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Molecular identification of chilli leaf curl disease in Sikkim

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Abstract
Cherry Pepper (*Capsicum annum var. cerasiforme*) is a valuable cash crop of Sikkim and can be found in almost all parts of the state. The fruits are small in size but have high pungency, because of this it have a good demand in national and international market. No doubt that a healthy crop of cherry pepper can give a handsome amount of return to the grower, but the crop is susceptible to various disease infestation. Chilli Leaf Curl Virus and Chilli vein mottle virus are the most devastating one and causes an un-repairable loss to the farmer. Considering the facts a two year extensive experiment was conducted during 2013- 14 and 2014-15 to study and identify the chilli leaf curl virus in Sikkim. The results revealed that begomovirus is the main pathogen associated with chilli leaf curl in Sikkim as, the virus isolate under study showed maximum 99% similarity with Chilli leaf curl virus which was found in Palampur isolate. The sequence data obtained in this experiment can be utilized in future to develop sensitive diagnostic kits for the early detection and control of this viral disease.

Keywords: chilli, leaf curl, virus, Sikkim, begomovirus

Introduction
Chilli (*Capsicum annum*) of family Solanaceae is an important spice crop in India. It comes in various shapes, sizes and colours. But the main reason for its demand in market is due to its intense pungent flavour or the oleoresin "capsaicin". India is the world's largest chilli producer and a lot of genetic variability can be seen throughout the Indian sub-continent. Most of the harvest gets consumed within the county, which is 95% of the total production and only 5% is exported, which is around 80,000–1.0 lakh tonnes per year.

Capsicum annuum var. cerasiforme (cherry pepper) is a valuable cash crops of Sikkim and grown almost all over the state. It is known for its immense pungency and unique aroma. This perennial plant bears small to medium sized fruit with high pungent. The fruits are used in a multitude of food preparations and marketed in different regions.

But recently Leaf curl disease of Chilli has emerged as a serious problem in the various part of India. During December 2004, very high disease incidence (up to 100% of plants) was observed in farmers' fields in Narwa and Tinwari village (Senanayake, *et al*., 2007) [10]. After testing it was observed that, whitefly (*Bemisia tabaci*) was the main culprit behind this viral transmission. In India, the most important disease caused by viruses in chilli is yellow mosaic disease (YMD). Around 12-95% incidence was reported with yield losses as high as 65-75% (Bidari, *et al*., 1990; Narasimham, *et al*., 1986) [3, 8]. YMD in chilli is known to be of complex nature and eight viruses were identified in Karnataka and four viruses in Punjab (Kaur, *et al*., 1985; Bidari, *et al*., 1994) [5, 4]. Recently, it has been observed that the chilli leaf curl disease is spreading day by day in Sikkim and acquiring new areas (Annonymus, 2011) [2]. The increase in spread of this disease can be perceived the increase in whitefly population. If this problem is left unnoticed it may create havoc to the chilli growers of Sikkim. Thus, keeping in mind the well wishes for the farmers of Sikkim the present investigation was carried out for molecular identification of infecting chilli virus. So that an effective management strategy could be develop to control or eradicate the spread of the virus, after studying and comparing the present study with the previous cases of this disease.

Materials and Methods
Survey and collection of viral infected chilli plants in Sikkim
Various surveys at different parts of Sikkim viz., East, West, North and South were conducted

during the period of 2013–14 and 2014-15, to collect the infected chilli plants sample (two plants from each location). The samples exhibiting curling symptom were taken from open field of the farmers.

Maintenance of virus culture

All the collected infected samples were again planted under a net-house along with other healthy chilli and tomato plants, which could be an ideal host for the pathogen to spread. Here, under the net-house the virus was cultured for its biological and molecular characterization. The virus culture was maintained by planting new 3-4 leaf stage seedlings at every 25-30 days interval.

