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Survey and Molecular Identification of Begomovirus Associated with Chilli Leaf Curl Disease in Kurnool District of Andhra Pradesh

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ABSTRACT

Background: Chilli is an important spice and vegetable crop grown in tropical and sub-tropical climate. Recently, Chilli leaf curl disease has become a serious threat to chilli growing farmers.

Methods: The present study was aimed to know prevalence of leaf curl disease in chilli. For this, roving field survey was carried out in 63 randomly selected chilli fields covering 21 villages in seven *mandals* of Kurnool district in Andhra Pradesh during 2019-20. Attempts were also made on molecular diagnosis of begomovirus associated with chilli leaf curl disease.

Result: The average mean per cent incidence of chilli leaf curl virus in Kurnool district was 50.76% ranging from 25.0% to 82.2%. During the field survey, the highest mean leaf curl incidence was noticed in Peddakadubur *mandal* (71.1%) followed by Gonegandla (59.96%) and Nandavaram (52.27%) *mandals*, while the lowest leaf curl incidence was observed at Adoni (37.83%) *mandal* in Kurnool district. The amplified coat protein gene was sequenced and obtained 770bp sequence which was deposited in Gen Bank. The obtained sequence shared maximum identity of 99.7% with chilli leaf curl virus Guntur isolate (MN417112). As per species demarcation and nomenclature criteria of begomovirus, the virus associated with chilli leaf curl disease in Kurnool district shared > 94% sequence identity with Chilli leaf curl virus. Hence it was identified as chilli leaf curl virus (ChiLCV).

Key words: Begomovirus, Chilli, Kurnool, Leaf curl disease, Roving survey.

INTRODUCTION

Chilli (*Capsicum annuum* L.) is an important spice crop grown mainly in tropical and sub-tropical climate. India is the largest consumer, producer and exporter of the chilli in the world accounting for 13.76 million tonnes of production annually and it is cultivated in an area of 7.75 lakh hectares with a production of 14.92 lakh tonnes during 2014-15 (Geetha and Selvarani, 2017). Major chilli growing states in India are Andhra Pradesh, Assam, Gujarat, Maharashtra, Nagaland, Orissa, Rajasthan, Tamil Nadu and West Bengal. Various fungal, bacterial and viral diseases worsen the quality and quantity of chilli production. Chilli leaf curl disease is one of the major limiting factor in chilli production transmitted by whitefly (*Bemisia tabaci*) (Gennadius) (Hemiptera: Aleyrodidae) which can cause significant reductions in yield and quality of chilli (Kumar *et al*., 2006). Epidemics of ChiLCV can lead to cent per cent yield loss which results severe economic consequences (Senanayake *et al*., 2006). Since 2017-18, severe incidence of leaf curl disease was reported in Kurnool district of Andhra Pradesh.

In order to find out occurrence of viral disease and to assess the levels of resistance/tolerance and susceptibility of chilli cultivars grown in farmer's fields at Kurnool district, roving field survey was conducted in Kurnool district of Andhra Pradesh. The attempts were also made to identify the associated begomovirus species with the leaf curl disease of chilli in Kurnool district of Andhra Pradesh.

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MATERIALS AND METHODS

Survey

Roving field survey was under taken in major chilli growing *mandals* of Kurnool district Andhra Pradesh from July to

October months during 2019-20. Totally, seven *mandals* (Adoni, Aspari, Gonegandla, Kodumur, Nandavaram, Peddakadubur and Yemmiganur) were selected and from each *Mandal* three villages were selected. A minimum of three fields were selected randomly in each village for determining the disease status. Incidence of the disease, variability of disease symptoms and crop variety were recorded. The per cent disease incidence of chilli viral disease complex was recorded. The per cent disease incidence was calculated using the following formula:

Per cent disease incidence =

$$\frac{\text{Total number of infected plants}}{\text{Total number of plants observed}} \times 100$$

