MOLECULAR DETECTION AND CHARACTERIZATION OF LEAF CURL AND PHYLLODY ASSOCIATED WITH SUNFLOWER IN ANDHRA PRADESH

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Abstract—Total 163 sunflower plants samples showing leaf curling, phyllody and mixed infections of both were collected from different regions of Kurnool district of Andhra Pradesh state in India. The total DNA extracted from these sunflower samples was subjected to nested PCR using 16SrRNA primers and PCR with generic begomovirus specific primers. 11/163 sunflower samples showed positive amplification begomovirus and phytoplasma by PCR. No PCR products were observed for asymptomatic plants. The phytoplasma identified as a member of Peanut witches-broom phytoplasma16SrII (nt identity of 97.1%) and the virus is a member of previously described monopartite begomovirus which is closely related to Tomato leaf curl virus (ToLCV, nt identity of 99%) infecting sunflower in India respectively. Further the co-infection of virus and phytoplasma in eleven sunflower samples was successfully detected by multiplex PCR, which is more rapid and sensitive and specific detection of the phytoplasma and virus.

Keywords: Sunflower, mixed infection, phytoplasma, begomovirus, Duplex PCR, ToLCKV,

1. Introduction

Sunflower (*Helianthus annuus*L.), belongs to the member of compositae family is the most important edible oilseed crop in the world. The crop being cultivated over an area of 20 million hectares and production around 30 million tonnes. In India, sunflower is being grown over an area of 0.72 million hectares with a production of 0.50 million tones, which is next to groundnut and soybean for edible oil and it contributes about 14% of the total oilseed production from nine major oil seed crops.Due to its wider adaptability, high yield potential, shorter duration and profitability crop is being grown at different agro-climatic zones of India. Sunflower is a major source of vegetable oil in the world used for a variety of cooking purposes. The seed contains about 48- 53 percent edible oil and contain high level of linoleic acid and absence of linolenic acid, possesses good flavor. The cultivation of sunflower in India is hampered by many insects and diseases from germination to harvest which causes heavy economic losses. Among the different diseases, the viral and viral-like diseases are poses a major problem in southern part of India (Govindappa*et al*., 2011, Vanitha*et al*., 2013, Vindyashree et al., 2016, Anand *et al*., 2006; brunt *et al*., 1996). The genus begomoviruses belong to family Geminiviridae are second largest families of plant viruses and are characterized by circular, single-stranded DNA with characteristic of quasi icosahedral geminate particles. The family Geminiviridae is divided into seven genera, *Mastrevirus, Topocuvirus, Curtovirus, Begomovirus, Becurtovirus, Eragrovirus* and *Turncurtovirus* on the basis of host range, insect vector and genome structure (Brown *et al*., 2012, Varsani*et al*., 2014). The begomovirus genomes are composed of one (monopartite) or two (bipartite) single-stranded DNA molecules designated as DNA A and DNA B (Fauquet*et al*., 2008), The majority of begomoviruses are originating from old world viruses have only DNA A, which may be associated with satellite DNA components referred to as alpha and beta satellites (Briddon*et al*., 2003, 2008). The DNA-A component encodes viral movement and encapsidation and genes on the complementary sense strand responsible for replication (Rep), regulation of gene expression and suppression of post transcriptional gene silencing (PTGS). Component DNA-B encodes for nuclear transport (nuclear shuttle protein [NSP], BV1) and genes on the complementary sense strand encodes for cell-to-cell movement (movement protein, [MP] BC1) in the bipartite genomes, (Stanley *et al*., 2005). Both DNA A and DNA B, contains noncoding region of 150 nt length with high sequence similarity called common region.

The begomoviruses originating from the New World (NW) are bipartite, having genomes consisting of two components (known as DNA-A and DNA-B), both of which are required for virus infectivity. In the Old World (OW) few bipartite begomoviruses have been identified (**Padidam et al., 1995**) and majority are monoparatite begomovirus with a single genomic component, which is a homolog to the DNA-A of the bipartite begomovirus. The monopartite begomoviruses are associated with additional ssDNA molecules known as beta satellites and/or alpha satellites (DNA 1) which have become globally widespread (Mansoor *et* al., 1999, Nawaz-ul-Rehman and Fauquet, 2009). Beta satellites associated with the monopartite viruses are approximately half the size of their helper begomoviruses and required to induce typical disease symptoms in their original hosts (**Briddon et al, 2002)**. These satellites depend on their helper virus for replication, movement, encapsidation and vector transmission. Alpha satellites are self-replicating circular ssDNA molecules, depend on the helper virus for movement, encapsidation and vector transmission and play role in symptoms attenuation and reduction in beta satellite accumulation (**Briddon et al, 2006; Idris et al., 2011**)

Phytoplasma are cell-wall-less plant pathogenic bacteria belonging to the class Mollicutes, formerly known as mycoplasma-like organisms. These are causing numerous diseases on plant in different parts of the world. The wall less bacteria is transmitted naturally by leafhoppers (Families Cicadellidea), plant hoppers (Families Fulgoridea) and psyllids (Families Psyllidea) (Lee *et al*. 2000). Normally the phytoplasma infected plants exhibit different symptoms like phyllody, virescence, yellowing, witches' broom appearance, abnormal elongation of internodes, bunchy appearance, stunting, floral abnormalities and shoot proliferation (Lee *et al*.2000; Bertaccini and Duduk 2009). Phytoplasmas were recently added to a novel genus Candidatus (Ca.) Phytoplasmas within the Mollicutes class based on the percentage similarity of 16S rRNA gene sequence (IRPCM 2004; Bertaccini and Duduk 2009). Based on the analysis of 16SrRNA sequences, 31 groups and 100 subgroups of diverse phytoplasmas were identified (**Martini and Lee 2013**). These belong to 16SrI, 16SrII, 16SrV, 16SrVI, 16SrIX, 16SrXI and 16SrXIV groups. Among these, Aster yellows group (16SrI) is alone associated with more than 31

diseases and are reported from north-eastern parts of the country (**Mall et al., 2011**). So far, only few phytoplasma diseases were reported from Eastern, Western and Central parts of India.

