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Isolation and characterization of a virus infecting chilli in eastern Uttar Pardessh

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ABSTRACT

Chilli plants showing severe mosaic mottling on foliage and bud necrosis symptoms, were collected from different locations around Faizabad and the causal virus was purified. The purified virus samples reacted only with polyclonal antiserum raised against coat protein (cp) of Tobacco streak virus (TSV) isolate from India (TSV- SF) in direct antigen coated enzyme linked immunosorbent assay. The identity of the causal virus associated with chilli bud necrosis was further confirmed by reverse transcription polymerase chain reaction and sequence analysis. The CP gene was amplified and sequenced. The CP gene was 717 nucleotides long and could encode a protein of 238 amino acids. Comparative amino acid sequence analysis revealed that the virus infected chilli shared maximum identity both at nucleotide (98-99%) and amino acids (98%) levels with the corresponding region of TSV isolates.

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INTRODUCTION

Chilli (*Capsicum annum* L.) is one of the most important spice crop of family solanaceae. India is a major producer, consumer and exporter of chilli. It is extensively grown in all states of India including Andhra Pradesh, Maharastra, Karnataka, Tamil Nadu, Orrisa, Madhya Pradesh, West Bengal and Uttar Pradesh. The total area under chilli cultivation during 2007-2008 was about 9.9 Lacs hectare with total production of 11.0 lacs tones. India is a largest exporter of dried whole chilli in the world (Murugan, 1998). The production of chilli is adversely affected due to a large number of diseases incited by various biotic stresses but heavy losses are caused by viruses. Several viral disease attack this crop and induce mild to severe mosaic, yellow mosaic, mosaic mottle, leaf roll, bushystunt, necrosis along with leaf and bud necrosis symptoms. About seven viruses are reported to cause mosaic of chilli in India. These include chilli mosaic virus (Jha and Ray Chaudhary, 1956; Kamara and Duby, 1975), cucumber mosaic virus (Gahukar and Nariani, 1982), tobacco mosaic virus and its chilli and

bell paper strain, a strain in potato virus, potato virus X, a necrosis virus and a vein banding virus. The common mosaic in chilli is mostly caused by chilli mosaic virus and cucumber virus (Kamara and Duby, 1975). In addition to above described viruses, the virus causes leaf curl of tomato is responsible for leaf curl of chilli also and is quite common in India wherever this crop is grown (Capoor, 1967). Poty viruses naturally occurring on chilli in India are tobacco etch virus (TEV), pepper veinal mottle virus (PVMV) and pepper vein banding virus (PVBV) (Rishi and Dhawan, 1989; Bidari and Reddy, 1990) but virus producing leaf and bud necrosis symptoms on chilli has not been studied so for. Keeping this fact in to consideration the detailed studies regarding diagnosis of the causal virus were conducted.

MATERIAL AND METHODS

The plant samples showing severe disease symptoms were collected from the field and brought to the laboratory for the isolation and identification of the casual virus. All the specimens were preserved with proper labeling and were kept in both dry (in blotter paper) and wet (in liquid nitrogen at – 90 ^{0}C) conditions for further studies. The characterization of causal virus was done following the method of Awasthi and Singh (2006).

Transmission (Mechanical sap transmission):

Symptomatic young leaves collected from diseased plants were washed properly and gently blotted dry with tissue/ blotting paper. Phosphate butter (0.1m and pH 7.4) was taken equal to (w/v) the weight of the leaves and leaves were ground in sterilized pestle and mortar. After grinding the whole leaf pulp was pressed through double layered muslin cloth to get filtered standard extract of the leaves, Carborundum powder was lightly dusted to cause the injury on the leaves and cotton pad soaked in extract was rubbed on to the upper surface of leaves of test plants for inoculation. Inoculated plants were kept in insect free glass house at 30 ± 2^{0}C for observation.

Physical properties :

Dilution end point (DEP) :

The infected leaves were ground in mortar and extract was collected by passing through cheesecloth. The solution was centrifuged at 6000g for 15 minutes. The supernatant was collected and the dilutions were made as undiluted, 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5},10^{-6} and 10^{-7}. Eight test tubes were kept in a row in a test tubes rack and 7 test tubes were field with 9 ml. of sterile water with the help of a pipette. 1ml sap was poured in the second test tube for making the dilution 10^{-1} and mixed thoroughly and again 1 ml sap was added to the dilution (10^{-1}) to the third test tube to make the dilution 10^{-2} and this process was repeated till 10^{-7}. Each sample was then inoculated separately on the leaves of test host to find out infectivity of the samples.

