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RESEARCH ARTICLE

Molecular detection and prevalence of tomato leaf curl disease in tomato

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SUMMARY

Whitefly transmitted geminiviruses are emerging threat to several crops in tropical and subtropical regions of the world. Tomato (*Solanum lycopersicum* L.) is the world's largest vegetable crop after potato and sweet potato. Leaf curl of tomato is one of the most destructive disease caused by begomoviruses triggering substantial yield losses. Plant showed typical leaf curling, puckering and stunted growth due to viral infection. A severe leaf curl symptom with significantly high disease incidence ranges 25-38 per cent was observed during the survey of different locations of Bhagalpur during *Rabi* 2016-17. The PCR assay was carried out using whitefly transmitted geminivirus and *Tomato leaf curl New Delhi virus* specific primers. Out of 94 tested plant samples, 60 were found for positive begomovirus. Among the positive samples, 45 and 39 were found positive for ToLCNDV F/R and AVI gene specific primers. Thetemporal dynamic of leaf curl was assessed, it was progressively increases up to the middle March along with whitefly population.A positive correlation of whitefly and leaf curl incidence was observed in linear regression with $R^2 = 0.095$. The generated informationunder the study will helpful in understanding the present viral population in tomato crop. Moreover, it will helpful in understanding the epidemic factors and sustainable disease management options.

Key Words : Begomovirus, PCR, Tomato leaf curl, Whitefly

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Several economically important crops are challenged by begomoviruses, which includes more than 300 species (Zerbini *et al.*, 2017). The member of genus Begomovirus are vectored by whitefly (*Bemisia tabaci*) and cause severe diseases in tropical and subtropical regions particularly dicot host plants. The begomoviruses affecting tomato in India are the most devastating. In the northern part of India, bipartite begomoviruses genome (DNA-A and DNA-B) are

common whereas in southern parts monopartite begomoviruses (DNA-A and beta satellite) infecting various crops (Kirthi *et al.,* 2002 and Muniyappa *et al.*, 2000). Both DNA-A and DNA-B genomes of approximately 2.7 kb size (Vasudeva and Samraj, 1948). Among the viruses of tomato, Tomato leaf curl Bangalore virus (ToLCBV), Tomato leaf curl New Delhi virus (ToLCNDV), Tomato leaf curl Karnataka Virus (ToLCKV), Tomato leaf curl Gujarat virus (ToLCGV) and Tomato leaf curl Joydebpur virus are the important begomoviruses which limit the tomato production (Ansar *et al.,* 2019 and Agnihotri *et al.,* 2019). The leaf curl disease in susceptible cultivars causes more than 90 per cent yield loss (Saikia and Muniyappa, 1989). The disease may cause upto 75 per cent or more reduction in fruit yield due to its devastating nature. Considering its distressing nature leaf curl has become a national problem (Sastri and Singh, 1973 and Saikia and Muniyappa, 1989). The present investigation deals with various aspects of tomato leaf curl disease which causes severe problems in early grown tomato. The disease was characterized by the curling and twisting of leaves followed by a marked reduction in size. The study covered molecular detection (PCR assay) of virus in suspected samples, prevalence of disease and relationship between whitefly and leaf curl.

MATERIAL AND METHODS

Sample collection and DNA isolation:

Leaf samples of infected tomato plants were collected from different locations of Bhagalpur and surrounding areas e.g. Naugachia, Rangrachowk, Sabour, Akbernagar, Nathnagar, Ibrahimpur, Kahalgaon and Ghogha during *Rabi* season (2016-17). Total genomic DNA extracted using 100 mg symptomatic leaves sample using GeneJET Genomic DNA Purification Kit (Thermo Scientific, USA). From each location, a healthy plant also considered for DNA isolation. The isolated DNA was confirmed in gel electrophoresis.

PCR assay of collected samples:

For the confirmation of suspected virus, PCR was performed using three sets of primers. A specific primer pair Deng 541F/540R (Deng *et al.,* 1994) was used to confirm the presence of whitefly-transmitted geminivirus. Moreover, two sets of ToLCNDV specific primer was also used. The PCR was performed in a Master Cycler (Nexus, Eppendorf, Germany) programmed with one step of preheating at 95°C for 3 min, 35 cycles of denaturation for 30 sec at 95°C, annealing for 30 sec at 49°C for Deng540/ Deng541, 50°C for ToLCNDV-F/R and at 52°C for ToLCNDV-AF/AR (Table A) and 1 min extension at 72°C, followed by a one step final extension at 72°C for 10 min. The PCR tests were performed using Dream Taq Green Master Mix (2X) (Thermo, USA) in the total reaction mixture of 25µl which consisted of 2µl (20 ng/µl) DNA template, 1µl of each primer (20 pmol), 12.5 µl 2X Taq Green Master Mix and 8.5µl of nuclease free water. Amplified products were analysed in 1 per cent agarose gel with 1X TAE buffer containing 0.1 per cent ethidium bromide and visualized in the gel documentation system (UNITEC, Cambridge).