Virus transmission

White fly (*Bemisia tabaci*) was used as a vector of transmission. Therefore, 20-30 non viruliferous whiteflies were introduced into the net house for 24 hrs so that they can transfer the virus from the curled infected plant to the healthy newly planted seedlings. Then the plants were kept under insect proof nets till further development of the disease symptoms.

Agarose gel electrophoresis

Double stranded DNA was analyzed by electrophoresis in submarine horizontal agarose slab gel apparatus as described by (Sambrook, *et al*., 1989) [9]. Gels of different strength were prepared depending on the size of DNA to be analyzed.

Elution of DNA from agarose gel

DNA was eluted from 1-1.5% agarose gel using QI Aquick Gel extraction kit protocol (Qiagen Inc., GmbH, Germany) as per manufacture's instruction.

Sequence of the RCA products

All the sequence of RCA products was made through Genei Pvt. Ltd., India, Chromas (Chromas Biotech Pvt. Ltd., India) and MWG, Germany. For every sequence, three samples were sequenced and consensus data of three identical sequences were determined with single ORF.

Computational analysis of sequence data

Reserve complementation of nucleotide sequence was done using program, Reserve complement (http://www.ualberta.ca/stothardy/javascript/rev_comp.html).

The sequence data obtained through sequencing result was analyzed for consensus data remaining no ambiguities and submitted in National Centre for Biotechnology Information GenBank database (NCBI, http://www.ncbi.nlm.nih.gov/Bankit). The open reading frame (ORFs) in sequenced data were predicted by bioinformatics tool: ORF finder (www.ncbi.nlm.nih.gov/projects/gorf/) to find in frame AUG (ATG)-start and UAG (TAG)-termination condon. ORFs were translated into amino acid using Expasy tool. To observe the nucleotide identity within and with other reported strains of leaf curl disease, basis local alignment search tool (BLAST) searches were performed with all available database using the NCBI-BLAST sever (www.ncbi.nlm.nih.gov). The database were compared within with other reports strains of leaf curl sequences obtained with the Entrez program using the BLAST (NCBI, Bethesda, USA, (http://www.ncbi.nlm.nih.gov/blast). Phylogenetic analyses were perused using NCBI program with 100 replicates bootstrapping and phylogram were generated with Neighbour–joining method.

Results and Discussion

During the survey around different parts of Sikkim, the natural occurrence of severe leaf curl disease was observed in various chilli growing area of the state during 2013-14 and 2014-15. The symptoms were upward and downward curling of the leaves accompanied with puckering, blistering of inter-venial areas and thickening/swelling of the leaf veins. The Infected plants showed stunted growth, reduced or lack of flowering and deformed fruits as compared to the healthy plants. The severely infected plants did not bear any flowers or fruits resulting in 25-30% of total yield loss in various locations.

The presence of whiteflies (*Bemisia tabaci*) in the vicinity of growing area and on the symptoms bearing chilli plants, clearly strengthened the assumption of it being the main vector of the disease which transmitted virus (begomovirus) as earlier reported (Khan, *et al*., 2006) [6]. Later this assumption was confirmed by whitefly transmission tests which was carried out on chilli and tomato plants.

Virus transmission tests through whiteflies were performed with samples of chilli collected from different parts of Sikkim (two plants from each location which was maintained in net-house). Host range studies were also conducted on a few test plants as shown in fig 1. In each whitefly transmission tests, approximately 6 viruliferous whiteflies were transferred to healthy seedlings of *Capsicum annuum* and *Solanum lycopersicon* for inoculation as described in materials and methods (MM). The inoculated seedlings were maintained in insect proof net-house and observed periodically for symptom development for a period of 6 weeks. The disease was successfully transmitted from naturally infected *C. annuum* to healthy *C. annuum*, *S. Lycopersicon* which developed severe and mild leaf curl symptoms by 25-30 days post inoculation. Therefore, the virus culture/s was maintained on *C. annuum* seedling in a net- house through whitefly transmission.

