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Prevalence and Molecular Characterization Coat Protein Gene of *Tobacco streak virus* Causing Peanut Stem Necrosis Disease in Coastal and Rayalaseema Districts of Andhra Pradesh, South-India

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ABSTRACT

Background: Peanut stem necrosis disease (PSND) caused by *Tobacco streak virus* (TSV) is a major constraint for groundnut production in Andhra Pradesh (A.P.). However, studies on prevalence and spread of the disease confined to only few districts of A.P. with this background current study focused on incidence and spread of the disease in entire state of A.P. Further an isolate of TSV occurring in A.P. characterized on the basis of genetic features by comparing with other TSV isolates originated from different hosts and locations from world.

Methods: Roving survey was conducted during *kharif* 2017-18 in groundnut growing districts of Andhra Pradesh (A.P.) for peanut stem necrosis disease incidence. Groundnut plants showing PSND symptoms were collected and tested with direct antigen coating enzyme linked immunosorbent assay (DAC-ELISA). Groundnut samples found positive by ELISA once again tested by reverse transcription polymerase chain reaction (RT-PCR). The representative TSV-GN-INDVP groundnut isolate from Prakasham district was maintained on cowpea seedlings by standard sap inoculation method in glasshouse for further molecular characterization. The Phylogenetic tree for coat protein (CP) gene was constructed using aligned sequences with 1000 bootstrap replicates following neighbor-joining phylogeny.

Result: Thirty-eight (52.7%) of seventy-two groundnut samples collected from different locations in A.P were given positive reaction to TSV by DAC-ELISA. For the first time, PSND incidence observed in coastal districts (Krishna, Guntur, Sri Pottisriramulu Nellore, Prakasham) of A.P. Maximum PSND incidence recorded from Bathalapalli (22.2%) and the minimum incidence in Mulakalacheruvu (4.1%). The coat protein (CP) gene of TSV-GN-INDVP groundnut isolate was amplified by RT-PCR and it shared maximum per cent nucleotide identity (97.51-98.62%) with TSV isolates from groundnut and other different crops reported in India. All Indian isolates cluster together irrespective of crop and location based on the phylogenetic analysis.

Key words: Coat protein (CP) gene, Groundnut, PSND, Survey, TSV.

INTRODUCTION

Groundnut (*Arachis hypogaea* L.) is an annual oil seed legume crop, widely grown in tropical and semi-arid tropical regions of the world. It commonly cultivated for edible oil, vegetable protein, fodder, medicinal and cosmetic products. It is also used to improve soil fertility by fixing atmospheric nitrogen. Groundnut is grown in an area of 26.4 M. ha with a total production of 46.1 M.M.T (million metric ton) (USDA, 2020). China and India are the world's largest groundnut producers, accounting for 38 percent and 11 per cent, respectively. India ranked second in production with 5.5 M.M.T of shell groundnuts (USDA 2020). In India, Gujarat ranked first in groundnut production, followed by Rajasthan, Andhra Pradesh and Karnataka (APEDA 2018-19). However, the groundnut productivity was very low in India as compared to world groundnut productivity, Major constraint for not attaining higher productivity of groundnut was due to biotic (viral and fungal diseases), abiotic conditions and rain fed nature of crop.

Among the viral diseases infecting groundnut, peanut stem necrosis disease (PSND) caused by *Tobacco streak*