In this background, we surveyed different sunflower-growing regions of Kurnool district of Andhra Pradesh state in India during 2015-2016. The incidence of disease was significant in almost all the farmers' fields and symptoms consisted of extensive mosaic, leaf curl, and phyllody and mixed infection of leaf curl and phyllody were predominately observed in different farmers' fields. This diverse morphogenic symptom makes me difficult to assess the exact virus involved in causing diverse symptoms in sunflower. Although the leaf curl and mosaic disease of sunflower is caused by tomato leaf curl virus (Vindyashree et al., 2016) and tobacco streak virus (Anand and Halakeri 206), but mixed infection of leaf curl and phyllody was first recorded in the famers field. Therefore the presently study was undertaken to detect and characterize the type of Phytoplasma and begomovirus associated with mixed type of symptomatic sunflower plants in Andhra Pradesh state of India.

2. Material and Methods

The roving survey was conducted during 2015-2016 in fifty different farmer fields in Kurnool district, Andhra Pradesh state of India, to estimate the alone and mixed incidence of begomovirus, Tobacco streak virus and phytoplama on sunflower. The disease incidence (% of plants showing severe mosaic, leaf curl and phyllody alone and mixed type of symptoms) was estimated in each field by visual examination of 1,000 plants following a W pattern (by crossing the rows) as a sampling procedure. Total 163 leaf samples from sunflower plants showing distinct symptoms like severe mosaic, leaf curl and phyllody, mixed infection of leaf curl and mosaic or leaf curl and phyllody, plants are stunted growth and producing few flowers and seeds (Fig.1) were collected from different location of the district. One sample each from both the location without any symptoms of sunflower plants was also collected.

Fig1: A,B infected sunflower Plant showing mixed infection of leaf curl and phytoplasma,leaf curl and mosaic.

A.Detection of viruses

Total 163 sunflower plants samples were collected during survey, which were showing different kinds of symptoms (severe mosaic, leaf curl and phyllody, mixed infection of leaf curl and mosaic or leaf curl and phyllody) and brought to Plant Pathology laboratory, Indian Institute of Horticultural Research, Bangaluru. Initially the samples were tested by DAS-ELISA using different known antibodies such as Groundnut bud necrosis virus (GBNV), Cucumber mosaic virus (CMV) and Tobacco streak virus (TSV) (DSMZ, Germany) to conformed the type of viruses associated with sunflower. Mean while the begomovirus and phytoplasma infection was confirmed by PCR using specific primers to know the type virus and phytoplasma present in the mixed infected plant under field conditions.

B. DNA isolation, PCR-mediated amplification and sequencing

I.DNA isolation

Total nucleic acids were isolated from begomovirus infected as well as mixed symptomatic sunflower plants by CTAB method (**Doyle and Doyle 1990**) were initially confirmed for the presence of a begomovirus in the samples by PCR using begomovirus genome (DNA-A component) specific primers (2395F/680R) as described by **Venkataravanappa et al. (2012).** On the basis of the determined sequences (1.2kb fragment), the eleven samples are associated a member of a previously described monoparitebegomovirus species which is closely related to ToLCV. Therefore one sample from each crop was selected for full-length amplification of begomovirus genomes (DNA A and DNA B) by rolling circle amplification method using an IllustraTempliPhi 100 Amplification kit (Amersham Biosciences, Piscataway, NJ, USA) following the manufacturer's instructions. The RCA products were digested with different restriction endonucleases (*EcoRI, Xba1, Bam HI* and *Hind III*) and cloned into BamHI-linearized pUC19 plasmid as described by **Venkataravanappa et al (2015)**. The confirmed clones were sequenced in both orientations from Medaxin DNA Sequencing facility, Bangalore, Karnataka, India. Further the samples were also confirmed by begomovirus associated betasatellites specific primers beta01/beta02 (**Briddon et al., 2002**) and Alphasatellites (**Kumar et al, 2010**) as described previously for begomovirussubgenomic detection.

II.Uniplex and Duplex PCR used for detection of begomovirus and Phytoplasma

The isolated DNA from mixed type symptomatic sunflower plants (leaf curl and phyllody) was amplified by uniplex and duplex PCR by using begomovirus specific primers (**Venkataravanappa et al. 2012**) and Phytoplasma-specific 16S rDNA primers P1/P7 (Deng and Hiruki 1991; Schneider *et al.,* 1995) for simultaneous amplification of DNA fragments of phytoplasma and begomoviruses. PCR conditions for uniplex and duplex PCR have been optimized ensuring the sensitive detection of the phytoplasma as well as ToLCV.

I. ***Sequence analysis***

Sequence similarity searches were performed by comparing sequence to all sequences available in the GenBank database using BlastN (**Altschul et al., 1990**) (Supplementary table 1).Sequences showing the highest identity scores with the present isolates (sunflower) were aligned using the Muscle method implemented in SDT version 1.0 (**Muhire*et al.*, 2014**) and per-cent pairwise identity of the identified sequences and the representative sequences were generated. A phylogenetic tree was generated using MEGA 6.01 software (**Tamura *et al.*, 2013**) using the Neighbour-Joining method with 1000 bootstrapped replications to estimate evolutionary distances between all pairs of sequences simultaneously.