Longevity in vitro (LIV):

It may be defined as the time expressed in days, weeks or hours for which the virus in crude sap kept at room temperature remains infectious. Eight test tubes were taken and 2 ml. of sap was pipetted into each test tube. The test tubes were kept close to a stopper or aluminum foil. The test tubes were preserved at different times at room temp (25-28^{0}C). After 10 minutes, the healthy test plants were inoculated with the treated sap of virus. The number of lesions and sum for each sample were counted and the longevity of the virus *in vitro* were recorded.

Thermal Inactivation point (TIP) :

The desirable quantities of infected leaves were ground in a mortar and sap was passed through cheesecloth. The partially purified sap was poured in a shallow dish and then 2ml of sample was pippeted in 8 test tubes. Water was filled into the water bath and the level of water in the water bath was kept at 3 cm above the level of the sap the test tube. Water in the water was heated to different temperature *viz.* untreated (about 20^{0}C) 40^{0}C, 50^{0}C, 60^{0}C, 70^{0}C, 80^{0}C, 90^{0}C and 100^{0}C and one tube is kept in the water bath when the temperature of water in water bath reaches to the lowest temperature. A thermometer was kept close the tube at the same level and by adjusting the flame temperature was maintained for 10 minutes. After 10 minutes the tube from the bath was removed and cooled with running water. After heating the bath to the next temperature second test tube was kept and treated in the same manner. When all the tubes have been treated the healthy plant was inoculated with different sap treated at different temperature. The number of lesions and sum of each sample were counted and thermal inactivation point was noted.

Isolation and purification of virus :

The isolation and purification of the causal virus was done by differential centrifugation method (Scott, 1963) and as modified by Awasthi and Singh (2006). One hundred gram of leaves showing disease symptoms were ground in 0.5 M potassium phosphate buffer (pH 7.0). Slurry was squeezed through double layers of muslin cloth. The debris was discarded and filtrate was centrifuged at 3000g for 15 minutes. Pellets were discarded and supernatant was collected. The supernatant was clarified by ultracentrifugation at 120000g for 90 minutes. The pellets were collected and supernatant discorded. Pellets were dissolved in phosphate buffer and the virus was precipitate with a saturated solution of ammonium sulphate. The precipitate was collected and dissolved in the same buffer. The supernatant containing traces of ammonium sulphate was removed by dialysis for 24 hours under running water. The partially purified sample was layered on sucrose gradient column containing 4 per cent sucrose solution in distilled water and centrifuged at 59000g for 90 minutes in a swinging bucket type SW93L rotor. The light scattering Zones were harvested with syringe and used for further studies.

Serology :

The infected sample was first assayed for the presence of TSV using direct antigen -coated enzyme-linked immunosorbent assay.

(DAC-ELISA) :

Antiserum: Tobacco Streak llarvirus

Protocol:

200 µl test/ control samples was added and extracted /diluted in coating buffer containing 2 per cent (w/v) PVP to each well of the microtitre plate and the plate was covered and incubate at 37^0C for ½ to 1 hour. The plate was emptied and washed by flooding the wells with PBST for about 3 minutes. Wash and soak operations was repeated thrice, then plates was emptied and residual liquid was shake out by draining on a proper towel. 200 µl blocking solution was added to each well and incubated at 37^0C for 1 hour to block polystyrene well's reactive surfaces. The plates were washed three times as in step 2. 200µl crude antiserum (primary antibody) was added, diluted in PBS-TPO to each well and incubated at 37^0C for 2 hour. 200µl enzyme-labeled (Alkaline phosphate, ALP) antirabit lgG (Secondary antibody) was added, diluted in PBT-TPO to each well and incubated at 37^0C for 2 hour. 200µl freshly prepared substrate (p-Nitro phenyl phosphate, PNPP, 0.5-1 mg/ml) was added to each well and incubated at room temperature for 1-2 hours.