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virus (TSV) is a major constraint for groundnut production in south Indian states. Thrips species serves as vectors for TSV and carry the infected pollens from weed hosts to crop plants. TSV cannot spread by seed from infected plants to healthy groundnut plants under Indian conditions (Vemana and Jain, 2010). *Parthenium hysterophorus* weed was a non-symptomatic host and considered as principal source of TSV-infected pollen leading to disease epidemics in nearby crops (Prasada Rao *et al*., 2003). TSV on groundnut was first reported in Ananthapuram district of Andhra Pradesh (A.P.), India, TSV caused an epidemic on groundnut crop in 2000 resulting in a loss estimated to the tune of Rs.300 crore (Reddy *et al*., 2002). Since then, the occurrence of PSND has frequently been reported in A.P (2005-14.9%, 2006-21.0%, 2007-11.4%, 2011-7.9%, 2013-4.4%) (Anonymous 2005, 2006, 2007, 2011 and 2013). Later, its occurrence was detected in the adjacent groundnut growing states of Karnataka and Tamil Nadu (T.N). TSV belongs to the family *Bromoviridae* and the genus Ilarvirus having quasi isometric shape. TSV genome has RNA 1 (3.5 kb), RNA 2 (2.9 kb) and RNA 3 (2.2 kb) (Fauquet *et al*., 2005). RNA 1 encodes for RNA dependent RNA polymerase responsible for virus replication. RNA 2 encodes the nonstructural 2a protein necessary for viral replication and an overlapping 2b protein. RNA 3 genome encodes for coat protein (CP) and movement protein (MP). PSND is a serious problem of groundnut cultivation in A.P. However, studies on incidence and spread of TSV confined to Rayalaseema (Anatapuramu, Y.S.R. Kadapa, Kurnool, Chittoor) districts of A.P.

Hence, present study was taken up for the first time to identify the incidence and spread of TSV on groundnut in coastal and Rayalaseema districts A.P. Further, molecular characterization of coat protein (CP) was made for the test isolate by comparing with other TSV isolates originated from different hosts and locations.

MATERIALS AND METHODS

Glasshouse facility and molecular laboratory studies were carried out at Agricultural Research Station, Kadiri, All India Coordinated Research Project on Groundnut (AICRP-G) centre, A.P.

Survey

Roving survey was conducted in major groundnut growing districts of A.P during *kharif* 2017-18 to know per cent disease incidence of PSND. TSV infected groundnut plant samples were collected from different locations of A.P. (Table 1). Randomly five spots selected in each field by walking across the field and counted the number of TSV suspected plants vs total number plants per square meter area. Later, the per cent disease incidence was calculated using following formula:

$$\text{Per cent disease incidence (\%)} = \frac{\text{Number of infected plants}}{\text{Total number of plants}} \times 100$$

Diseased leaf samples from all surveyed locations were stored in plastic bags and brought to the laboratory. The suspected TSV samples exhibiting characteristic symptoms such as small necrotic rings on leaf, severe necrosis on leaf lamina, petioles and plants with peg necrosis, were collected and tested by direct antigen coating enzyme linked immunosorbent assay (DAC-ELISA) using TSV specific antisera (Clark and Joseph, 1984) supplied by International Crops Research Institute for the Semi-Arid Tropics (ICRISAT).

RT-PCR

The TSV positive samples confirmed by DAC-ELISA were once again tested by reverse transcription polymerase chain reaction (RT-PCR). Total RNA was extracted from the collected leaf samples using RNeasy Plant Mini Kit (Qiagen Inc., Valencia, CA). First strand cDNA was synthesized using revert aid first strand cDNA synthesis kit (Thermo fisher) according to manufacturer's instructions. Synthesized cDNA was subjected to PCR using primer pair TSV1 (forward) (5′-ATGAATACTTTGATCCAAGGTCCN-3′) and TSV2 (5′-TCAGTCTTGATTCACCAGGAAAN-3′) (reverse) specific to CP gene of TSV. The PCR amplifications were carried out in thermal cycler (Eppendorf master cycler gradient). The reaction mixture contained 2 µl of cDNA template (50-60 ng), 1 µl each of 10 µM forward and reverse primers, 5 µl of 10 X PCR buffer, 3 µl 25 mM $MgCl_2$,1 µl of 10 mM dNTPs, 0.5 µl of Taq DNA polymerase (5 U/µl) and added RNase free water to make up final volume up to 50 µl. All the above reaction components were procured from Thermo scientific company. PCR profile consists of initial denaturation at 94°C for 5 min, followed by 35 cycles of denaturation at 94°C for 45 sec, annealing at 50°C for 45 sec and extension for 1 min at 72°C. Final extension was allowed for 30 min at 72°C (Daliyamol *et al*., 2019). Subsequently PCR product was run on 1 per cent agarose gel for 1 hour at 50 volts and product of ~700 bp was amplified in all the samples (Fig 1).