3. Results

A. Roving survey and incidence of viral disease on sunflower

The roving survey was conducted during cropping season 2015-2016 in different farmer fields around in Kurnool district, Andhra Pradesh state of India. Total 50 field in different location of the district was surveyed both vegetative stage and flowering stage of the crops. The highest incidence (>50 to 60%) of mosaic was noticed across followed by leaf curl disease (30-40%) at vegetative stage and flowering stage. The incidence of both leaf curl and phyllody was about 5 to 10% and leaf curl, phyllody and mosaic about 5 % in different farmer's field at both the stage. The sunflower plants showing these kinds of diverse symptoms were collected along with one non-symptomatic sample from each location.

B.Detection of different viruses and phytoplasma

The viruses infected sunflower samples (163 samples) collected from field are showing different kinds of symptoms were tested by DAS-ELISA using polyclonal antibodies of different viruses (Table.1).117 out of 163 samples are reacted with Tobacco streak virus (TSV), 23/163 is detected with only begomovirus and 7/163 is detected with phytoplasma alone. The remaining 11/163 samples are detected with both begomovirus and phytoplasma and 5/163 are reacted with Tobacco streak virus (TSV), begomovirus and phytoplasma respectively.Since the aim our study is know the type of begomovirus and phytoplasma associated leaf curl and phyllody disease of sunflower. Therefore the samples showed positive to the begomovirus and phytoplasma were amplified by PCR using degenerate primer (Venkataravanappa et al., 2012). On the basis of the determined sequences (1.2kb fragment) analysis showed that, 11/163 samples are associated a member of a previously described monopartite begomovirus species which is closely related to ToLCV (nucleotide sequence identity is more than 99% among the isolates). Therefore one isolate was (Snf-1) selected for full length amplification of genomic components (DNA-A-like sequence) of the begomovirusby RCA method. The sub genomic components such as alpha satellite and beta satellite were successfully amplified from the virus and phytoplasma infected sunflower plant.

I.Analysis of 16SrRNA sequence of sunflower phyllody phytoplasma

Comparison of F2n/R2 primed 16SrRNA sequences of Sunflower phyllody phytoplasma (acc. No KX236092) with corresponding region of different groups of phytoplasma retrieved from database. The Sunflower phyllody phytoplasma showed maximum nt identity of 97.1% Peanut witches-broom phytoplasma-16SrII-A (L33765) followed by 96 % with Crotalaria phyllody phytoplasma-16SrII-C (EF193355) and Cactus witches'-broom phytoplasma-(16SrII-F EU099556, 16SrII-H-EU099569) respectively.Based on the classification of phytoplasma groups and subgroups, the 16SrRNA sequence identity between two distinct groups of phytoplasmas was ranged from 88 to 94% (Lee et al., 1993, 2000). Since the 16SrRNA gene sequence similarity of Sunflower phyllody phytoplasma with members of Peanut witches-broom group(16SrII) was above the threshold level of 94%, it is proposed that Sunflower phyllody phytoplasma should be regarded as a member of Peanut witches-broom group (16Sr II). This result also supported by a phylogenetic analysis which shows that the 16SrRNA gene of sunflower phyllody is closely clusters with Tomato witches-broom-16SrII-D (HM584815), Chickpea phyllody-16SrII (FJ870549), Ca.P.austrlasia-16SrII-D (Y10097), Peanut witches-broom phytoplasma-16SrII-A (L33765) belongs to themembers of Peanut witches-broom (16Sr II) infecting different crops (Fig 2).

