cherry plants in China (Zhou *et al.*, 2013). In India CNRMV has been detected in sweet cherry (Noorani *et al.*, 2013) and 21 out of 35 tested varieties of bamboos (Awasthi *et al.*, 2014).

The concurrence of the CNRMV infected bamboo and the Hwc tree both of which belong to two distinct classes of flowering plants out ruling any possibility of grafting or budding, which are till date the only reported means of transmission of CNRMV infection, instigated us to assess the mode of spread of infection in these plants. Aphids are important vectors that been implicated in the spread of >50% of viral diseases (Celetti *et al.*, 2002). Aphids acquire the virus through feeding-related activities and then transmit it to nearby healthy plants. Although the efficiency of transmission largely depends upon the virus strain, the host species, the cultivar and the aphid species, migrant aphid species that do not colonize stone fruit or *Prunus* spp. are considered important virus transmission vectors (Celetti *et al.*, 2002).

Delphacids are small (2–10 mm in length), brown or greenish plant hoppers in the family *Delphacidae*, that feed on monocots such as rice, wheat, corn and sugarcane and are reported to be important pest species because of their role as vectors of plant viruses. Delphacids belonging to the genera: *Laodelphax*, *Peregrinus*, *Perkinsiella* and *Nilaparvata* in the *Delphacidea* family have been implicated in the transmission of viruses of the family *Rhabdoviridae* (*Maize mosaic virus* and *Barley yellow striate mosaic virus*), *Reoviridea* (*Fiji disease virus*, *Mal del Rio Cuarto virus* and *Rice ragged stunt virus*) and an unassigned genus *Tenuivirus* (*Rice stripe virus* and *Maize stripe virus*) (Matthews, 1991 & Capinera, 2008). *Bambisuphaga* spp. have been described to exclusively feed on bamboos (*Bambusoideae*) (Liu & Chen, 2008) and have not been described for the transmission of plant viruses.

In this article we present first report of Hwc as a new natural host of CNRMV and provide coat protein (CP) gene sequence information of two isolates. Additionally, CNRMV has also been detected for the first time in three insect vectors namely *Astegopteryx bambusae* Buckton, *Tinocalloides montanus* Basu and *Bambusiphaga* sp., which possibly act as virus vectors.

Material and methods

Plant material

In order to check for the presence of CNRMV, symptomatic leaf samples were collected and analyzed by reverse transcription-polymerase chain reaction (RT-PCR) using the detection primers that are targeted to ~190 nt region from the 3′-UTR of CNRMV genome (Noorani *et al.*, 2013). Further, to assess the prevalence of CNRMV in Hwc, four different adjoining areas of Kangra district. Kangra namely Dharamshala, Kangra, CSK HPKV and CSIR-IHBT campus, Palampur were surveyed and a total of 40 symptomatic and asymptomatic leaf samples were collected.

Double antibody sandwich-enzyme linked immunosorbent assay (DAS-ELISA) and dot blot hybridisation

The collected samples were subjected to DAS-ELISA as described by Clark & Adams (1977) using the indigenously raised antisera against the CP of CNRMV (Noorani *et al.*, 2013). To further confirm the results, the samples were tested by dot blot hybridisation. RNA from all the collected samples was extracted using the cetyltrimethyl-ammonium bromide (CTAB) method (Zeng & Yang, 2002) and blotted on to the positively charged nylon membrane (Ambion, Foster City, California, USA) in a Vacuublot system (Hoefer, GE Healthcare) according to the standard protocol (Sambrook *et al.*, 1989). The CNRMV CP antisense DIG-labelled riboprobe was prepared using the DIG RNA labelling kit (Roche Diagnostics, Mannheim, Germany). The membrane was hybridised with the riboprobe at 65 °C. Chemiluminescent detection was done using CDP star (Applied Biosystems, Foster City, CA, USA) as per the manufacturer's instructions.

Detection of CNRMV in insect vectors

The predominant insect species found on the infected trees were collected (group of 50–60 insects). Total RNA was isolated by CTAB method (Zeng & Yang, 2002) and checked for the presence of the virus by RT-PCR (using the detection primers) and dot blot hybridisation as described earlier. The isolates were named based upon the host plant from which they were collected.