Maintenance of TSV culture

The representative groundnut TSV isolate collected from Vetapalem in Prakasam district A.P. (TSV-GN-VPIND) was selected for further study by maintaining on cowpea (*Vigna unguiculata* cv. PUSA KOMAL) by following standard sap inoculation method in glass house at 25±2°C. Five to eight seeds were sown in each plastic pot (4" diameter) allowed to germinate. Seven day old seedlings with uniform growth were selected for inoculation. Infected as well as healthy tissues from field samples were macerated separately using sterilized chilled pestle and motor in 0.1M phosphate buffer (pH 7.2, 1:1 w/v) containing 0.1% mercaptoethanol as an extraction buffer (EB). The sap was kept on ice till the inoculation was completed. The collected sap was rub-inoculated on surface of young leaves of 7-day-old cowpea seedlings previously dusted with celite (diatomaceous earth). The excess inoculum was washed off with distilled water after three min and kept in the insect-proof glass house. Inoculations were preferably carried out in the evening. The inoculated seedlings were observed for symptom

Table 1: Incidence of PSND in groundnut growing districts of Andhra Pradesh during *Kharif*-2017-18.

District	Mandal (village)	GPS coordinates	% PSND /m²	TSV DAC-ELISA-A405 nm ;1 h		Predominant weed hosts
				O.D	Range	
Ananthapuramu	Kadiri	14°6′34.187′N 78°8′42.503"E	20.8	2.24	1.21-2.24	*Parthenium hysterophorus, Ageratum conyzoides, Celosia argentea*
	Bathalapalli (Nallaboinapalli)	14°26′8.915"N 77°54′7.21"E	22.2	1.27		
	Uravakonda (Chinnamusturu)	15°26′.617′N 080°10.193′E	16.6	1.21		
	Vajrakarur	15°02′62.58"N 77°38′60.99′E	13.0	1.26		
	Bathalapalli (Sanjeevapuram)	14°32′27.16′N 77°44′18.04"E	15.3	2.21		
YSR Kadapa	Pulivendula	14.39253′N 78.2199′E	18.5	1.23	0.75-0.91	*Parthenium hysterophorus, Celosia argentea*
	Gandi Village	14.3107′N 78.4922′E	8.7	0.82		
	Vempalli	14.35353′N 78.46113′E	12.5	0.75		
Chittoor	Mulakalacheruvu	13.78905′N 78.27806′E	4.1	0.91	0.23-1.24	*Parthenium hysterophorus, Celosia argentea, Cleome viscosa*
	Madanapalle	13.64752′N 78.46525′E	4.1	0.82		
	S.V. Agriculture College, Tirupathi.	13.6247′N 79.3714′E	8.3	1.24		
Kurnool	Bethamcherla	15.59226′N 78.17602′E	-	0.23	0.76-1.21	*Parthenium hysterophorus, Celosia argentea*
	Allagadda	15.68783′N 78.41737′E	12.5	1.21		
	Krishnagiri	15.55153′N 77.84649′E	8.4	0.92		
	Aluru	15.50401′N 80.13231′E	8.6	0.85		
Prakasham	Kothpatnam	15°26.460′N 080°10.255′E	_	_	0.12-0.92	*Leucas aspera, Cleome viscosa, Parthenium hysterophorus*
	Kothpatnam (Aluru)	15°26.617′N 080°10.193′E	_	_		

Table 1: Continue...