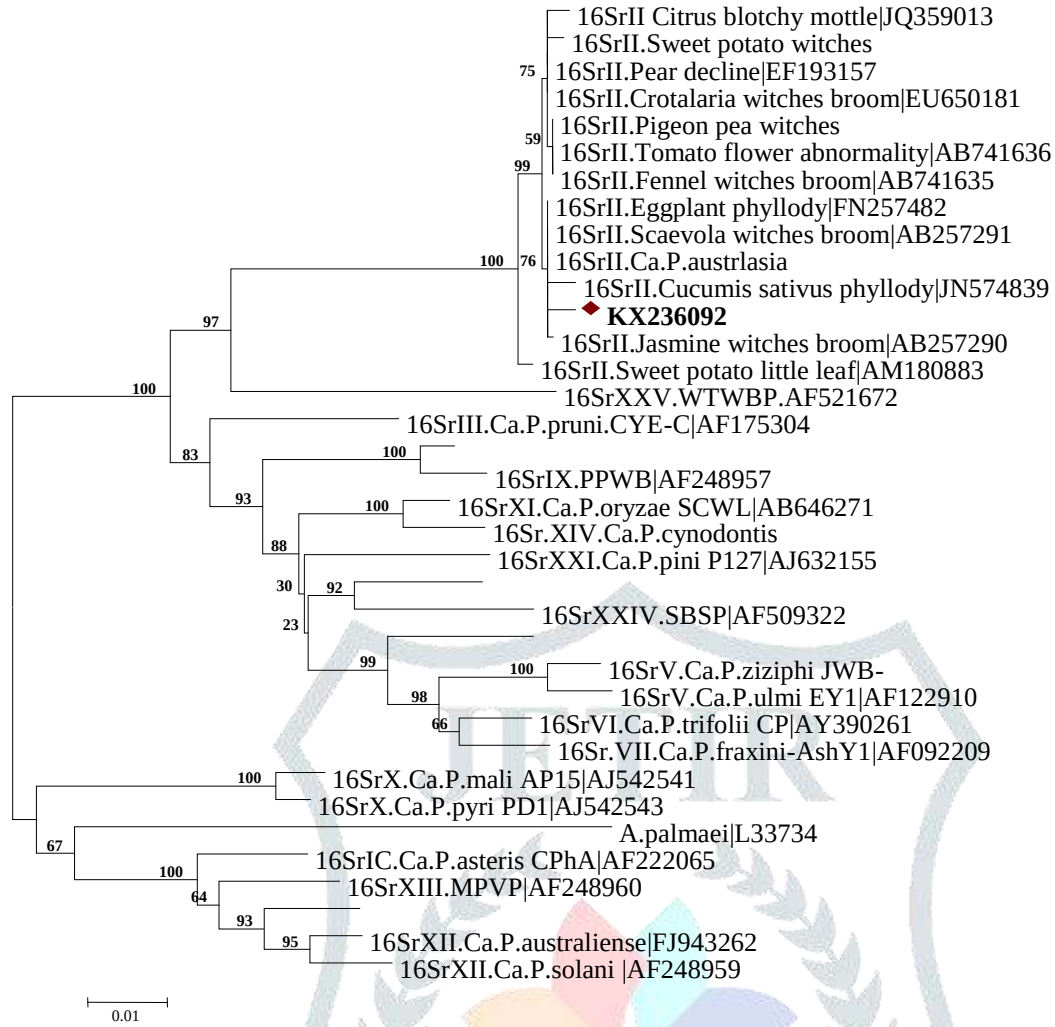

Fig. 2 . Phylogenetic tree obtained by the Neighbor-Joining method of 16S -23S rRNA spacer region sequences from phytoplasmas belonging to different groups and a clone of the sunflower phytoplasma identified in Andhra Pradesh. Roman numerals and letters represent 16S rDNA RFLP groups and subgroups, respectively.

II.Genomic organization and affinities of the DNA-A -like sequence

The complete DNA-A-like sequence of sunflower clone (Snf-1)was 2761nt in length respectively. Sequence alignment followed by pairwise comparisons using a muscle method implemented in SDT program, revealed that the Snf-1clones are shared 98 - 99 percent nt sequence identity among themselves, indicating that they are single species of ToLCVas per the presently applicable species demarcation criteria for begomoviruses (91%; **Brown et al., 2015**). The Snf-1clones have a typical features like other monopartite begomoviral genomes, based on the size and the characteristic organization of the six ORFs (V1, V2, C1, C2, C3 and C4), and the conserved IR. The IRs contained one directly repeated sequence, or iteron, the TATA-box, and the stem-loop and nanonucleotide sequence, TAATATTAC, required for transcription and viral genome replication.

The sequences were used to search the database using BLASTn to identify the most closely related viruses and other distinct taxa, which were selected and used as reference sequences (Table.2) for pair wise distance and phylogenetic analyses. The nt sequence identity of Snf-1 clone was comparison revealed that the clone was shared nt identity of 99.2 % with the DNA-A-like sequence of ToLCV infecting sunflower (JX678965) in India (Table.2). This result is well supported by a phylogenetic analysis, the Snf-1 clones is closely grouped with ToLCV infecting sunflower (Fig.5). ORF wise sequence identities at protein level of Snf-1 clone showed highest nt identity of coat protein (CP), Pre-coat (AV2) Rep (C1), TrAP (C2), REn (C3) and C4 with an isolate of ToLCV infecting sunflower.