Detection of virus

Total DNA was isolated from diseased chilli leaf samples using cetyl trimethylammonium bromide (CTAB) method. The presence of chilli leaf curl virus was confirmed by PCR amplification with coat protein specific primers 5′-AGAATTATGTCCAAGCGACCA-3′ and 5′-AAGCGTTGG GGATACACAAA-3′ (Sinha *et al.,* 2013). The PCR amplification was carried out in a thermal cycler. The PCR reaction mixture contained 2 µl DNA sample, 1 µl of each primers (Conc. 10 mM), 0.5 µl dNTPs (10 mM), 2 µl $Mgcl_2$ (25 mM), 2.5 µl 10× reaction buffer, 0.2 µl Taq polymerase and 15.8 µl sterile HPLC-H_2O which gave final volume of 25 µl PCR reaction. The conditions for PCR reaction were, an initial denaturation at 94°C for 5.00 minute followed by 34 cycles of denaturation at 94°C for 30 sec., annealing at 55°C for 30 sec and extension for 1minute at 72°C. Then final extension at 72°C for 7 minute was included. Amplified PCR product were separated by electrophoresis on 1.5% agarose gel and documented by Gel documentation system. The amplified PCR product of 750 bp was purified using PCR Purification Kit and sequenced. Database search with study sequence was done in BLAST at NCBI website. The multiple sequence alignments were performed using Clustal W programme in BioEdit software and phylogenetic analysis was done with Mega 7 Using neighbour joining method with 1000 bootstrap replicates.

RESULTS AND DISCUSSION

Survey, symptomatology and disease incidence

The major disease symptoms observed in most of the fields surveyed were severe upward leaf curling with puckering, crinkling of leaves and reduced intermodal length. In few locations, other characteristic symptoms *i.e* reduced leaf size, leaf distortion, blistering, stunting and smaller fruits were also observed (Table 1 and Fig 1). The characteristic field symptoms of chilli leaf curl virus were upward leaf curling, puckering, reduced leaf size with no or less number of fruits (Senanayake *et al.,* 2007).

The results of field survey indicated the presence of leaf curl disease in all seven *mandals* of Kurnool district that were surveyed. The data collected during filed survey was presented in Table 2. Maximum leaf curl incidence of 82.2 per cent was recorded in Peddakadubur village and minimum incidence of 25.0 per cent was recorded in Nagarur village of Aspari *mandal*. The *mandal* wise mean incidence of leaf curl disease revealed that maximum leaf curl incidence was observed in Peddakadubur *mandal* (71.1%) followed by Gonegandla (59.96%), Nandavaram (52.27%), Yemmiganur (48.03%), Aspari (44.63%), Kodumur (41.50%) and with minimum leaf curl incidence in Adoni (37.83%) *mandal* of Kurnool district. Similarly, 40-70% of per cent disease incidence of leaf curl was recorded in Kurnool district of Andhra Pradesh (Bagavatha Devi *et al.,* 2019).

The variation of symptomatology of plant in different *mandals* indicated the presence of mixed infections of begomoviruses and infestation of sucking pests like thrips and mites. During the survey it was observed that severely affected plants produced no fruits or with significantly reduced fruits. The reasons for higher leaf curl disease incidence were continuous cultivation of chilli crop over the years (Monocropping), seasonal weather conditions which influence the sucking pest population, more number of sprayings with neonicotinoid insecticides during initial stages of crop which increases the resurgence of white fly population (vector) and non-adoption of integrated management practices. The sucking pests (whiteflies and thrips) were invariably present in all surveyed fields. Leaf curl due to sucking pest is reversible and can be managed through vector control, but leaf curl due to virus is irreversible and cannot be controlled (Dore *et al.,* 2017).

Information regarding chilli hybrids was collected from chilli growers during survey. It was observed that, majority of the farmers in the surveyed fields cultivated Teja, Syngenta and Deluxe hybrids (Devanur deluxe, Kohinoor deluxe, Nuziveedu deluxe and Super deluxe). Among all these, Devanur deluxe and Super deluxe were the major chilli hybrids grown in the surveyed fields. The chilli hybrids

Table 1: Types of viral symptoms observed in different *mandals* of Kurnool district.