Molecular characterisation of CP gene

In order to characterize the CP gene of CNRMV from Hwc, two ELISA and dot blot positive samples from different locations (Kangra and CSIR-IHBT campus, Palampur, India) were randomly selected and analyzed using the CP primers (Noorani *et al.*, 2013), RNA was isolated using CTAB method (Zeng & Yang, 2002). First strand cDNA synthesis was carried out in 25 µL reaction containing 500 ng of total RNA, 200 U of reverse transcriptase enzyme (M-MLV; USB, USA), 5 µL of 5× RT

buffer, 2.5 mM dNTPs and 10 pmol of downstream primer, CNR8432L: 5′-CTCTCGTAGAAAACTGAAGGA-3′. The reaction was incubated at 42 °C for 60 min followed by 80 °C for 5 min in a GeneAmp PCR System 9700 (Applied Biosystems).

PCR was carried out in 50 µL reaction mix containing 5 µL *Taq* Buffer A (10×) (Bangalore Genei, Bangaluru, India), 1.5 µL dNTP mix (10 mM), 1 µL (10 pmol/µL) each of downstream and upstream primers, CPU: 5′-GTGTGTGAGCTTTCAAGTTTA-3′ and CPL: 5′-CAGAGGTTTATCATTCATCACC-3′, 1.5 µL of 25 mM Magnesium chloride, 0.5 µL *Taq* DNA polymerase (3 U/µL) (Bangalore Genei) and 5 µL cDNA. The PCR reaction was performed with initial denaturation at 94 °C for 3 min; followed by 35 cycles of denaturation at 94 °C for 30 s; primer annealing at 53 °C for 30 s; extension at 72 °C for 1 min with final extension at 72 °C for 10 min. PCR products were electrophoresed on 1% agarose gel at 80 V. Gel was stained with ethidium bromide (1 µg/mL) and visualised under UV transilluminator. The amplicons were purified from the gel using GeneJET Gel Extraction kit (Thermo Scientific, Waltham, MA, USA) and cloned into pGEM-T Easy vector (Promega, Madison, WI, USA). Recombinant plasmids were purified using Plasmid DNA Miniprep kit (MDI, Ambala Cantt, India) and sequenced with an automated DNA sequencer (ABI PRISM 3130xl Genetic Analyzer) using ABI prism Big Dye Terminator v3.1 Ready Reaction Cycle Sequencing kit (Applied Biosystems), sequencing both strands.

Phylogenetic analysis

Sequences obtained were analyzed with the help of BLAST and were compared with sequences of other CNRMV isolates. Multiple alignments were carried out with the help of MultAlin software available online at http://bioinfo.genopole-toulouse.prd.fr/multalin/multalin.html (Corpet, 1988). Percentage Identity matrix was created using ClustalW2-Phylogeny version 2.1 (http://www.ebi.ac.uk/Tools/phylogeny/clustalw2_phylogeny/). Phylogenetic tree was constructed based on nucleotide sequence using the Maximum Likelihood method (implemented in MEGA5.2, best fit model: Kimura-2-parameter using a discrete gamma distribution and 1000 bootstrap replicates).

The CNRMV sequences used for the phylogenetic analysis of the 3′-UTR of CNRMV were as follows: Flowering cherry (EU188438, EU188439; Japan), Sweet cherry (FN546178, India; AF237816, Germany; KF030828, KF030832, KF030875, KF030844, KF030843, KF030839, KF030835, KC218930, USA).

The CNRMV sequences used for the phylogenetic analysis of the CP gene were as follows: Bamboo (HG796178 and HG796179, India), Sweet cherry (KC432621, Chile; AB822637, Korea; JX491635, China; KC136845, Poland; KC218930, USA; AF237816, Germany; FN546178, India), Sour cherry (JX491636, China; FJ462741, Poland) Flowering cherry (EU188439, Japan), Apricot (JX491637, China), Peach (JX648205, China) and Plum (JX648206, China). *Bamboo mosaic virus* (BaMV), acc. no. X57581 from Taiwan was taken as an outgroup.