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	Vetapalem) (Akkaipalem	17°48′24.103′N 80°21′33.08′E	12.0	0.92		
	Chirala (Prasadanagaram)	15°49′37.59′N 80°22′46.22′E	_	0.12		
Sri Potti Sriramulu	Muthukuru (Buchareddy palem)	14°16.579′E 080°04.481′N	_	0.12	0.12-0.72	*Parthenium hysterophorus, Ageratum conyzoides, Euphorbia hirta*
Nellore District	Kavali (Thummalapenta)	14°53.610′E 080°03.788′N	8.3	0.72		
Guntur	Bapatla	15°57′12.88′N 80°32′25.56′E	8.4	1.25	0.09-1.25	*Parthenium hysterophorus, Celosia argentea*
	Karlapalem	15°56′42.202′N 80°32′47.352′E	_	0.21		
	Karlapalem (Yazali)	15°58′13.869′N 80°34′40.232′E		0.09		
Krishna	Vunguturu (Avitipalli)	14°26.285′E 079°58.288′N	_	0.12	0.12-0.61	*Parthenium hysterophorus, Celosia argentea,*
	Machilipatnam (Kothapatnam)	16°13.064′E 081°11.434′N	12.5	0.61		
West Godavari	Nallajerla (Dubacherla)	16°55.860′N 081°20.996′E	_	0.13	0.13	*Euphorbia hirta, Abutilon indica*
Visakhapatnam	Hukumpeta (Marripalem)	17°52.728′N 082°20.667′E	_	0.12	0.09-0.17	*Celosia argentea, Euphorbia hirta*
	Chintapalli (Asarada)	17°54.728′N 082°24.666′E	_	0.09		
	Chintapalli	17°56.710′N 082°24.662′E 17°52.728′N	_	0.17		
*Vizi*anagaram	*Vizi*anagaram (Gajularega)	18°07.482′N 083°23.884′E	_	0.11	0.11-0.18	*Parthenium hysterophorus, Celosia argentea*
	Dattirajeru (Gadasam)	18°20.369′N 082°24.840′E 18°07.482′N	_	0.18		
Srikakulam	Ponduru (Kesuvadasupuram)	18°10.597′N 083°47.466′E	_	0.08	0.08-0.21	*Parthenium hysterophorus, Celosia argentea, Cleome viscosa*
	Laveru (Gumadam)	18°10.900′N 083°47.078′E	_	0.12		
	Ranasthalam (Ranasthalam)	18°07.475′N 083°23.883′E	_	0.21		

DAC-ELISA: Direct Antigen coated enzyme linked immunosorbent assay; Positive control O.D value: 2.4; Negative control (Healthy) O.D value: 0.20.

development. Typical symptoms of necrotic lesions observed on cowpea after three days' post inoculation (PDI) and subsequently TSV culture mass multiplied by transferring single lesion to fresh cowpea seedlings. Similar symptoms were observed on healthy groundnut seedlings upon re-inoculation using above TSV culture and same was used for further molecular study.

Molecular characterization of CP gene of TSV

The TSV-Vetapalem (TSV-GN-INDVP) groundnut isolate from A.P. was characterized on the basis of coat protein (CP) gene to confirm its molecular identity with already reported TSV isolates. The extracted total RNA from artificially inoculated cowpea leaf was used as template for RT-PCR amplification of CP using primer pair TSV1 and TSV2. In RT-PCR, amplicon of ~ 700 bp was amplified and it was gel-purifed (Gene JET, Fermentas, India) and sent for sequencing facility at Bangalore (Eurofins Genomics Bangalore India). Low quality nucleotide sequences of coat protein (CP) gene of TSV-GN-INDVP trimmed both ends in chromas (version 2.6.6). Trimmed sequences assembled using bioedit (version 7.0.5.3). Assembled sequence of partial CP gene (443bp) first evaluated using BLASTN program from the NCBI website (www.ncbi.nlm.nih.gov) to know the identity and homology with other reported TSV isolates. The partial sequence obtained was deposited in the GenBank. For further analysis, reference sequences of CP gene sequences of TSV available in database were downloaded from NCBI website (Table 2). *Cucumber mosaic virus* (CMV) was used as an out-group member. Partial CP gene of TSV was compared with thirty reported TSV isolates from different parts of the world infecting oilseeds, vegetables, pulses, ornamentals and weed host. All the sequences were aligned using MUSCLE algorithm of MEGAX (Version 10.1.1). The phylogenetic tree for CP gene was constructed using aligned sequences with 1000 bootstrap replicates following neighbor-joining phylogeny of MEGAX (Sudhir *et al*. 2018).