Mandals	Symptoms
Adoni	Upward leaf curling, puckering and leaf size reduction.
Aspari	Upward leaf curling, stunted growth and bushy appearance.
Gonegandla	Upward leaf curling, leaf crinkling, puckering and small fruits.
Kodumur	Upward leaf curling, stunted growth and bushy appearance.
Nandavaram	Upward leaf curling, leaf blistering and bushy appearance.
Peddakadubur	Upward leaf curling, leaf blistering, stunted growth and bushy appearance.
Yemmiganur	Upward leaf curling, stunted growth and bushy appearance.

were compared based on percent disease incidence recorded during survey and found that Teja and Syngenta cultivars recorded less PDI (25.00-45.2%) of leaf curl disease compared to deluxe cultivars (30.6-82.2%) which might be due to broader leaf size of deluxe hybrids which favours sucking pest infestation, apart from tolerance levels are compared to Teja chilli hybrid.

Detection of virus and characterization of virus associated with chilli leaf curl disease

The genomic DNA was isolated from leaf samples with characteristic symptoms of begomo virus infection from Kodumur *mandal* were subjected to polymerase chain reaction (PCR) using ChiLCV coat protein specific primers 5′-AGAATTATGTCCAAGCGACCA-3′ and 5′-AAGCGTTG

Table 2: Incidence of leaf curl virus infecting chilli in Kurnool district of Andhra Pradesh.

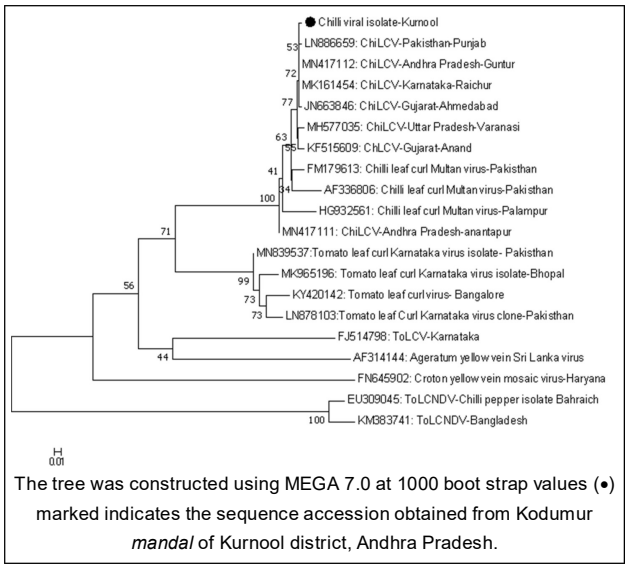
Mandal	Village	Variety/Hybrid	PDI (%)	Mean PDI (%)
Adoni	Arekal	Teja	38.1	37.83
	Bychigeri	Deluxe	30.6	
	Pesalabanda	Deluxe, Teja	44.8	
Aspari	Billekallu	Deluxe	60.0	44.63
	Chinnahotthur	Deluxe, Teja	48.9	
	Nagarur	Syngenta	25.0	
Gonegandla	Gonegandla	Deluxe	53.3	59.96
	Peddamariveedu	Deluxe, Byadiga	57.7	
	Kulumala	Deluxe	68.9	
Kodumur	Kodumur	Deluxe	31.1	41.50
	Laddagiri	Deluxe	55.6	
	Pyalakurthy	Syngenta	37.8	
Nandavaram	Nandavaram	Deluxe, Teja	45.2	52.27
	Joharapuram	Deluxe,	58.9	
	Kanakaveedu	Deluxe	52.7	
Peddakadubur	Peddakadubur	Deluxe	82.2	71.1
	H.Muravari	Deluxe	64.4	
	Kambaladinne	Deluxe	66.7	
Yemmiganur	Errakota	Deluxe, Syngenta	38.2	48.03
	Hanumapuram	Deluxe	52.6	
	Kalugotla	Deluxe	53.3	

Table 3: The details of begomoviruses used for sequence analysis and per cent nucleotide similarity of study isolate with other begomoviruses.