Polymerase Chain Reaction (PCR), Bhat et al. (2002):

The total RNA from the infected sample was isolated using plant Rneasy mini Kit (Quagen), by using TSV specific primers (ATGAATACTTTGATCCAAGG' and TCAGTCTTCACCAG'), the CP gene was amplified by RT- PCR, 10X buffer 10 ml, 5X Q 20 ml, 0.1 M DDT 10 ml, 0.1M dNTPs 2 ml, RKJ 11 ml, RKJ 21 ml, RNA inhibitor 0.5 ml, RT 0.5 ml, Taq polymease 1 ml, Template RNA 22 ml Total - 100 ml. The mixture placed in a thermal cycler set at the amplification of 42^0 C-45 min for RT, 94^0 C-30s for denaturation, 48^0 C-1 min for annealing, 72^0 C-1 min for extension these were repeated for 40 cycles 72^0 C-1 hr for final extension Following PCR, amplicons wee analyzed by electrophoresis in 1 per cent Agarose gel. Following the agarose gel electophoresis, the DNA band of 750 bp was extracted using the QI A quick Gel extraction Kit and ligated in pDrive PCR cloning Vector. The ligation reaction was set up as PDrive vector 1 ml, 2X ligase buffer 5 ml, PCR product 4 ml The ligation mixture was incubated overnight at 4^0 C. The ligated product was transformed to E. coli and transformed colonies were screened. The positive clone was sequenced commercially.

RESULTS AND DISCUSSION

The findings of the present study as well as relevant discussion have been presented under the following heads:

Mode of transmission :

Mechanical transmission:

The causal virus produced different types of symptoms when inoculated mechanically in Chilli, tomato, brinjal and other common indicator hosts *viz. Vinga anguiculata* and *Cyamopsis tetraganalaba* (Table 1). The causal virus was not transmitted with insect vectors (Aphid, whiteflies and bugs), dodder and grafting.

Table 1 : Transmission of causal virus: Mechanical (sap) transmission

Sr. No.	Name of host	Type of symptoms
1.	*Capsicum annuum*	Necrosis of leaves
2.	*Vigna anguiculata*	Necrotic local lesions
3.	*Cyamopsis tetragonaloba*	Small dark local lesions
4.	*Lycopersicum esculentum*	No symptoms
5.	*Solanum melongema*	No symptoms

Host-range :

The causal produced necrotic local lesions on *Cyamopsis tetragonoloba, Phaseolus vulgais, Vigna anguicula* and *Nicotiana tobacum* var. *Turkish* plants.

Physical properties :

Since the causal virus produced local lesions in *Cyamposis tetragonolob* and *Vigna unguiculata* plants, the virus sample after physical properties like dilution end point (DTP), thermal inactivation point (TIP) and longevity *in vitro* studies were assayed in these test hosts.

Dilution end point (DEP) :

Results presented in Table 2 have revealed that samples diluted to 10^{-1}, 10^{-2}, 10^{-3} and 10^{-4}, produced local lesions on leaves of *Vigna uniguiculata* plants no lesion were observed by the dilutions 10^{-5}, 10^{-6}, and 10^{-7} samples Thus the diluation end point (DEP) of casual virus was 10^{-4} (1:10000).

Table 2 : Physical properties (Dilution end point) of the causal virus

Sr. No.	Dilutions	Average no. of local lesions per leaf
1.	1:1	21.6
2.	1:10	20.0
3.	1:100	16.4
4.	1:1000	12.4
5.	1:10000	7.6*
6.	1:100000	0.0
7.	1:1000000	0.0
8.	1:10000000	0.0

Longevity in vitro (LIV):

Results presented in Table 3 have revealed that the causal virus remained infective till 36hr of preservation of sap. The LIV of causal virus was 36 hrs.

Thermal inactivation point (TIP) :

Results or in Table 4 revealed that the causal virus

Table 3 : Longevity *in vitro*

Sr. No.	Dilutions	Average no. of local lesions per leaf
1.	0.00	20.00
2.	6.00	20.00
3.	12.00	18.40
4.	18.00	16.20
5.	24.00	14.60
6.	30.00	14.60
7.	36.00*	12.80
8.	42.00	0.00
9.	48.00	0.00

remained infective at 60^0C thus TIP of casual virus was 60^0C.