Table 2: List of accession numbers for coat protein (CP) gene of TSV isolates used for comparison.

Accession no	Location	Host	Isolate code
* MW014340	India: Andhra Pradesh: Vetapalem	*Arachis hypogaea*	TSV-GN-INDVP
HM770023	India: Tamil Nadu	*Arachis hypogaea*	TSV-GN-INDCO
MH744487	India: Kadiri, Andhra Pradesh	*Arachis hypogaea*	TSV-GN-INDKD
HM622157	India: Bangalore, Karnataka	*Arachis hypogaea*	TSV-GN-INDBN
FJ447357	India: Tirupati, Andhra Pradesh	*Arachis hypogaea*	TSV-GN-INDTPT
DQ864453	India: Kurnool, Andhra Pradesh	*Arachis hypogaea*	TSV-GN-INDKU
KC683810	India: Tirupati, Andhra Pradesh	*Ricinus communis*	TSV-CA-INDTPT
JN254782	India: Tirupati, Andhra Pradesh	*Hibiscus cannabinus*	TSV-HC-INDTPT
MN071396	India:	*Solanum lycopersicum*	TSV-TO-IND
KU378234	India: Tamil Nadu, Coimbatore	*Gossypium hirsutum*	TSV-CO-INDCO
JQ269831	India: Tiruapti, Andhara Pradesh	*Cyamopsis tetragonoloba*	TSV-GU-INDTPT
FJ655170	India: Bangalore	*Cucurbita pepo*	TSV-SQ-INDBN
AY501483	India: Gokulwadi, Maharashtra	*Corchorus olitorius*	TSV-JU-INDGK
AF515825	India: New Delhi	*Crotalaria juncea*	TSV-SH-INDND
AY505076	India: Parbhani, Maharashtra	*Helianthus annuus*	TSV-SU-INDPB
MF555191	India: Coimbatore, Tamil Nadu	*Parthenium hysterophorus*	TSV-PA-INDCO
KY176873	India: Tumkur, Karnataka,	*Cucumis sativus*	TSV-CU-INDTU
KF220492	India: Kadiri, Andhra Pradesh	*Cajanus cajan*	TSV-RG-INDKD
KC996727	India: Anatapur, Andhara Pradesh	*Jasminum sambac*	TSV-JS-INDATP
FJ655173	India: Bangalore	*Abelmoschus esculentus*	TSV-OK-INDBN
JQ269830	India: Kadapa, Andhra Pradesh	*Allium cepa*	TSV-ON-INDKP
FJ655171	India: Hessaraghatta, Karnataka	*Vigna radiata*	TSV-GG-INDHS
DQ225172	India: Coimbature, Tamil Nadu,	Vigna mungo	TSV-BG-INDCO
KJ825822	India: Tamil Nadu, Coimbatore	*Glycine max*	TSV-SO-INDCO
KC683812	India: Tirupati, Andhra Pradesh	*Macrotyloma uniflorum*	TSV-HG-INDTPT
AM933669	Sudan	*Vicia faba*	TSV-FB-SUD
JX463349	Australia	*Xanthium occidentale*	TSV-X0-AUS
LC030108	Japan	*Dahlia variabilis*	TSV-DA-JAP
MF784819	South Korea	*Tagetes minuta*	TSV-MA-SK
FJ403377	USA	*Glycine max*	TSV-SB-USA
MK73294	Argentina	*Helianthus annuus*	TSV-SU-ARG

*MW014340-Present study isolate.