Results

The Hwc tree under consideration in this study, growing near an already infected bamboo clump, was found to exhibit severe virus-like symptoms on the leaves which included: marginal chlorosis, mosaic, shot holes and necrotic spots (Fig. 1). The primers which were used to detect the presence of CNRMV in this tree amplified a 190 nt DNA fragment which was cloned and sequenced and was found to be 3′-UTR of the CNRMV genome (accession number: LK392370) thereby confirming the presence of CNRMV. In order to understand the incidence of this virus on wild cherry, a survey was carried out at four different locations in the Kangra district and 40 samples were collected. It was observed that 60% of Hwc trees sampled had necrotic spots and chlorosis on the leaves while 5% of these displayed additional symptoms such as marginal chlorosis, mosaic, shot holes and leaf curling, whereas 40% trees were symptomless. The samples were tested through DAS-ELISA and dot blot hybridisation. Out of the 40 plants tested 22 were found positive with optical density (OD) values 2.5× to 3× the negative control. The samples were further confirmed by dot blot hybridisation. In hybridisation, 23 samples came out to be positive which include 20 ELISA positive samples and three additional samples which were not detected by DAS-ELISA. The results of DAS-ELISA and dot blot hybridisation were further validated by RT-PCR (for a few samples). The combined results of the three detection techniques confirmed presence of CNRMV in 23 out of 40 samples thereby reporting 57% incidence of CNRMV in Hwc. About 83% of the symptomatic trees were found to be infected with CNRMV while 8% of the symptomless trees were also found to be carriers of the virus.

In case of CNRMV, grafting and budding are the only known modes of transmission of viral infection. Although Hwc rootstock is often used for the propagation of cherry, the spread of infection between a dicotyledonous Hwc tree and a monocotyledonous bamboo plant suggests that the virus also employs alternate mode of transmission besides grafting. As the wild cherry tree was growing in close proximity with the already reported CNRMV infected *D. hamiltonii* (‘maggar’ bamboo), it was hypothesised that the insects present on these could have a role in

