

Characterization of the Nucleocapsid protein gene of *Groundnut bud necrosis virus* (GBNV) in Tamil Nadu and its phylogeny phylogenetic relationships

ABSTRACT

The nucleocapsid protein (*N*) gene of *Groundnut bud necrosis virus* (GBNV) associated with bud blight of tomato plants from different locations of Tamil Nadu viz., Coimbatore, Krishnagiri, Dharmapuri and Madurai were was cloned and sequenced. The *N* gene comprised of 831 nucleotides and 277 aminoacids. The nucleotide sequence identities of the present isolates of the study ranged from 97.1% to 100%. Coimbatore isolate (MK032858) shared maximum sequence identity of 100% with Dharmapuri (MK032860) and Coimbatore isolates (AY618561) of tomato. Krishnagiri isolate (MK032859) shared maximum nucleotide sequence identity of 100% with Andhra Pradesh tomato isolate (AY510133). The aminoacid sequence identities of our isolate ranged from 97.8% to 100%. Krishnagiri isolate (MK032859) shared maximum aminoacid sequence identity of 100% with Andhra Pradesh isolate (AY510133). On this basis, it is concluded that the GBNV isolates from different crops and different agro-ecological zones have not much variations in nucleotide and aminoacid levels.

Keywords: *Tospovirus*, GBNV, Nucleocapsid protein gene

1. INTRODUCTION

Tomato (*Solanum lycopersicum*) is the second most important vegetable crop. standsing next to potato, and belongs to the family *Solanaceae*. It is highly sensitive to viral diseases viz., *Tomato spotted wilt virus* (TSWV) (Brittlebank, 1919), *Tomato leaf curl New Delhi virus* (ToLCNDV) (Padidam *et al.*, 1995), *Tomato torrado virus* (Verbeek *et al.*, 2007), *Tomato marchitez virus* (Verbeek *et al.*, 2008), *Tomato infectious chlorosis virus* (Duffus *et al.*, 1996), *Tomato chlorosis virus* (Wisler *et al.*, 1998b), *Tomato necrotic spot virus* (Batuman *et al.*, 2009), *Tomato pelargonium zonate spot virus* (Gallitelli, 1982), *Tomato yellow ring spot virus* (Hassani-Mehraban *et al.*, 2005), *Capsicum chlorosis virus* (McMicheal *et al.*, 2002), *Tomato*

24 *necrotic spot virus* (Dong *et al.*, 2008), *Pepino mosaic virus* (Van der Vlugt *et al.*, 2002) and
 25 *Tomato yellow leaf curl virus* and related species (Cohen and Harpaz 1964). In the Indian
 26 subcontinent, *Groundnut bud necrosis virus* (GBNV) is one of the most destructive viruses in
 27 tomato. GBNV belongs to the family *Bunyaviridae*, composed of 19 species (Fauquet *et al.*,
 28 2005). Among them, 14 have been identified in Asia (Pappu *et al.*, 2009). Six tospoviruses
 29 have been reported from India viz., *Groundnut bud necrosis virus* (Reddy *et al.*, 1992,
 30 Sathyanarayana *et al.*, 1996a), *Groundnut yellow ringspot virus* (Sathyanarayana *et al.*,
 31 1998), *Watermelon bud necrosis virus* (Jain *et al.*, 1998), *Iris yellow spot virus* (Ravi *et al.*,
 32 2005), *Capsicum chlorosis virus* (Kunkalikar *et al.*, 2007, 2008, Krishanreddy *et al.*, 2008)
 33 and *Tomato spotted wilt virus* (Renukadevi *et al.*, 2015). Tospoviruses belong to the family
 34 Thysanoptera and are transmitted through Thrips thrips belongs to the family
 35 Thysanoptera by circulative and propagative manner (Whitfield *et al.*, 2005a). Among
 36 various tospoviruses, The GBNV particles are enveloped, quasi-spherical in shape and 80-
 37 110 nm in diameter with a tripartite single-stranded RNA virusgenome. The large (L) RNA
 38 had has a single open reading frame (ORF) and encoded encodes for RNA dependent RNA
 39 polymerase (RdRp); the M- RNA has two ORFs encoded in ambisense strategy encoded for
 40 glycoprotein precursors for in a viral complementary sense and nonstructural movement
 41 (NSm) protein in a viral sense. S- RNA also has an ambisense strategy, two ORFs encoded
 42 for Nucleocapsid protein (N) in a viral complementary sense and nonstructural small (NSs) in
 43 a viral sense.