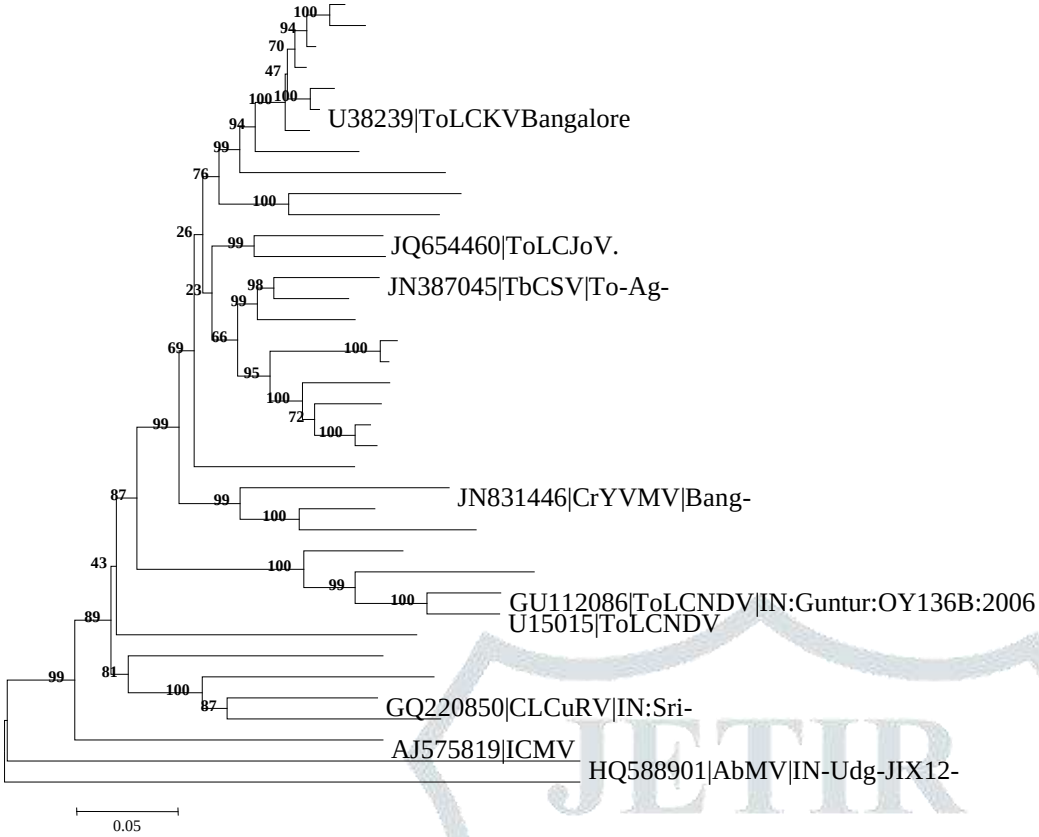

Fig 5. Phylogenetic tree of complete nucleotide sequences of the DNA-A of the Tomato leaf curl Karnataka virus (ToLCKV) isolate originating from with other begomoviruses available in the databases. The ToLCKV) isolate originating from Andhra Pradesh is highlighted. The numbers at the nodes indicate the bootstrap confidence values (1000 replicates). For each isolate the database accession numbers are given.

III.Genome organization of alphasatellite and betasatellite and their sequence affinities to other satellites

The alphasatellite and betasatellite isolated from sunflower was determined and it was ranged from 1351 to 1373nt in length respectively. Which are available in the NCBI database (under accession numbers KX236091, KX219745 respectively). The sequences of alphasatellite and betasatellite contain typical features of other alphasatellites (Briddon et al., 2004) betasatellites (Briddon*et al*., 2002; Venkataravanappa et al, 2011). The alphasatellite and betasatellite characterized as part of this study showed maximum per cent nt identity of 98.2% and 98.8% with sunflower leaf curl virus alpha satellite (JX569789) and Tomato leaf curl Karnataka betasatellite(JX678964) respectively. Phylogenetic analysis, supported the betasatellites and alphasatellites closely grouped with ToLCKV infecting sunflower andSLCKA infecting tomato (Fig.6,7)

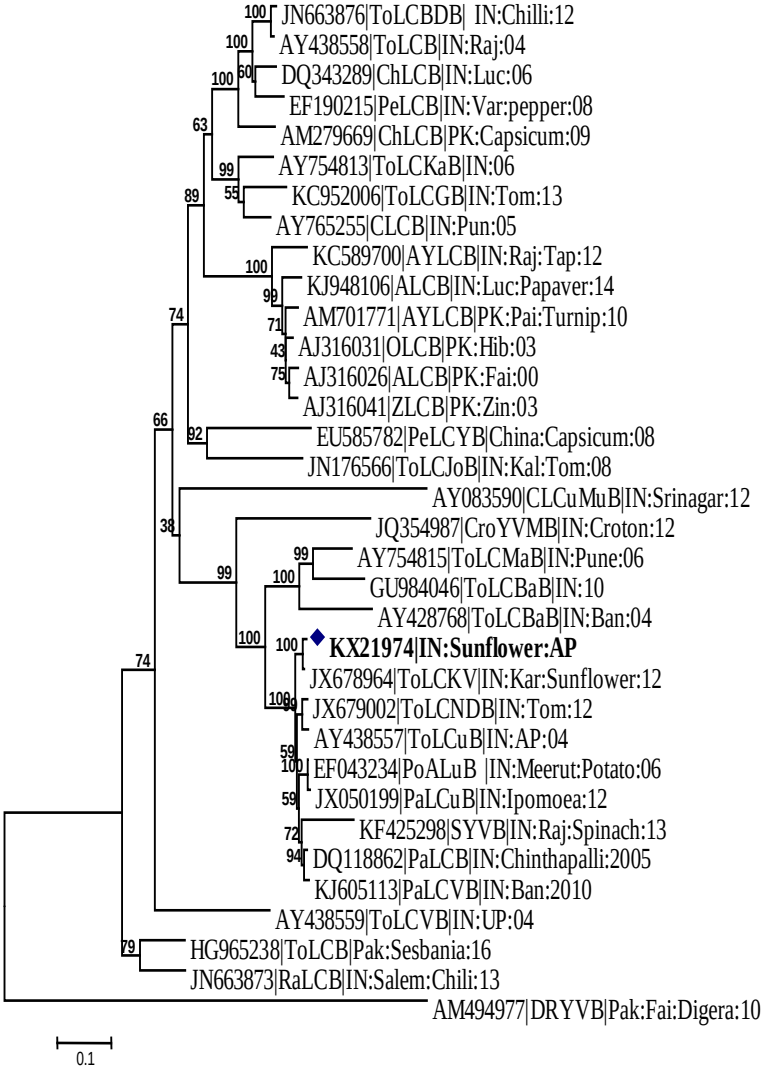

Fig. 6 Phylogenetic tree constructed from aligned complete nucleotide sequences of ToLCKB [IN:sunflower :AP:15] betasatellite with other betasatellites retrieved from the database using Neighbor-joining algorithm. The ToLCKV) isolate originating from Andhra Pradesh is highlighted. The numbers at the nodes indicate the bootstrap confidence values (1000 replicates). For each isolate the database accession numbers are given.