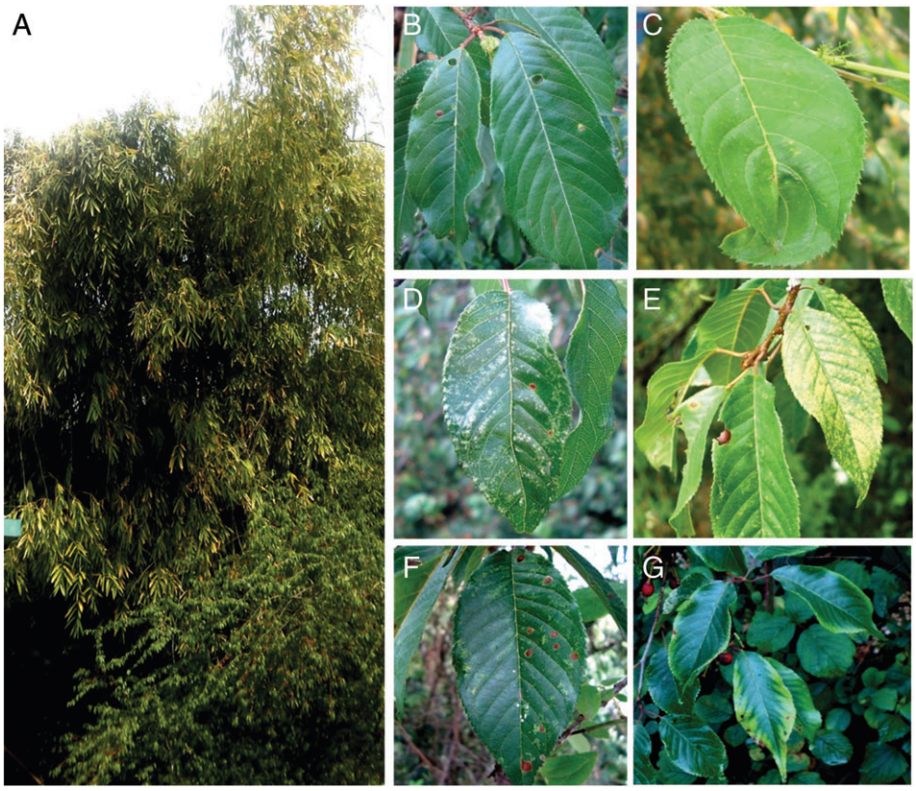

Figure 1 Virus-like symptoms observed on a Himalayan wild cherry tree growing in the CSIR-IHBT campus. (a) Himalayan cherry tree growing in the vicinity of a *Cherry necrotic rusty mottle virus* (CNRMV)-infected *Dendrocalamus hamiltonii* clump; (b–g) Virus-like symptoms on the leaves of Himalayan wild cherry tree (b: shot holes, c: leaf curling, d: chlorosis, e: mosaic, f: necrotic spots and g : marginal chlorosis along the leaf margin).

transmission of CNRMV infection among unrelated plant species. The trees were observed for 2 years, through complete growing season for colonisation of insect vectors and were found to harbour four different types of colonizing as well as non colonizing insects which included three aphid species namely: *Pseudoregma bambusicola* Takahashi, *A. bambusae* and *T. montanus* and one delphacid of *Bambusiphaga* sp. (Fig. 2).

The period of inhabitance was different for each of the four insects sampled from CNRMV infected trees (Table 1). The *P. bambusicola* aphid colonised the bamboo clumps early in November and were seen till February whereas *A. bambusae* and *T. montanus* colonised bamboo and Hwc plants, respectively only during December till early March. The Delphacid, *Bambusiphaga* sp. appeared in late March and was seen till July not only on bamboo and Hwc but also on a variety of other wild plants and trees growing at that site viz, fig, *Albizia chinensis*, wild rose, rubus, morning glory, etc.

These insects were checked for the presence of CNRMV by dot blot hybridisation and RT-PCR (using the detection primers) as described earlier. In dot blot, *A. bambusae*, *Bambusiphaga* sp. and *T. montanus* were found positive for CNRMV whereas *P. bambusicola* which was collected from the stem of CNRMV infected *B. bambos* and *D. hamiltonii* was negative for the virus. Aphids from uninfected healthy plant were taken as negative control and no signal was observed in these samples.

When analyzed by RT-PCR, positive amplification of a DNA fragment of ~190 nt was obtained from two aphid species namely *A. bambusae*, *T. montanus* and a delphacid, *Bambusiphaga* sp. collected from the sampling site. The amplicons were found to encompass 3′-UTR of CNRMV (as expected). The sequences were submitted to the Genbank database with the accession numbers: LK392372 (BAMAPH isolate from *A. bambusae*), LK392373 (Delph isolate from the delphacid: *Bambusiphaga* sp.) and LK392374 (HWCAPH isolate from *T. montanus*).

The length of 3′-UTR amplified from *Bambusiphaga* sp. was 184 nt whereas from *A. bambusae* and *T. montanus* it was 189 nt long. Multiple sequence alignment of 3′-UTR sequences from the insects and all other isolates of CNRMV showed that there is maximum variability in the nucleotide sequence between positions 21 and 29. In the sequence from bamboo aphids and Hwc aphids,

Table 1 Insects collected from CNRMV infected Himalayan wild cherry and bamboo plants along with the season during which the insects were seen on their host trees. Dot blot hybridization and RT-PCR based diagnosis results

Insect name	Host plant	Inhabitance and season	Dot blot	RT-PCR
Pseudoregma bambusicola	Bamboo (*Bambusa bambos*)	November–February	–	–
Pseudoregma bambusicola	Bamboo (*Dendrocalamus hamiltonii*)	November–February	–	–
Astegopteryx bambusae	Bamboo (*Dendrocalamus hamiltonii*)	December–March	+	+
Bambusiphaga sp.	Bamboo (*Dendrocalamus hamiltonii*), Himalayan wild cherry, adjoining plants and trees	March–July	+	+
Tinocalloides montanus	Himalayan wild cherry	December–March	+	+
Healthy insects	Uninfected plant	April	–	–

CNRMV, Cherry necrotic rusty mottle virus; RT-PCR, reverse transcription-polymerase chain reaction.