Distribution and incidence:

To assess the prevalence of leaf curl virus disease a roving survey was conducted. Eight locations of Bhagalpur and surrounding areas e.g Naugachia, Rangrachowk, Sabour, Akbernagar, Nathnagar, Ibrahimpur, Kahalgaon and Ghogha were taken into consideration during *Rabi* season of 2016-17. At each location, three plots were considered with an average150 plant population. Plants showed curly stunted growth were counted among total observed plants from each plot. The disease incidence (DI) percentage was calculated using the following formula:

$$\text{Disease incidence (\%)} = \frac{\text{Number of infected tomato plants}}{\text{Total number of observed tomato plant}} \times 100$$

Table A : Primer pairs used for diagnosis of tomato leaf curl disease in PCR assay

Primer I D	Primer sequence (5'-3')	Annealing temperature (^{o}C)	Expected size of amplicon
Deng 541F	TAATATTACCKGWKGVCCSC	49	~500 bp
Deng 540R	TGGACYTTRCAWGGBCCTTCACA		
ToLCNDV-F	ATGAACAGAAAACCCAGAATATAC	50	~600 bp
ToLCNDV-R	TTAATTTGTTACCGAATCATAGAAAT		
ToLCNDV-AF	TACGATCTTGTCCGAGATCTCA	53	~1000bp
ToLCNDV-AR	ACCCAGGTCCTTAAGTACCT		

Whitefly and leaf curl dynamics in tomato crop:

For establishing the relationship between whitefly population and leaf curl incidence, an experiment was designed in vegetable farm at Bihar Agriculture University, Sabour consisting of three tomato cultivars like BRDT-1, Kashi Vishesh and indeterminant cherry tomato in 2.5 x 2.5 m^2 plot. Each plot contains 30 plants maintained upto the whole cropping period. Data of leaf curl incidence and whitefly population were recorded at 15 days interval after 45 days of transplanting. Whitefly population recorded in all three varieties of tomato plants by considering five leaves per plant (2 middle, 2 lower, and 1 top leaf). The incidence of leaf curl was assessed as earlier described formula. A linear regression analysis was performed to correlate the whitefly population and leaf curl.

RESULTS AND DISCUSSION

The results obtained from the present investigation as well as relevant discussion have been summarized under following heads :

DNA isolation and PCR assay:

A total of 94 symptomatic tomato leaves were collected from eight different locations of the Bhagalpur and surrounding locations. All samples were processed for DNA isolation and PCR assay to confirm the presence of the virus. Positive amplification was found in 60 symptomatic leaves for whitefly transmitted geminivirus. Further, these samples were again tested for ToLCNDV-F/R (partial CP gene) and ToLCNDV-AF/AR (full AV1 gene) primer, among them 45 were found positive with partial CP gene primer (ToLCNDV-F/R) whereas 39 with AV1 gene primer (Table 1). All healthy leaves sample of each location was found negative in PCR assay (Fig. 1).

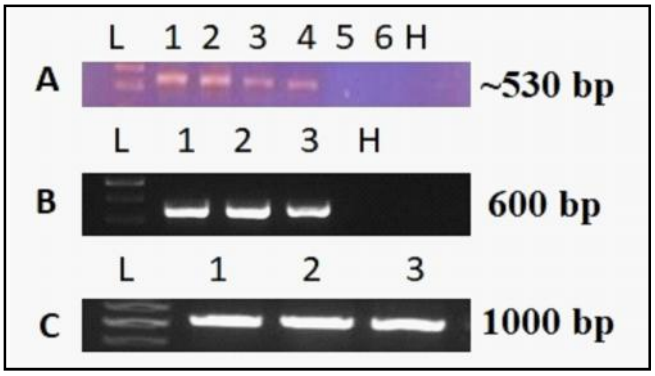

Fig. 1: The gel electrophoresis of PCR amplified products; whitefly transmitted geminivirus specific primer (Deng541F/Deng540R)(A);ToLCNDV-F/R (B); ToLCNDV-AF/AR (C)

Distribution and incidence:

The incidence of leaf curl was assessed at eight different locations of Bhagalpur and surrounding areas.