The molecular identification was established through RCR/RCA technique, the total genomic DNA was extracted from naturally infected chilli plants collected from different locations of Sikkim. RCA reaction was performed as per manufacture's instruction. The DNA concatamers produced in the RCA reaction were monomerized by *Eco* RI, *Eco RV*, *Apa*I, *Bam* HI, *Sal* I, *Not* I and *Hind* III enzymes and the bands corresponding to ~ 2.7kb genomes were purified and cloned in the respective sites fig 2. The sequence obtained was analyzed through NCBI BLAST. DNA A is important for taxonomic classifications as DNA A is responsible for replication and other important viral proteins. The nucleotide sequencing results the 2.7 kb genome. The BLAST analyses fig 3 revealed the association of begomovirus with the leaf curl disease of chilli as DNA-A component showed 99% identity with the DNA A of *Chilli leaf curl Palampur virus*. The phylogenetic analyses fig 4 also revealed a close homology with the other reported strains of begomovirus. There was only some report of molecular characterization of virus causing leaf curl disease of chilli in India, therefore, molecular identification of chilli disease was attempted to understand the origin and diversity of the virus responsible for leaf curl disease of chilli.

Typical leaf curl symptoms induced on chilli plants in Net-hose through whitefly (*B. tabaci*) transmission from chilli sample collected from various locations of Sikkim

Fig 1: Total genomic DNA of chilli

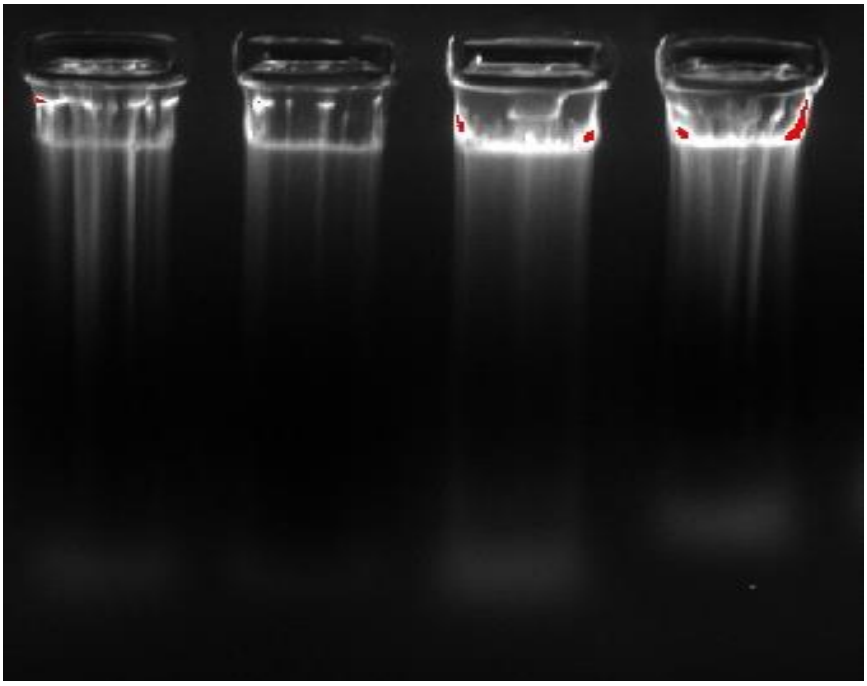
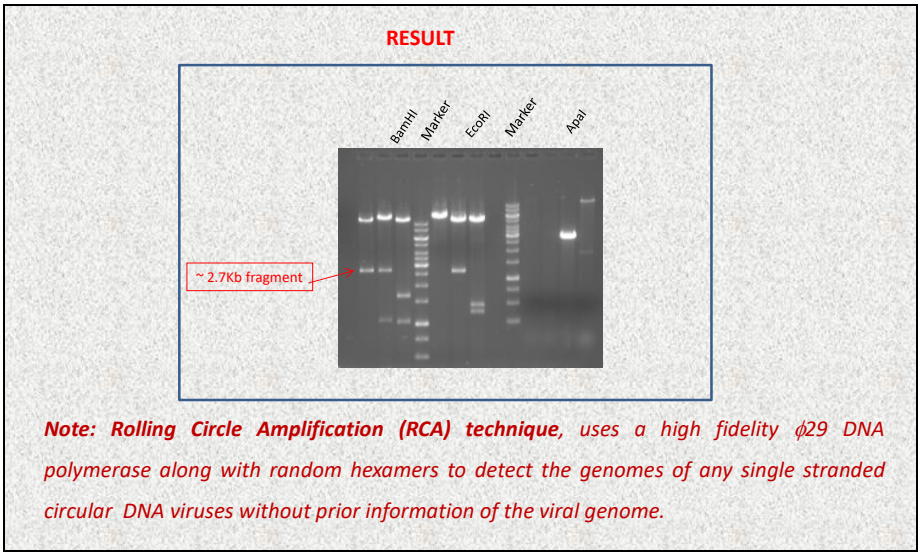