'AT' was missing (25–26) in comparison to the sequence characterised from plants with the exception of sequence from delphacid, where the 'AT' is present but four preceding nucleotides are missing. In the phylogenetic analysis of the 3′-UTR, all the Indian isolates (Sweet cherry, Bamboo, Hwc and the three insects) grouped together and were most closely related to the German and American isolates from sweet cherry. The percentage identity matrix computed for the 3′-UTR sequences revealed that the Indian isolates of CNRMV shared 97.8–100% identity amongst themselves whereas overall sequence identity between the Indian isolates and all other isolates characterised from different parts of the world was 77–98.4%.

CP gene of CNRMV was amplified from two randomly selected samples of Hwc. The amplicon sequence matched with the CP gene of CNRMV, thereby further confirming presence of the virus. The two isolates were named HPHWC7 and HPHWC29 and were submitted to the Genbank database with accession numbers LK392371 and LK392370, respectively. Multiple sequence alignment of the CP gene sequences of Hwc isolates with other already characterised isolates showed that the CP gene is 801 nt in length, consistent with the Indian isolates as compared to all other isolates characterised from other parts of the world, where these were 804 nt long. The sequence of the CP gene is also highly conserved amongst the Indian isolates with variability at just four nucleotide positions viz. 71, 173, 648 and 794. The two isolates of CNRMV characterised from Hwc (HPDH7 and HPDH29) were 99.7% similar to each other while their identity with other Indian isolates was 99.8%, 99.7% and 99.6% with HPDH36 (bamboo isolate), JK10 (Sweet cherry isolate) and HPDH12 (bamboo isolate), respectively. Similarity of Hwc isolates with Chilean sweet cherry isolate was 97% followed by 95% with flowering cherry isolate from Japan and 93% with the Korean sweet cherry isolate. The German and Chinese isolates from sweet cherry had 90% identity to the Hwc isolates. In comparison to all the CNRMV isolates from different hosts and geographical areas, Indian isolates characterised from Hwc, bamboo and sweet cherry, shared an overall 84–97% identity.

In the phylogenetic analysis based upon the CP gene, the eight isolates of CNRMV – Hwc (LK392371 and LK392370, India); Bamboo (HG796178 and HG796179, India); Flowering cherry (EU188439, Japan); Sweet cherry (KC432621, Chile; AB822637, Korea; FN546178, India) – grouped in a clade different from other isolates of the world (Fig. 3). The Indian isolates formed a close cluster with Chilean sweet cherry isolate, being the nearest neighbour.

Discussion

CNRMV has been detected in wild cherry growing abundantly in the Himalayas. Seedlings of this variety are a commonly used rootstock for the propagation of sweet cherries in many stone fruit growing areas of the country. In this study it has been established that besides being a symptomatic host, Hwc is also a symptomless carrier of the virus; therefore using infected planting material for further propagation of cultivars can lead to extensive spread of infection to the commercial plantations. Virus tested and certified planting material should be used by the growers for raising nursery trees. Further studies are necessary to establish whether CNRMV is seed-transmitted in Hwc.

Phylogenetic analysis based upon the CP gene showed that the Indian isolates which have been characterised from different areas in the states of Himachal Pradesh (Bamboo and Hwc isolates) and Jammu Kashmir (Sweet cherry isolate) are distinct from all other isolates characterised from different parts of the world and form a separate cluster irrespective of the host plant and geographical origin, suggesting that they are polyphetic. The Indian isolates are most closely related to the sweet cherry isolate from Chile followed by the Japanese and the Korean isolate which suggests that probably there has