44 Phylogenetic analysis of nucleocapsid protein gene sequences s revealed a classification
 45 of tospovirus based on serogroup s. Isolates of serogroup I (TSWV) and serogroup III (INSV)
 46 were closely related with each other. Serogroup II has been distinct from *Tomato chlorotic*
 47 *spot virus* and *Groundnut ring spot virus* (De Avila *et al.*, 1993). *Watermelon bud necrosis*
 48 *virus* (WBNV), *Groundnut bud necrosis virus* (GBNV) and *Watermelon silver mottle*
 49 *tospovirus* were related to serogroup IV. Serological classification in Tospoviruses was
 50 based on the nucleocapsid protein sequence. Bhat *et al.* (2002) explained that sequence
 51 identity in the nucleocapsid protein gene sequences from GBNV causing bud blight of
 52 soybean caused by GBNV has is been showed 98% identical to that of sequence identity
 53 in nucleocapsid protein gene sequences with other isolates of GBNV.

54 During the field survey conducted in different districts of Tamil Nadu viz., Dharmapuri,
 55 Krishnagiri, Coimbatore and Madurai, various symptoms were observed on tomato plants.
 56 Symptoms of GBNV on tomato were chlorotic and necrotic spots on young leaves, necrotic
 57 streaks on the stem, bud blight on young buds, bronzing, stunting and wilting of entire plants.
 58 The per cent GBNV disease incidence in tomato was ranged from 18% to 35% in different
 59 fields of Tamil Nadu (Suganyadevi *et al.*, 2018). Understanding the genetic diversity of

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GBNV will be helpful to develop strategies for management of bud necrosis disease of tomato. Nucleocapsid sequences are highly conserved for identification of Tospoviruses. The present study was carried out to investigate the variability of the nucleocapsid protein gene GBNV in Tamil Nadu. Nucleocapsid protein gene sequences were used for sequence analysis to study genetic diversity and phylogenetic analysis. The information on the variability of nucleocapsid protein gene may help to generate GBNV resistant transgenic plants through pathogen-derived resistance.

2. MATERIAL AND METHODS

2. 1. RNA extraction and RT- PCR analysis

Leaf samples from tomato plants showing characteristic symptoms of GBNV viz., chlorotic and necrotic spots on infected leaves, stem necrosis, bronzing, bud blight, wilting and stunting of the infected plant were collected from different parts of Tamil Nadu viz., Coimbatore, Dharmapuri, Krishnagiri, Salem and Madurai. Collected GBNV infected tomato leaves were inoculated on cowpea plant cv. CO5 under insect-proof glass house condition. Extraction of the virus was done by macerating GBNV infected fresh leaves with 0.1 M of sodium phosphate buffer pH 7.0 at the ratio of 1:2 (infected leaf tissue: buffer volume) using ice tray. The cowpea plants were used for propagating the virus since they produce characteristic local lesion symptoms within 3-4 days after inoculation. Inoculation was carried out by gentle rubbing with inoculum using the broad end of the pestle on the cotyledonary leaves of six-day-old cowpea plants previously incubated in the dark for 12 hrs and dusted with 600 mesh carborundum powder. The excess inoculum was washed with a jet of sterile distilled water using a wash bottle. The inoculated plants were kept under observation for 4-5 days for the expression of symptoms (Subramanian and Narayansamy, 1973).

The total RNAs were extracted from cowpea leaf samples (100 mg) showing local lesions of GBNV using the Trizol method by TRI reagent developed by Chomczynski and Sacchi (1987). The plant samples showing symptoms along with healthy samples were homogenized separately using liquid nitrogen. One ml of Trizol reagent (Sigma- Aldrich now Merck) was added, transferred to a 1.5 ml centrifuge tube and incubated at under room temperature for 2 min and centrifuged @ 12,000 rpm for 10 min at 4°C. The supernatant was transferred to a new 1.5 ml centrifuge tube and 250 µl of chloroform was added, mixed well and the centrifuged @ 13,000 rpm for 15 min. The aqueous layer was transferred to another new tube and 250 µl of isopropanol and 250 µl NaCl was added. Then it was incubated over the ice for 10 min and then centrifuged @ 13,000 rpm for 15 min. The supernatant was discarded and the pellets retained in the tube were washed with 75%