Fig 2: Cloned in the respective sites



***Note:* Rolling Circle Amplification (RCA) technique**, *uses a high fidelity ϕ29 DNA polymerase along with random hexamers to detect the genomes of any single stranded circular DNA viruses without prior information of the viral genome.*

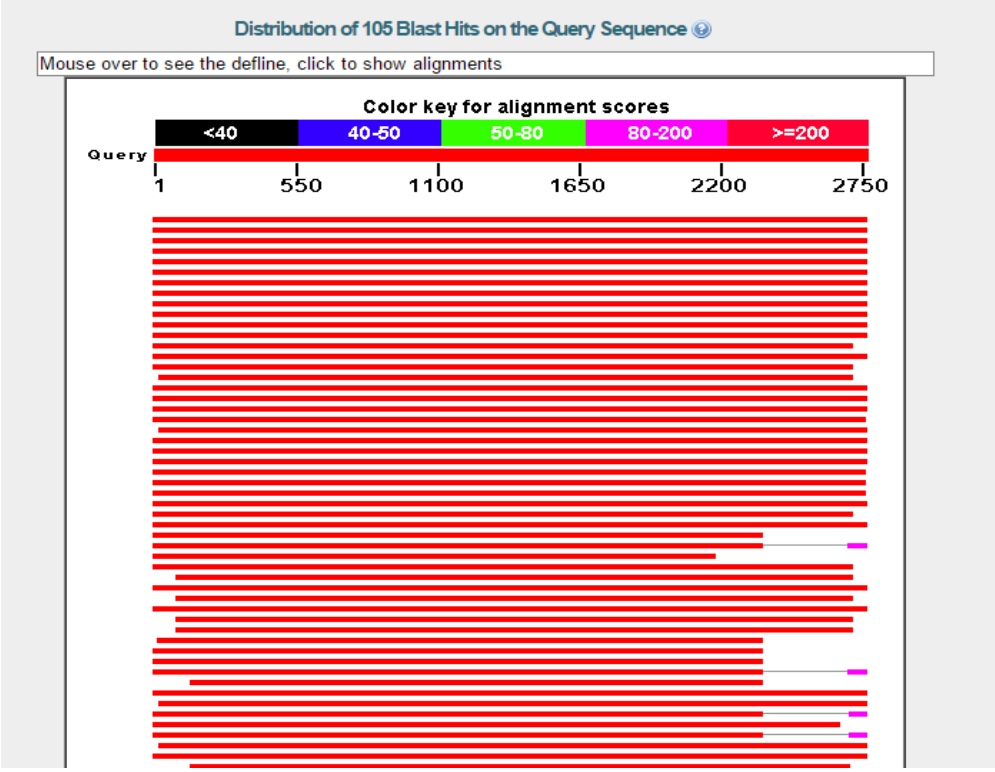


Fig 3: Phylogenetic analysis

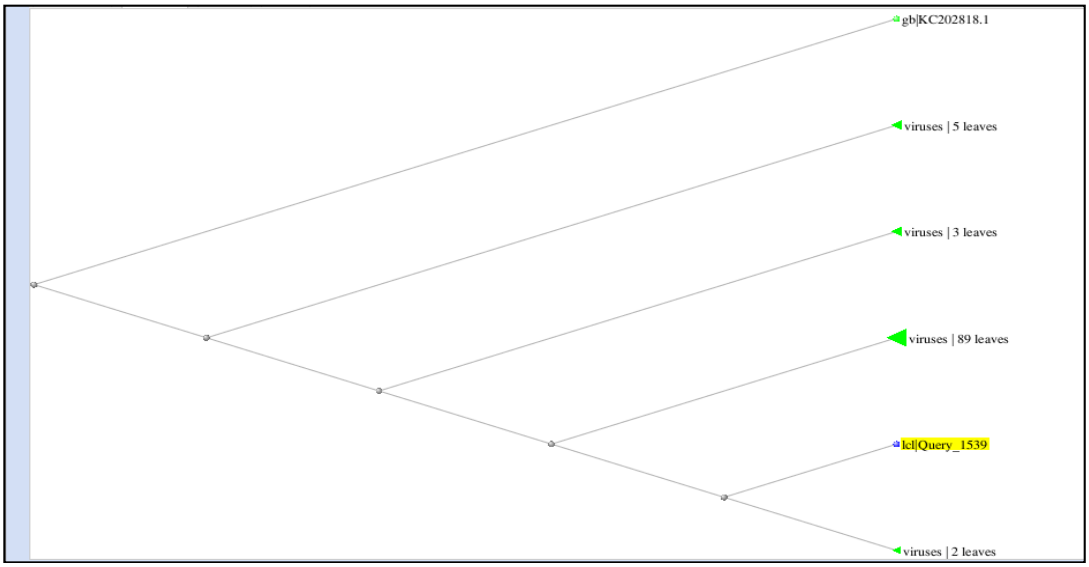


Fig 4: The phylogenetic analyses

Conclusion

The present study identified the chilli leaf curl virus in Sikkim for the first time at molecular level. The generated sequences data of various virus isolates confirmed the presence of begomovirus association with chilli in Sikkim. Further this data may be considered as a beacon of light for study of genetic diversity and evolutionary relationships among the chilli viruses in North East India. The findings obtained from the present study will also help in detection of leaf curl disease at early stage which can be used for the management and control of the viral disease of chilli before reaches severe stage.

Acknowledgements

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References

1. Agrios GN. Plant Pathology. 2nd edition. Academic Press, Inc., San Diego, California, 1978, 466-470.