RESULTS AND DISCUSSION

Roving survey

Extensive roving survey was conducted during *kharif* 2017-18 to record the incidence of PSND in groundnut crop from 12 districts of Andhra Pradesh (Table 1). TSV incidence was observed in 8 out of 12 districts surveyed. PSND incidence was observed in all Rayalaseema districts (Anathapuramu, YSR Kadapa, Kurnool and Chittoor) of A.P. In coastal A.P, only 4 districts (Krishna, Guntur, Sri Pottisriramulu Nellore and Prakasam) showed PSND incidence and no incidence were observed in the remaining districts (Srikakulam, *Viz*ianagaram, Visakhapatnam and West Godavari). During survey typical symptoms of TSV were observed on groundnut crop *viz*., severe necrosis on leaf lamina, necrotic streaks on petioles stem and peg necrosis, necrotic spots on pods and stunted growth with axillary shoot proliferation (Fig 1). Maximum TSV incidence was recorded from Bathalapalli (22.2%) and minimum incidence was in Mulakalacheruvu (4.1%). Thirty-eight out of seventy-two ground nut samples collected from different locations during survey in A.P were found positive by DAC-ELISA. The absorbance values (A_{405} nm;1 h) ranged from 0.09 -2.24 (Table 1). During survey predominant weed host observed around the surveyed fields were mentioned in the Table 1. Positively tested DAC-ELISA samples once again confirmed by RT-PCR assay and got amplification using coat protein (CP) gene specific primers (Fig 2).

In India, TSV was first recorded on sunflower in Karnataka in the year 1997 (Annual Progress Report 1997; Singh *et al*., 1997) Later, incidence of TSV was first observed on groundnut in 2000 (Reddy *et al*., 2002) at Ananthapuramu district of A.P and since then it has been frequently recorded in A.P. on groundnut (2005-14.9%, 2006-21.0%, 2007-11.4%, 2011-7.9%, 2013-4.4%) (Anonymous 2005, 2006, 2007, 2011 and 2013). Subsequently, TSV expands its host range by infecting 16 different crop species, 4 ornamentals and 11 weed hosts (Krishna Reddy *et al*., 2017). Recently, Sunil Kumar *et al.,* 2018 surveyed in Ananthapuramu and Kurnool districts for TSV infection in agricultural and horticultural crops in A.P and recorded 9-28 per cent in groundnut, 6-18 per cent in sunflower and 5-22 per cent in cucumber.



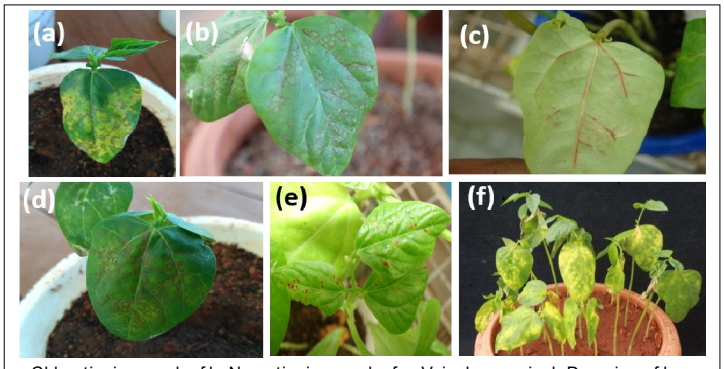
a: Necrosis on leaf lamina, b: Systemic infection c: Auxiliary shoot proliferation d: Peg necrosis e: Necrosis on pod f: Death of plants in isolated patches.

Fig 1: Typical symptoms of TSV on groundnut crop under field conditions.



M =1 kb DNA ladder; Lane 1-8: (1-Guntur,2-Nellore,3-Krishna,4-Prakasham, 5-Anatapuramu, 6-Chittoor, 7-Kadapa, 8-Kurnool, 9-Negative control, 10-Positive control).

Fig 2: RT-PCR amplification of Coat protein (CP) gene of TSV groundnut isolates collected from different locations in Andhra Pradesh.



a: Chlorotic rings on leaf b: Necrotic rings on leaf c: Veinal necrosis d: Drooping of leaves e: Systemic infection (Necrotic spots) f: Wilting of plants.