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ethanol. The pellets were air dried and dissolved in 30 µl of RNase free water under the sterile conditions and stored at -80°C. First strand cDNA synthesis was carried out **by** using **the** first strand cDNA synthesis kit (Thermo scientific RevertAid first strand cDNA synthesis kit, USA) as per manufacturer's instruction. The reaction was performed at 42°C for 60 min followed by incubation at 70°C for 5 min.

RT-PCR was carried out with the GBNV nucleocapsid protein gene primer pair, GK-GBNV-F (5'-ATGTCTAACGTYAAGCAGCTC-3') and GK-GBNV-R (5'-TTACAATTCCAGCGAAGGAC-3') (Nagendran *et al.*, 2018) corresponding to complete nucleocapsid protein gene of GBNV. The PCR was carried out with the master mix (Smart prime, India) in 50 µl reaction volume containing master mix- 25 µl; GK-GBNV-F- 5 µl; GK-GBNV-R- 5µl; distilled water- 10 µl; cDNA- 5 µl. The PCR setting comprised **with** an initial denaturation for 94°C for 2 min, 35 cycles of denaturation at 94°C for 1 min, annealing at 54°C for 1 min, the extension at 72°C for 1 min and a final extension of 72°C for 10 min. The RT-PCR product was then analysed on 1.2% agarose gel, stained with ethidium bromide and viewed under transilluminator.

2. 2. Cloning, sequencing and sequence analysis

The amplicon of complete nucleocapsid protein gene was purified using GenJET PCR purification kit (Thermo Fischer Scientific Inc.,) and ligated into the pGEM-T Easy vector (Promega, Madison, WI, USA) at 4°C following the manufacturer's instructions. The ligated product was transformed into *Escherichia coli* DH5α competent cells by following standard molecular biology procedures (Sambrook, 1989). Plasmid DNA was isolated from the potential recombinant clones using GenJET plasmid Midiprep kit (Thermo Fischer Scientific Inc.,) according to the manufacturer's protocol. Potential recombinant clones were identified by restriction digestion analysis using *EcoRI* enzyme. Two independent clones were sequenced in both the orientations by using universal sequencing primers M13 (forward and reverse) and sequencing was done with M/s Chromous Biotech Pvt. Ltd., Bangalore. The PCR products were sequenced and the sequence results were analysed in the BLASTn search (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>). To investigate the phylogenetic relationship among GBNV isolates of Tamil Nadu were compared with other isolates of India. Closely related nucleocapsid protein gene sequences were obtained from NCBI as mentioned in Table 1. The consensus sequences of the nucleocapsid protein gene of GBNV isolates were analysed and compared by using CLUSTAL W. The phylogenetic trees at nucleotide and aminoacid levels were constructed by using MEGA version 4.0. and Bioedit 5 software.

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3. RESULTS AND DISCUSSION

3. 1. Sequence determination and data analysis

The GBNV infected tomato leaf samples were identified by the presence of chlorotic and necrotic spots on leaves (Fig. 1) from different parts of Tamil Nadu. Symptomatic tomato leaf samples were inoculated on cow pea cv. CO5 leaves on cotyledonary leaves under insect-proof condition. Cowpea leaves exhibited chlorotic to necrotic lesions of inoculated cotyledonary leaves (Fig. 2). Though samples were collected from both Dharmapuri (DMP) and Salem (SLM) districts, they were considered one isolate as Dharmapuri (DMP) for sequencing, since these districts are very closer and have similar agro-climatic conditions. RNA extracted from symptomatic cowpea and non-symptomatic leaves were subjected for RT-PCR assay with GBNV nucleocapsid protein gene-specific primers, GK-PBNV-CP-F (5'-ATGTCTAACGTYAAGCAGCTC-3') and GK-PBNV-CP-R (5'-TTACAATTCCAGCGAAGGAC-3'). The symptomatic leaf samples resulted in the amplification of an approximately 831 bp amplicon and there was no amplification from non-symptomatic leaves (Fig. 3). The nucleocapsid protein gene sequences of four isolates were determined, analyzed and deposited in the GenBank database (accession numbers MK032858 (CBE); MK032859 (KGI); MK032860 (DMP); MK032861 (MDU)).