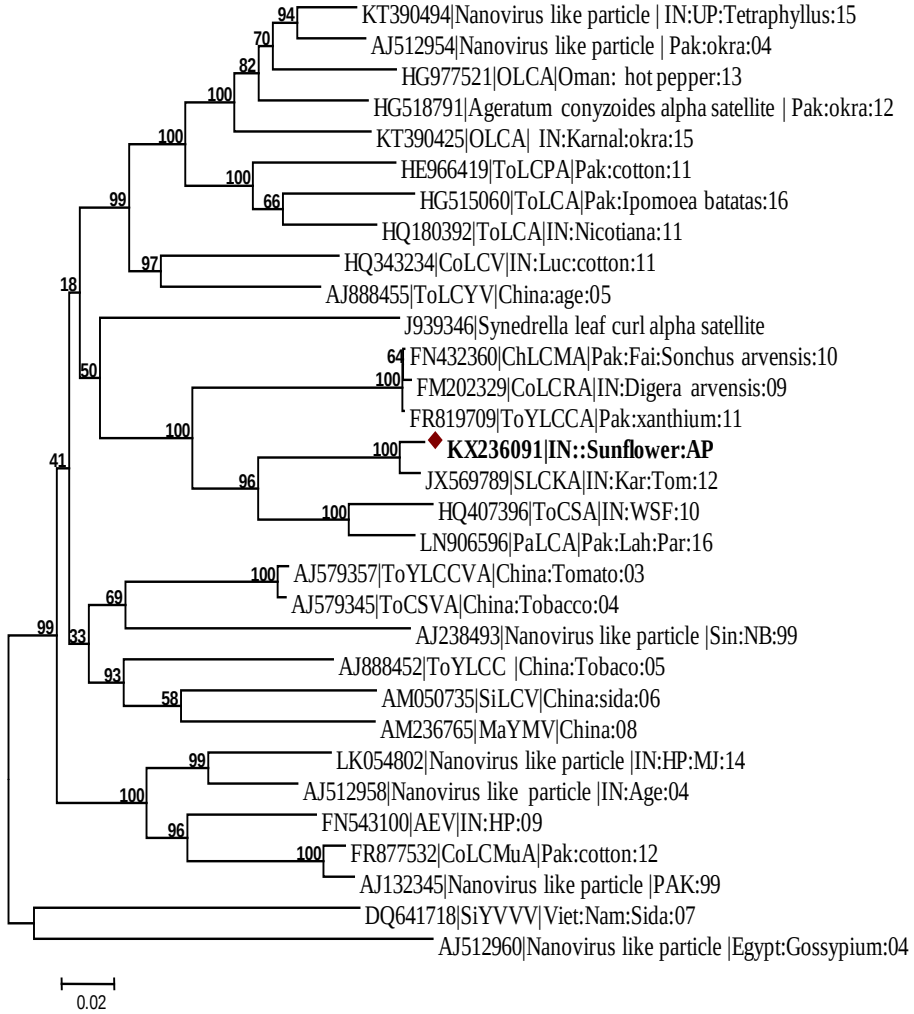

Fig. 7 Phylogenetic tree constructed from aligned complete nucleotide sequences of SLCKA[IN:sunflower :AP:15]alpha satellite with other alpha satellites retrieved from the database using Neighbor-joining algorithm. The SLCKV) isolate originating from Andhra Pradesh is highlighted. The numbers at the nodes indicate the bootstrap confidence values (1000 replicates). For each isolate the database accession numbers are given.

IV.Duplex PCR used for detection of begomovirus and Phytoplasma

Co-infection of virus and phytoplasma is common in many crops, as well in sunflower. Therefore for rapid and simultaneous detection of virus and phytoplasma in sunflower, the total DNA of eleven sunflower samples were amplified by duplex PCR using specific primers of begomovirus and phytoplasma. The resulted PCR amplificon of 1.7 kb and 1.2 kb were obtained by primers P1/ P7 and 310F/1455R for phytoplasma and begomovirus in symptomatic plants respectively (Fig. 3). There was no amplification in the samples collected from non-symptomatic plants. In this PCR assay the concentrations of the main reagents, such as primers, MgCl2, dNTPs, Taq DNA polymerase and PCR parameters were optimized to get expected fragments size to ensuring simultaneous, sensitive and specific detection of phytoplasma and begomovirus (Fig. 4).

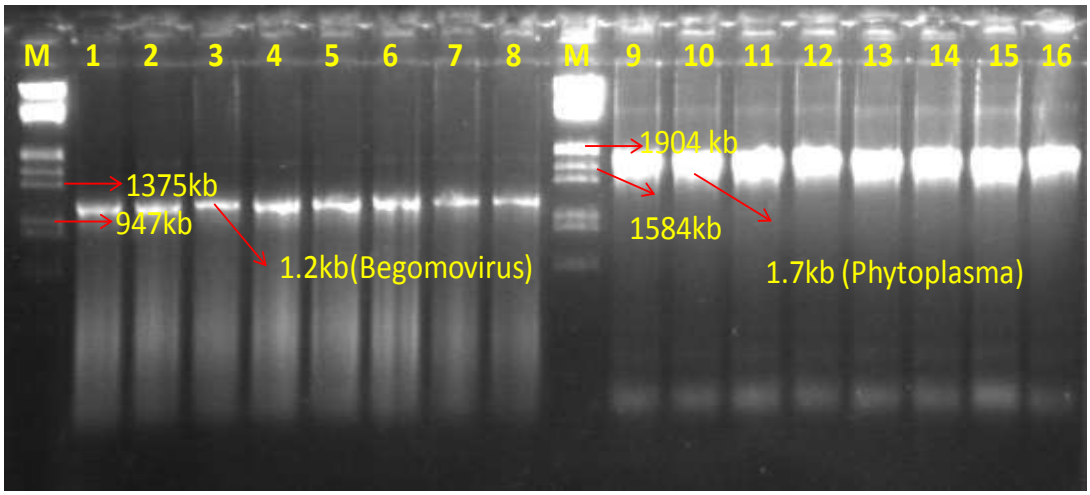

Fig 3: Gel picture showing Uniplex PCR amplification of begomovirus (1.2 kb) and phytoplasma samples (1.7 kb)
M: Molecular weight marker
Lanes 1-8: Amplification of begomovirus
Lanes 9- 16: Amplification of Phytoplasma