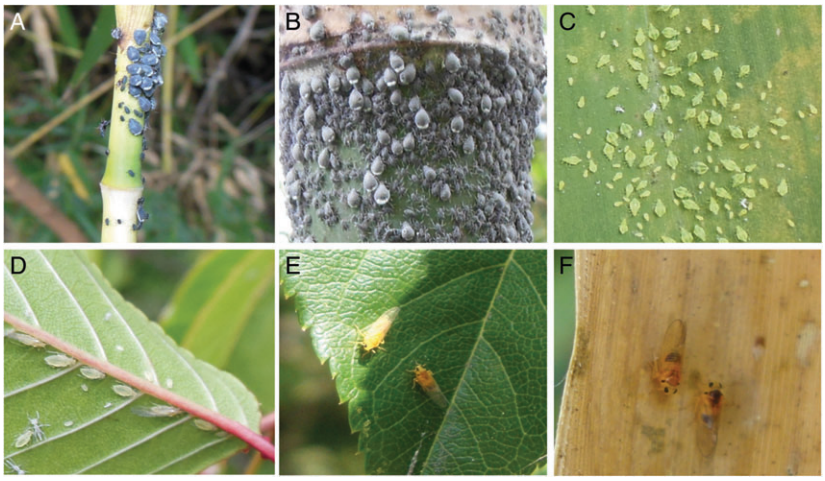

Figure 2 Insect population colonizing on the *Cherry necrotic rusty mottle virus* (CNRMV) infected Himalayan wild cherry and bamboo plants. (a) *Pseudoregma bambusicola* aphid on *B. bambos*, (b) *P. bambusicola* aphid on *Dendrocalamus hamiltonii*, (c) *Astegopteryx bambusae* aphid on *D. hamiltonii*, (d) *Tinocalloides montanus* aphid on Himalayan wild cherry, (e, f) *Bambusiphaga* sp. on the leaf of Himalayan wild cherry and *D. hamiltonii* respectively.

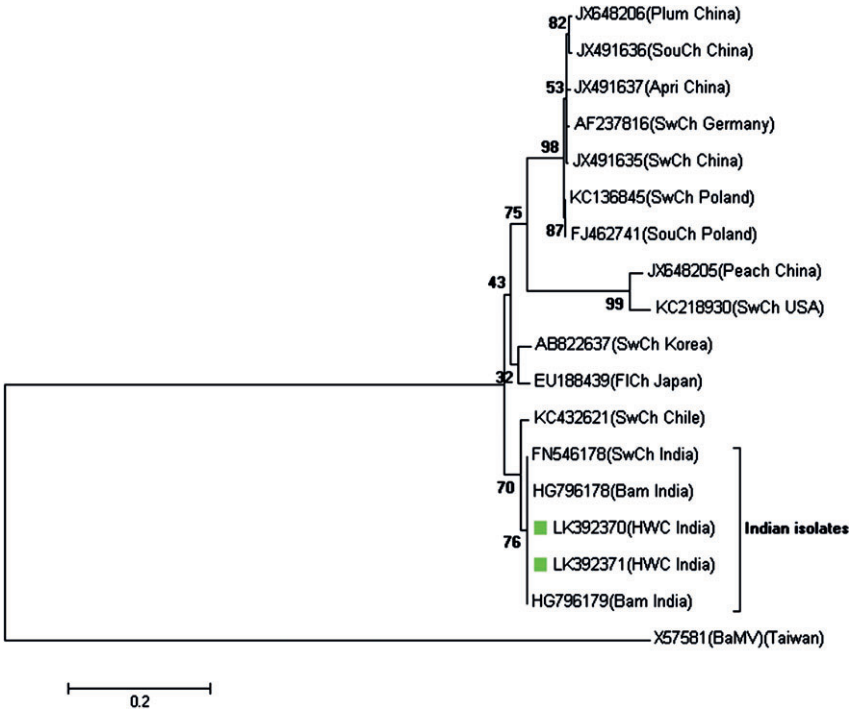

Figure 3 Phylogenetic tree constructed using the Maximum Likelihood method (implemented in MEGA5.2, best fit model: Kimura-2-parameter using a discrete gamma distribution and 1000 bootstrap replicates), showing genetic relationship of coat protein (CP) gene of the present *Cherry necrotic rusty mottle virus* (CNRMV) (Himalayan wild cherry) isolates with other CNRMV isolates (Bamboo, sweet cherry, sour cherry, peach, plum, apricot and flowering cherry) taken for the study. Apri, Apricot; Bam, Bamboo; FlCh, Flowering cherry; HWC: Himalayan wild cherry, SwCh: Sweet cherry, SouCh: Sour cherry.

been mixing and movement of isolates (and hence planting material) within and among these regions (Fig. 3).