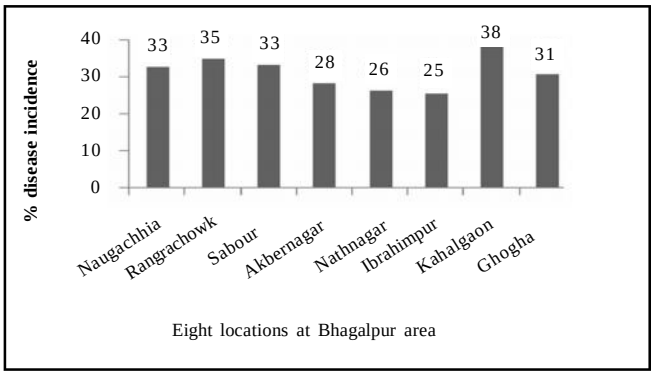

Fig. 2: Incidence of tomato leaf curl disease at different lo cation of Bhagalpur and surrounding areas

Table 1 : Details of sample collected at Bhagalpur and surrounding areas for PCR analysis

Location	PCR assay results		
	Whitefly transmitted geminivirus (Deng541F/Deng540R)	ToLCNDV F/R (Partial CP gene)	ToLCNDV-AF/AR (AV1 gene)
Naugachhia	8/14	7/8	7/8
Rangrachowk	9/12	7/9	5/9
Sabour	9/13	7/9	7/9
Akbernagar	6/10	5/6	4/6
Nathnagar	5/13	3/5	3/5
Ibrahimpur	5/08	2/5	2/5
Kahalgaon	10/13	8/10	6/10
Ghogha	8/11	6/8	5/8
Total	60/94	45/60	39/60

The disease was recorded at each location with a varying range of incidence (25-38%). The incidence was recorded 31-35 per cent at Ghogha, Sabour, Naugachia, and Rangrachowk. Maximum incidence being recorded at Kahalgaon with 38 per cent incidence. However, Ibrahimpur, Nathnagar and Akbernager the incidence noticed between 25-28 per cent (Fig. 2).

Relationship between whitefly population and tomato leaf curl incidence:

To establish the relationship between whitefly and leaf curl incidence, data was recorded during cropping period. The first appearance of symptoms noticed at 45 DAT along with whitefly infestation. The infestation of whitefly was progressively increased with crop growth. However, the population was found to decline in December and January. As a result of whitefly infestation, the incidence of leaf curl progressively increased (39.4 to 42%) upto the last week of February to and middle March in all three varieties. The correlation between whitefly density and disease incidence was assessed. The positive correlation of whitefly and leaf curl incidence was observed in linear regression with R^2= 0.095.