Table 4 : Thermal inactivation point

Sr. No.	Temperature (^{0}C)	Average no. of local lesions per leaf
1.	40	20.0
2.	45	18.4
3.	50	11.2
4.	55	5.8
5.	60*	0.0
6.	65	0.0
7.	70	0.0
8.	75	0.0

Serology :

Extracts from the field samples reacted only with polyclonal antiserum raised against coat protein (CP) of *Tobacco streak virus* (TSV) isolate from India (TSV-SF) in direct antigen-coated enzyme linked immunosorbent assay (A 405nm: 0.24-0.57). The identity of the virus associated with chilli was further confirmed by reverse transcription and polymerase chain reaction and sequence analysis. By using TSV specific primers (5ATGAATACTTTGATCCAAGG3) and (5'TCAGT CTTGATTCA CCAG3'), the CP gene was amplified and sequenced. The CP gene was 717 nucleotides long and could encode a protein of 238 amino acids. Comparative amino acid sequence analysis revealed that the virus infecting chilli shared maximum identity both at nuceotide (98-99%) and amino acid (98%) levels with the corresponding region of TSV isolates originating from multiple hosts and locations suggesting that the virus infecting chilli is a strain of TSV and be designated as TSV-CH. To our knowledge, this is the first report of natural infection of chilli by TSV in parts of northern

India. We have observed that the causal virus could be transmitted mechanically in chilli and produced systemic disease symptoms in the form of the necrosis of leaves. It produced necrotic local lesion on the inoculated leaves of *Vigna unguiculata* and *Cyamopsis tetragonoloba*. Similar findings were reported with tobacco streak virus (Fulton, 1981). The tobacco streak virus also produced necrotic local lesions on the inoculated leaves of *Vigna unguiculata*, *Cyamopsis tetragonoloba*, *Phaseolus vulgaris* and *Nicotiana tobacum* var. Turkish. However, for the first time we have been able to transmit tobacco streak virus by mechanical sap inoculation on chilli plants. As for as authors are aware this is the first report of the mechanical transmission of tobacco streak virus in chilli. Similar findings were reported earlier by Brunt *et al.* (1989). The causal virus had thermal inactivation point at 60^0C, dilution end point was 10^{-4} (1:10000) and longevity in *vitro* 36 hour on the other hand *tobacco streak llarvirus* has thermal inactivation points 64^0C, dilution end point 10^{-4} and longevity *in vitro* 1.5 days (Brunt *et al.*, 1989). Further studies like serology and Ploymerase chain reaction (RT-PCR) revealed that the casual virus was serologically related to *llarvirus* group. In direct antigen – coated enzyme linked immunosorbent assay (DAC-ELISA), the causal virus reacted positively only with the polyclonal antiserum raised against coat protein (CP) of tobacco streak virus (TSV), and thus it is confirmed that the virus under study is a strain of tobacco streak virus.

REFERENCES

Awasthi, L.P. and Singh, S. (2006). A severs mosaic disease of grain Amaranths from India. *Indian J. Virol.,* **17** (2): 102-105.

Bhat, A.I., Jain, R.K., Kumar, A., Ramiah, M. and Verma, A. (2002). Serological and coat protein sequnce studies suggest that necrosis disease on sunflower in India is caused by a strain of Tobacco treak Illarvirus. *Arch. Virol.,* **147**: 651-658.

Bidari, V.B. and Reddy, H. R. (1990). Identification of naturally occurring viruses on commercial cultivars of chilli. *Mysore J. Agri. Sci.,* **24**: 45-51.

Brunt, A., Crabtree, K., Dallwitz, M., Gibbs, A. and Watson, L. (1989). Viruses of plants: Descriptions and Lists from the VIDE Database. 393: 394 C.A.B. International, U.K.

Capoor, S.P. (1967). Important virus diseases of field & garden crops in India and their control. *I.C.A.R. Tech. Bull. (Agric*.) **12** : 79.

Fulton, R.W. (1981). Illarviruses. In : *Handbook of plant virus infectious and comparative diagnosis*. Elsevier, North Holland press. pp. 377-385.

Gahukar, K.B. and Nariani, T.K. (1982). Studies on an aphid borne mosaic disease of chilli. *Indian Phytopath.,* **35** :73-79.

Jha, A. and Raychaudhari, S.P. (1956). Mosaic disease of chilli. *Indian J. Agri. Sci.,* **26**: 217-222.

Kamara, B.J. and Dubey, G.S. (1975). Studies on mosaic disease of chilli. *Indian Phytopath*., **20** : 212-217.

Murugan, A.P. (1998). Production outlook for chillies. *Indian spices*, **35** (2) : 14- 17.

Rishi, N. and Dhawan, P. (1989). Characterization and identification of viruses causing mosaic disease of *Capsicum* in nature. *Indian Phytopath.,* **42** : 221-226.

Scott, H. (1963). Properties of cucumber mosaic virus. *Virol.,* **20** : 103-206.