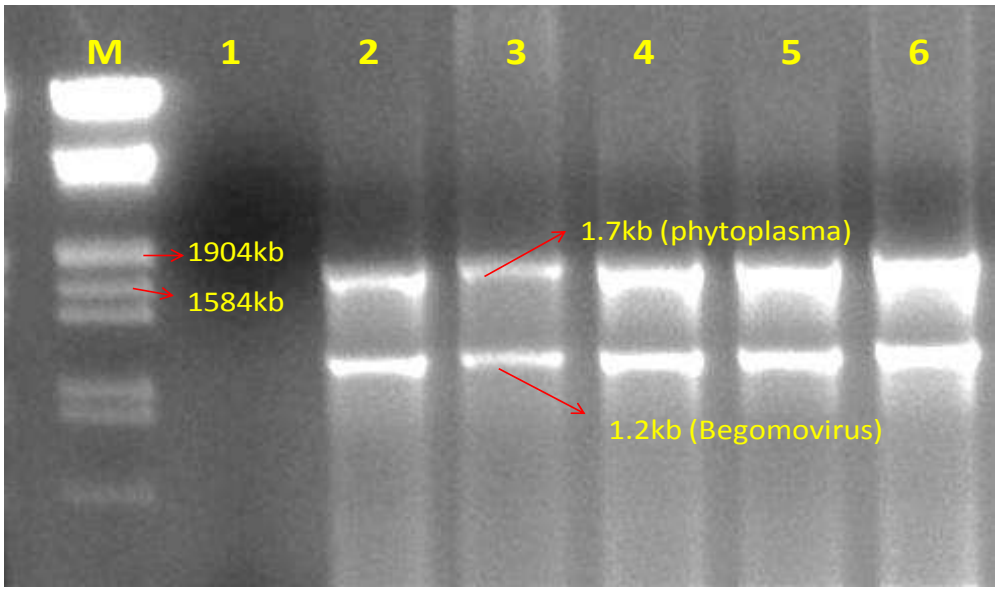

Fig 4: Gel picture showing duplex PCR amplification of begomovirus (1.2 kb) and phytoplasma samples (1.7 kb)
M: Molecular weight marker
Lanes 1: Amplification of asymptomatic plant
Lanes 2-6: Amplification of Begomovirus and Phytoplasma

4. DISCUSSION

Sunflower leaf curl disease caused by Tomato leaf curl virus was first recorded in Raichur district, Karnataka state of India (**Govindappa*et al*., 2011, Vanitha*et al*., 2013, Vindyashree et al., 2016**). Presently the virus is moving to adjoining state of Andhra Pradesh and cause an epidemic in different locations of the state. The roving sampling survey conducted in the present study focused on major sunflower growing regions of Andhra Pradesh state. During these surveys, it was found that several sunflower fields, the plants are displayed severe mosaic, leaf curl as well as mixed infection of mosaic, leaf curl and phyllody symptoms were predominantly observed across the

field. The field samples collected from the different location was confirmed by DAS-ELISA and PCR for different pathogens associated with sunflower leaf curl, mosaic and phyllody. Since the aim of present study is to know the type begomovirus and phytoplasma associated with leaf curl and phyllody mixed infection in sunflower. Therefore the mixed infected sunflower samples are tested by PCR, indicated that 11/163 samples are infected both phytoplama and begomovirus, which are belongs to the Peanut witches-broom phytoplasma (16SrII) (more than 97.1% nt identity) and ToLCV (more than 98% nt identity) respectively.Similarly phytoplasma and viruses mixed infections have been reported in a wide range of plant species (Arocha*et al*., 2009), tomato and pepper in Mexico (**Lebsky et al., 2011**) and Potyvirus with phytoplasmas in clover, (**Franova et al., 2004**). However, there are no earlier reports on the coexistence of begomovirus and phytoplasmas in sunflower in India or elsewhere. These types of mixed infections need to be analyzed in more detail for understanding pathogens interaction in the host.

The alphastellite and betasatellite associated with sunflower in the present study was also reported in the begomoviruses infecting sunflower (**Govindappa*et al*., 2011**) tomato (Chowda Reddy et al., 2005) and Parthenium (Kumar et al., 2016) in India.

The co-infection of virus and phytoplasma in sunflower samples was successfully detected by mulplex PCR, which is more rapid and sensitive and specific detection of the phytoplasma and the virus. Similar technique were followed for detection of co-infection in host by virus and phytoplasma in sugarcane (**Parmessur et al. 2002**), grapes (**Matus et al. 2008**) and white jute (**Biswas et al., 2013**).

With above facts the present study provides the evidence for the co-existence of phytoplasma and begomovirus in sunflower. Therefore further studies are required to understand recombination and synergism between the virus and phytoplasma in mixed infection under natural conditions. Other factors that include mechanism of coexistence, epidemiological implications and role of geographic location and climate conditions on disease development are required to be studied. In addition, long-term transmission experiments are necessary to explain the contribution of either phytoplasma or begomovirus, and their vectors, to symptom development and disease spread in sunflower.