Fig 3: Typical symptoms of TSV on cowpea (*Vigna unguiculata* cv. PUSA KOMAL) under glass house conditions.

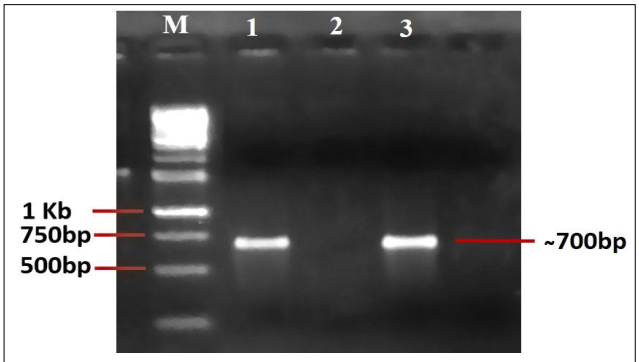
In the present study, the PSND incidence was observed whenever alternative weed host *Parthenium hysterophorus* exists around the field in most surveyed places (Table 1). Parthenium is widely distributed and is a symptom less carrier of TSV. It plays a major role in perpetuation and spread of the disease by supplying infected pollen as TSV is pollen borne (Prasada Rao *et al*., 2003). Screening technique was developed to screen groundnut genotypes using parthenium infector border growing around the screening plot in advance to groundnut crop that result in high disease pressure of PSND (90.0%) (Vemana *et al*., 2016). Kanchan *et al*. (1980) also suggested that large quantities of parthenium pollen are air borne and could move significant distances. In some areas disease incidence is low because farmers have acquired knowledge on the spread of disease and removed Parthenium well advance to groundnut crop.

TSV symptomatology in glasshouse

The representative TSV-GN-INDVP groundnut isolate was sap inoculated on seven-day old cowpea seedlings in glasshouse and expressed typical symptoms of TSV within the 3-5 days DPI. Symptoms appeared on leaves include *viz*., chlorotic rings, venal necrosis, necrotic rings, necrotic spots on systemic leaves (Fig 3). Systemic infection was observed on young leaves after seven DPI.

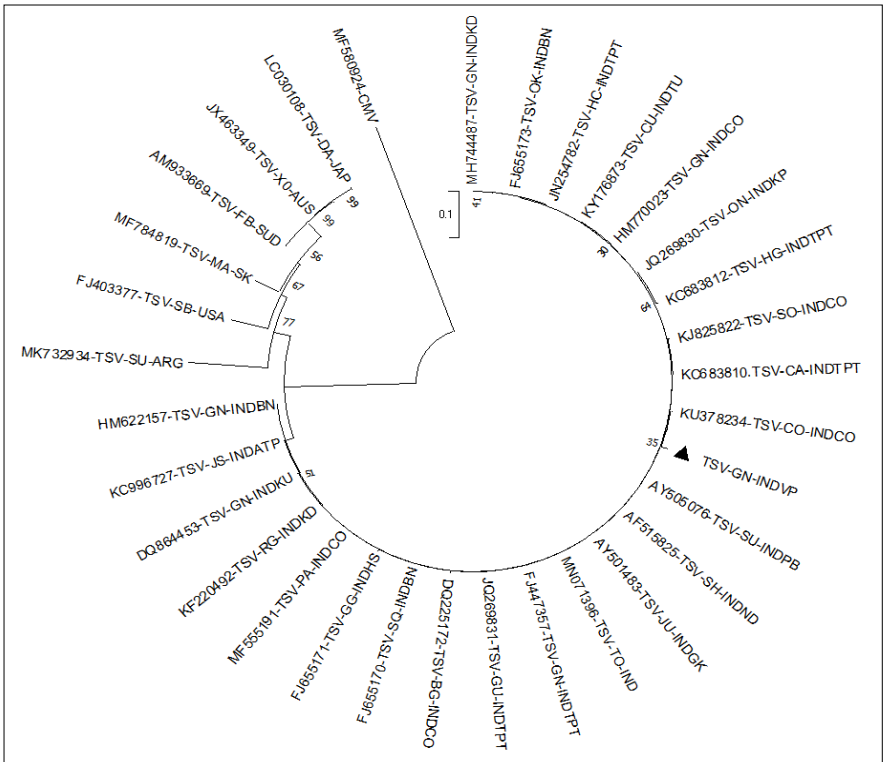
Molecular characterization of CP gene of TSV-INDVP