2. Anonymous. Sikkim annual report, 2011.
3. Bidari BDA, Reddy HR. Identification of naturally occurring virus on commercial cultivars of chilli. Mysore Journal of Agriculture Science. 1990; 24:42-51.
4. Bidari BD, Reddy HR. A survey on mosaic viruses and their distribution in different chilli cultivars in Karnataka. Mysore J Agriculture Science. 1994; 28:52-59.
5. Kaur S, Singh J. Resistance to fruit rot disease under field conditions in pepper (*Capsicum annuum* L.). Capsicum News Letter. 1985; 4:67.
6. Khan MS, Raj SK, Singh R. First report of Tomato leaf curl New Delhi virus infecting chilli (*Capsicum annuum*) in India. Plant Pathology. 2006; 55:289.

7. Kumar Y, Hallan V, Zaidi AA Chilli leaf curl Palampur virus is a distinct begomovirus species associated with a betasatellite. Plant Pathology. 2011; 60:1040-1047.
8. Narasimhan V, Alagianagalingam MN. Potassium in the management of chilli mosaic disease. Journal of Potassium Research. 1986; 2:59-64.
9. Sambrook J, Fritsch EF, Maniatis T. Molecular cloning: A Laboratory manual. Second Edition, Volume I, II and III. Cold Spring Harbor Laboratory, New York, 1989.
10. Senanayake DMJB, Mandal B, Lodha S, Varma A. First report of chilli leaf curl virus affecting chilli in India. Plant Pathology. 2007; 56:343.