Tables

Place	No. of fields surveyed	No. of Samples collected	Stage of the crop	Samples detected positive for TSV	Samples positive for Begomovirus	Samples positive for phytoplasma	Samples positive for both begomovirus and photplasma	Samples positive for TSV, Begomovirus and Phytoplasma
Adoni	5	25	Flowering stage	16	4	3	1	1
Kodimuru	5	23	Vegetative stage	14	4	1	2	2
Gudur	5	20	Flower bud stage	14	4	-	1	1
Brahmanakotkur	5	13	Flowering stage	7	3	2	1	-
Atmakur	5	17	Vegetative stage	15	-	-	2	-
Pamulapadu	5	10	Flowering stage	9	-	-	1	-
Banaganapalli	5	14	Flower bud stage	11	2	-	-	1
Bethamcherla	5	18	Seed development	13	3	-	2	-
Dhone	5	14	Flowering stage	10	2	1	1	-
Velthurthi	5	9	Seed development stage	8	1	-	-	-
	50	163		117	23	7	11	5

Table1.The viruses infected sunflower samples (163 samples) collected from field are showing different kinds of symptoms

Begmoviruses	**Accession numbers**	**Genome**	**IR**						
				V2	**CP (V1)**	**Rep (C1)**	**TrAP (C2)**	**REn (C3)**	**C4**
ToLCV	JX678965	**99.2**	**99.6**	**97.4**	**100.0**	**97.7**	**98.5**	**100.0**	**94.8**
ToLCPuV	AY754814	80.8	70.7	70.3	91.7	84.7	86.5	81.3	71.1
ToLCRaV	DQ339117	78.6	78.0	94.9	99.2	78.6	54.0	63.2	31.9
ToLCBaV	AF165098	81.1	63.6	70.3	87.9	85.8	83.5	82.8	91.7
ToLCKeV	EU910141	86.5	71.4	93.2	94.1	80.6	92.5	93.2	46.3
ToLCPatV	EU862323	73.9	45.3	78.9	81.7	80.3	61.4	67.9	49.0
ToLCNDV	U15015	71.6	48.5	69.4	93.7	78.1	52.8	63.9	35.0
ToLCPalV	AM884015	70.2	51.4	65.2	91.0	75.2	52.8	66.1	37.1
ToLCBV	AF188481	83.8	50.8	95.7	96.4	84.2	85.8	86.5	78.3
PaLCuV	HM143914	83.3	64.3	98.3	98.8	73.9	82.8	87.3	34.0

AEV	JX436472	81.8	49.0	95.7	96.8	80.6	85.8	88.0	43.2
TbCSV	JN387045	83.2	55.6	94.9	94.1	85.8	79.1	86.5	79.3
ChiLCV	JQ654460	82.3	51.6	78.8	96.4	85.0	86.5	80.5	76.2
RaLCuV	GU732204	82.6	51.2	94.9	96.4	70.6	90.2	91.0	51.5
CLCuKoV	HM461862	77.2	47.4	94.9	96.0	77.6	67.9	79.1	50.0
CLCuBaV	AY705380	75.1	48.0	69.4	94.1	79.3	58.6	68.6	50.0
CLCuAlV	AJ002452	70.5	42.5	66.1	95.3	73.2	56.6	70.8	32.9
CLCuMuV	GQ220850	74.9	56.4	58.4	94.9	80.4	58.0	69.4	48.0
OELCuV	GU111999	70.9	44.7	66.1	76.9	81.8	54.0	67.9	46.0
BYVMV	AF241479	72.0	51.8	66.9	94.9	78.2	57.3	70.8	44.0
FbLCV	JQ866297	73.4	45.4	66.3	91.4	80.4	73.1	71.6	43.0
MYMIV	AF481865	62.2	26.6	38.6	74.7	72.3	43.3	41.7	60.6
PeLCV	AM948961	83.3	51.9	95.7	96.0	84.2	89.5	91.7	48.4

#The species are indicated Tomato leaf curl virus (ToLCV), Tomato leaf curl Pune virus *(*ToLCPuV*)*, Tomato leaf curl Rajasthan virus (ToLCRaV) Tomato leaf curl Bangalore virus - C (ToLCBaV), Tomato leaf curl Kerala virus (ToLCKeV), Tomato leaf curl Patna virus (ToLCPatV), Tomato leaf curl New Delhi virus (ToLCNDV), Tomato leaf curl Palampur virus (ToLCPalV), Tomato leaf curl Bangladesh virus (ToLCBV), Papaya leaf curl virusn (PaLCuV), Ageratum enation virus (AEV), Tobacco curly shoot virus (TbCSV), Chilli leaf curl virus (ChiLCV), Radish leaf curl virus (RaLCuV), Cotton leaf curl Kokhran virus (CLCuKoV) , Cotton leaf curl Bangalore virus (CLCuBaV), Cotton leaf curl Alabad virus (CLCuAlV), Cotton leaf curl Multan virus (CLCuMuV), Okra enation leaf curl virus (OELCuV), Bhendi yellow vein mosaic virus (BYVMV), French bean leaf curl virus (FbLCV) Munbean yellow mosaic virus(MYMV), Pedilanthus leaf curl virus (PeLCV) . **For each column the highest nucleotide identity value is underlined.**

Table 2. Pairwise percent amino acid sequence identities of encoded genes from the begomoviruses components isolated from sunflower and genes of closely related begomoviruses selected in the databases.

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