A distinguishing feature of the Indian isolates characterised so far has been a missing triplet GGT codon at position 793 near the 3′ end of the CP gene sequence that has lead to a Glycine (G) deletion. Owing to this deletion the CP of the Indian isolates is 266 amino acids instead of 267 as in case of all other isolates. In case of RNA viruses it is a well-known fact that the virus populations may be variable within a given plant because

of the constant occurrence of replication errors leading to changes, mutations and recombination (Gell, 2011). Although there was no recombination event detected in the Indian isolates (analyzed by RDP3; Martin *et al.*, 2010), this missing triplet could be a result of a deletion which has been selected in nature and is being passed on to further generations, probably suited to virus multiplication in this Hwc and bamboo (Awasthi *et al.*, 2014). The length and sequence of the 3′-UTR was also quite variable amongst all the isolates characterised so far. The 3′-UTR of positive sense RNA viruses remains highly variable and has multiple roles in the virus life cycle, including regulation of translation expression, providing a telomeric function, systemic movement, mediating viral assembly, functioning as subgenomic RNA promoter, binding site for RdRp etc. (Dreher, 1999; Li & Wong, 2007). It is also assumed that the 3′-UTR interacts with host factors to synthesize minus-strand RNAs (Dreher, 1999). Therefore, it is probable that the UTRs have evolved to support a wide range of virus-specific requirements for infection in different hosts and propagation in the insects.

CNRMV is a graft transmitted virus with no evidence of vector transmission till date. In this study, CNRMV has been detected in three insect species colonising on CNRMV infected trees. The detection of CNRMV in *A. bambusae*, *T. montanus* and in *Bambusiphaga* sp. indicates that these insects might have acquired the virus during feeding on the sap from the leaves of these trees and have become carriers. Non-winged colonizing aphids are not mobile enough to contribute substantially to the spread of the viruses while winged aphids moving through the orchard are more probable candidates responsible for transmission (Celetti *et al.*, 2002).

T. montanus is a wingless aphid that was found colonizing only on the undersides of leaves of the Hwc tree whereas *A. bambusae* which is also wingless was restricted to the undersides of bamboo (*D. hamiltonii*) leaves. As the trees were growing in close proximity to each other with overlapping foliage at many places, there are significantly high chances of aphid exchange between them. Interestingly, the delphacid, *Bambusiphaga* sp. which is a winged plant hopper and is otherwise known to feed exclusively on bamboos, was present abundantly on nearly all the trees growing at the site of sampling. This also indicates that these plant hoppers may be acquiring the virus from infected tree and transmitting it to the neighbouring trees. Few members of genera viz. *Laodelphax*, *Peregrinus*, *Perkinsiella* and *Nilaparvata* in the *Delphacidae* family of plant hoppers are known to act as vectors for some viruses in *Reoviridae* and *Rhabdoviridae* families (Matthews, 1991). The time of emergence of these plant hoppers was from March till July. Extremely high populations of the plant hoppers: *Bambusiphaga* sp. were seen on nearly all adjoining trees at that time.

With the availability of large number of infected trees (bamboo or Hwc and other unknown plants), chance for insects to acquire the virus and transmit it to other trees increases. During a previous study CNRMV was detected in 21 different species of bamboos belonging to five different genera and in this study we recorded 57% incidence of CNRMV on Hwc trees. The surveys made in the adjoining areas show a wide host range, scattered distribution and high incidence of CNRMV infected trees. These infected trees serve as source of virus and if these insects which have been found to carry the virus also transmit it, then the number of infected trees are expected to rise. The similar delphacid, *Bambusiphaga* sp., was also located on apple trees (*Malus domestica*) in the pome and stone fruit germplasm field of this institute which makes it even more vital to determine the vectoring efficiency of these carrier insects.

Further characterisation of CNRMV isolates from different areas in the world and different hosts will be necessary to see if there are subpopulations specific of certain geographic areas and whether the variability in the sequences of CP gene and 3′UTR have some implication in host specificity or vector transmissibility. The knowledge of CNRMV incidence in insect vectors can provide new grounds for research on the mode of transmission and will be useful in the management and control of this virus in commercial cherry plantations.

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