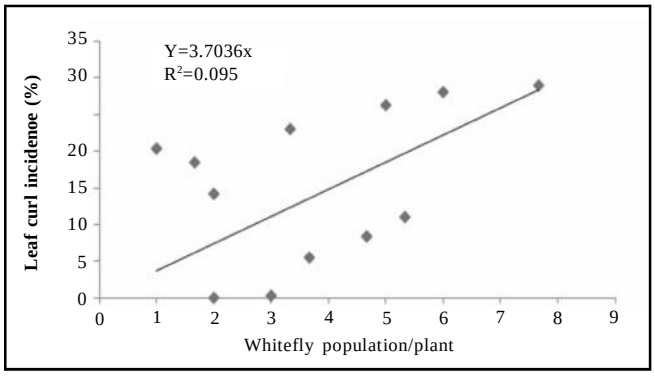

Fig. 3: Relationship of whitefly and leaf curl incidence in tomato

Tomato leaf curl disease is one of the most important viral diseases of tomato in the country, causes severe losses every year. The incidence of the disease varied from 24-100 per cent in tomato (Saikia and Muniyappa, 1989). The infected plant samples collected from the field and detected the leaf curl virus by PCR. Infected samples previously confirmed using whitefly transmitted geminivirus specific primer. Positive samples were again confirmed by ToLCNDV-CP gene specific primers and mostly were found positive. Since tomato crop affected by several mono and bipartite begomoviruses and it was confirmed by specific primer pairs (Anfoka *et al.*, 2005; Dennis and Narceo, 2007; Thakuria *et al.*, 2012 and Muniyappa *et al.*, 2000). Comprehensive observations of leaf curl in tomato plants were undertaken to find critical factors involved in the epidemiology of leaf curl virus. Under the investigation, the disease was found progressively increases upto the middle March along with the whitefly population. The findings of Reddy (2006) also explored the incidence of leaf curl influenced by whiteflies on tomato crops. A highly significant and positive correlation between viruliferous whitefly population and per cent diseases index of cotton leaf curl earlier reported in cotton (Kumar *et al.*, 2019). The generated information under the study will helpful in understanding the present viral population in tomato crops. Moreover, it will support in consideration of epidemic factors and sustainable disease management options.

REFERENCES

Agnihotri, A.K., Mishra, S.P., Ansar, M., Tripathi, R.C., Singh, R. and Akram, M. (2019). Molecular characterization of *Mungbean yellow mosaic India virus* infecting tomato (*Solanum lycopersicum* L.). *Australas. Plant. Pathol.*, DOI: https://doi.org/10.1007/s13313-018-0611-7.

Anfoka, G.H., Abhary, M. and Nakhla, M.K. (2005). Molecular identification of species of the *Tomato yellow leaf curl virus* complex in Jordan. *J. Plant Pathol.*, **87** : 65-70.

Ansar, M., Agnihotri, A.K., Akram, A. and Bhagat, A.P. (2019). First report of *Tomato leaf curl Joydebpur virus* infecting French bean (*Phaseolus vulgaris* L.). *J. Gen. Plant Pathol.*, DOI: http://doi.org/10.1007/s10327-019-00867-5.

Deng, A., Mc-Grath, P.F., Robinson, D.J. and Harrison, B.D. (1994). Detection and differentiation of whitefly-transmitted geminiviruses in plants and vector insects by the polymerase chain reaction with degenerate primers. *Ann. Appl. Biol.*, **125** : 327-336.

Dennis, D. and Narceo, B. (2007). Molecular detection of whitefly transmissible Geminiviruses (Family Geminiviridae, Genus Begomovirus) in the Philippines. *Philipp. J. Sci.*, **136** : 87-101.

Kirthi, N., Maiya, S.P., Murthy, M.R.N. and Savitri, H. (2002). Evidence of recombination among the *Tomato leaf curl virus* strains/species from Bangalore, India. *Arch. Virol.*, **147** : 225-272.

Kumar, A., Sain, S.K. and Monga, D. (2019). Study on Correlation between population of viruliferous whitefly and the per cent intensity of cotton leaf curl disease in cotton. *Int. J. Curr. Microbiol. App. Sci*., **8**:922-937 DOI: https://doi.org/10.20546/ijcmas. 2019.801.100.

Muniyappa, V., Venkatesh, H.M., Ramappa, H.K., Kulkarni, R.S., Zeidan, M., Tarba, C.Y., Ghanim, M. and Czosnek, H. (2000). *Tomato Leaf Curl Virus* from Bangalore (*ToLCV-Ban4*): sequence comparison with Indian *ToLCV* isolates, detection in plants and insects and vector relationships. *Arch. Virol*., **145** : 1583-1598.

Reddy, B.A. (2006). Molecular characterization, epidemiology and management of *Tomato leaf curl virus* (ToLCV) in northern Karnataka. Ph.D. Thesis, University of Agricultural Sciences, Dharwad, Karnataka (India).

Saikia, A.K. and Muniyappa, V. (1989). Epidemiology and control of *Tomato leaf curl virus* in Southern India. *Trop. Agric*., **66** : 350-354.

Sastry, K.S.M. and Singh, S.J. (1973). Assessment of loss in tomato by *Tomato leaf curl virus*. *Indian J. Mycol. Pl. Pathol*., **27** : 274-297.

Thakuria, B., Baruah, A.R. and Nath, P.D. (2012). Detection of *Tomato leaf curl virus* from Jorhat using PCR. *J. Mycol. Plant Pathol*., **42** : 230-233.

Vasudeva, R.S. and Samraj. (1948). Leaf curl disease of tomato. *Phytopathol*.,**18** : 364-369.

Zerbini, F.M., Briddon, R.W., Idris, A., Martin, D.P., Moriones, E., Navas-Castillo, J., Rivera-Bustamante, R., Roumagnac, P. and Varsani, A. (2017). ICTV Virus taxonomy profile: Geminiviridae. *J. Gen. Virol*., **98** : 131–133.