Virus Acronym	Accession number	Geographic origin	Host	% nucleotide identity
MN417112	ChiLCV	India-Guntur	Chilli	99.7
MK161454	ChiLCV	India-Raichur	Chilli	99.6
JN663846	ChiLCV	India-Gujarat	Chilli	99.6
LN886659	ChiLCV	Pakisthan	Chilli	99.4
MH577035	ChiLCV	India-Varanasi	Tomato	99.3
KF515609	ChiLCV	India-Gujarat	Tomato	99.2
MN417111	ChiLCV	India-Anatapur	Chilli	98.8
FM179613	ChiLCMV	Pakisthan	Marigold	98.7
AF336806	ChiLCMV	Pakisthan	Pepper	97.6
HG932561	ChiLCMV	India-Palampur	Tomato	97.5
MN839537	ToLCV	Pakisthan	Parthenium	91.5
MK965196	ToLCV	India-Bhopal	Zinnia	90.4
KY420142	ToLCV	India-Banglore	Cotton	89.8
LN878103	ToLCV	Pakisthan	Tomato	89.6
FJ514798	ToLCV	India-Karnataka	Mint	85.6
AF314144	AYVSV	Srilanka	-	84.3
EU309045	ToLCNDV	India-Behraich	Chilli	79.5
KM383741	ToLCNDV	Bangladesh	Tomato	79.4
FN645902	CYVMV	India-Haryana	Acalypha	79.0



Fig 1: Different symptoms of chilli leaf curl disease in surveyed fields.



The tree was constructed using MEGA 7.0 at 1000 boot strap values (•) marked indicates the sequence accession obtained from Kodumur *mandal* of Kurnool district, Andhra Pradesh.

Fig 2: Phylogenetic tree based upon alignments of the coat protein gene of Begomoviruses.

GGGATACACAAA-3′. PCR amplification of 775 bp was observed with all isolated DNA samples. The amplified product of 775 bp was subjected to PCR purification and sequenced. The nucleotide sequence of coat protein gene (772 bp) of study isolate was deposited in GenBank and obtained accession no (MZ691516). Whenever full length sequence of begomovirus is not available, the sequence of coat protein gene serves as an effective marker in begomovirus virus identification (Rybicki, 1994).

Different begomovirus sequences from NCBI site were used in comparative analysis. A BLAST search of GenBank revealed close sequence similarity with the Chilli leaf curl virus. The complete nucleotide sequence of coat protein gene of study isolate with other begomovirus from NCBI database revealed that it has 99.7% nucleotide similarity with chilli leaf curl virus-Guntur isolate followed by ChiLCV-Raichur isolate (Table 3) and least sequence similarity with Croton yellow vein mosaic virus (79%). In phylogeny analysis, tree forms three clusters consisting of ChiLCV and ChiLCMV (one cluster),ToLCV and ToLCNDV (Fig 2).

In India, Khan *et al.* (2006) reported the association of Tomato leaf curl New Delhi virus (ToLCNDV) with chilli leaf curl disease in Lucknow. Later in 2007, the association of chilli leaf curl virus with chilli leaf curl disease was reported in Jodhpur (Senenayake *et al.*, 2007). In the present study we confirmed the association of ChiLCV with leaf curl disease complex in Kurnool district of Andhra Pradesh.

CONCLUSION

In chilli field survey, it was observed that 25.00 to 82.20% percent disease incidence of leaf curl disease recorded in seven *mandals* of Kurnool district, Andhra Pradesh. Maximum leaf curl incidence was reported in Peddakadubur *mandal* (82.2%) and minimum disease incidence was recorded in

Aspari *mandal* (25.00%). In molecular characterization of coat protein gene of study viral isolate is identified as Chilli leaf curl virus (ChLCV).

Conflict of interest

The authors declare that they have no conflict of interest.

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