Total RNA isolated from TSV-GN-INDVP groundnut isolate maintained on cowpea and got amplification of ~700 bp by RT-PCR (Fig 4). Amplified product sequenced bidirectional and obtained sequence deposited in the GenBank (Accession No MW014340). The partial CP of TSV-GN-INDVP groundnut isolate shared wide range of nucleotide identities (80.72-98.62 %) with isolates reported globally.



M =1 kb DNA ladder; Lane 1-TSV-GN-INDVP groundnut isolate; Lane 2-Negative control, Lane 3-Positive control.

Fig 4: RT-PCR amplification of coat protein (CP) gene product of TSV-GN-INDVP groundnut isolate maintained on cowpea (*Vigna unguiculata* cv. PUSA KOMAL).



Note: 1. Each sequence is labeled with the NCBI accession number followed by isolate code.
2. Isolate TSV-GN-INDVP highlighted with triangle mark.

Fig 5: Phylogenetic comparison based on nucleotide sequences of coat protein (CP) gene of TSV-GN-INDVP isolate of groundnut with previously reported TSV isolates from India and other countries.

Present study isolate shared 97.97-98.51% nucleotide identities with groundnut isolates and 97.51-98.62% nucleotide identities with other crop TSV isolates from India. Among Indian isolates, TSV-CA-INDTPT (KC683810) showed the highest nucleotide identity (98.62%) with present study TSV-GN-INDVP groundnut isolate. On the other side, isolate TSV-GN-INDVP shared relatively less nucleotide identity with other country isolates (80.72-88.84%). Among other country isolates TSV-FB-SUD (TSV-*Vicia faba*) (AM933669.) shared highest nucleotide identity (88.84%) with isolate TSV-GN-INDVP. The nucleotide-based phylogenetic analysis showed all Indian TSV isolates clustered together irrespective of crop and location which indicated that the CP gene of Indian TSV isolates is highly conserved. Earlier studies also showed that the CP gene of TSV isolates occurring in India is highly conserved (Bhat *et al*., 2002; Krishna Reddy *et al*., 2003; Jain *et al*., 2005; Daliyamol *et al*., 2019). On the contrary, all other country's TSV isolates clustered together. Isolate TSV-GN-INDVP closely clustered with TSV-CO-INDCO (TSV-Cotton) isolate from Coimbatore. All the TSV isolates clustered separately to that of the CMV isolate as it is out group member (Fig 5).

CONCLUSION

All previous survey reports for TSV confined to Rayalaseema districts (Anathapuramu, Y.S.R. Kadapa, Kurnool, Chittoor) of A.P and there is no information about incidence of PSND in coastal Andhra Pradesh. The present study recorded PSND incidence for the first time from four coastal A.P. districts (Krishna, Guntur, Sri Pottisriramulu Nellore, Prakasham) with confirmation using DAC-ELISA and RT-PCR. The molecular characterization of TSV-GN-INDVP groundnut isolate revealed that TSV population in Indian subcontinent is highly conserved irrespective of hosts and locations based on CP gene sequences. The genetic information of TSV-GN-INDVP groundnut isolate from A.P can be used in the development of coat protein mediated resistance program in groundnut due to its highly conserved nature over the years. Overall the results on survey indicated incidence of PSND identified in coastal A.P. and control measures have to be taken to avoid epidemic occurrence on groundnut in future.

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Conflict of interest

On the behalf of all authors, I declare that, there is no conflict of interest.

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