3.2. Comparison of sequence and Phylogenetic analysis of GBNV isolates

Pair wise sequences identity of the complete nucleocapsid protein gene among four isolates ranged from 97.1% to 100% at the nucleotide level (Table 2). Coimbatore, Dharmapuri and Krishnagiri isolates were very closely related with each other showing sequence identities from 99.1% to 100%. The Madurai isolate had similar sequence identities varied from 97.1% to 97.4% with other isolates. The nucleocapsid protein gene of GBNV isolates of Tamil Nadu was compared with the corresponding gene from known GBNV isolates from other states of India at the nucleotide sequence levels. Sequences were obtained from the National Center for Biotechnology Information (AY618561, EU373768, AY510132, AY512648, AY173043, AY882000, AY510133, GQ844886, EU373786, EF179100, EU373791, AY727923, AY618563, KX244337) and were aligned with multiple sequence alignment (BioEdit 5). The nucleotide sequence identities of the present isolates ranged from 97.1% to 100%. Coimbatore isolate (MK032858) shared maximum sequence identity of 100% with Dharmapuri (MK032860) and Coimbatore isolate (AY618561) of tomato. The same isolate shared a minimum identity with Karnataka isolate (AY173043) of tomato as 99.6%. Krishnagiri isolate (MK032859) shared maximum nucleotide sequence identity of 99.6% with Tamil Nadu isolate (AY618561) of tomato, Maharashtra isolate (EU373768) of tomato, Karnataka isolate (AY512648) of brinjal and Karnataka isolate

(AY173043) of tomato. Madurai isolate (MK032861) shared maximum nucleotide sequence identity of 98.5% with Maharashtra isolate (AY510132) of tomato. The amino acid sequence identities of our isolate ranged from 97.8% to 100%. Coimbatore isolate (MK032858) shared maximum amino acid sequence identity of 100% with Dharmapuri (MK032860) and Coimbatore isolate (AY618561) of tomato. Krishnagiri isolate (MK032859) shared maximum amino acid sequence identity of 100% with Andhra Pradesh isolate (AY510133) of tomato. Madurai isolate (MK032861) shared maximum amino acid sequence identity of 98.3% with Maharashtra isolate (AY510132) of tomato.

The GBNV nucleocapsid protein gene sequences were subjected to phylogenetic analysis, with those of closely related GBNV isolates from India and outgroup as *Tobacco streak virus* (KR017710). The GBNV isolates viz., MK032858 (CBE), MK032860 (DMP), AY618561 ([Tomatotomato](#), Coimbatore), EU373768 ([Tomatotomato](#), Maharashtra), AY512648 (Brinjal, Karnataka), AY510132 ([Tomatotomato](#), Andhra Pradesh), MK032861 (MDU), AY173043 ([Tomatotomato](#), Bangalore, Karnataka), EF179100 (Peanut, Andhra Pradesh), AY882000 ([Tomatotomato](#), Maharashtra), GQ844886 (Brinjal, Maharashtra), EU373786 ([Tomatotomato](#), Maharashtra), AY510133 ([Tomatotomato](#), Andhra Pradesh) and MK032859 (KGI) formed a single cluster.

Tomato is one of the most important vegetable crops in India. Tospovirus is one of the most destructive diseases of tomato and has been recorded in almost all of the major tomato producing countries. According to our study, [the disease incidence ranges from 18% to 35% in the](#) major tomato growing areas of Tamil Nadu [were recorded with 18%- 35% of disease incidence](#) (Suganyadevi *et al.*, 2018). GBNV isolates of Tamilnadu along with other isolates of India were grouped into a single clade. The cause of Tomato spotted wilt disease in India was reported in 1985 (Prasad Rao *et al.*, 1985). However, the presence of TSWV in India has been reported in Chrysanthemum recently by Renukadevi *et al.* (2015). Isolates in the *Tospovirus* genus with greater than 90% nucleocapsid protein (N) gene sequence identity are delineated as strains of the same virus (Moyer *et al.*, 1999). Amino acid level variability in 76 GBNV isolates infecting different vegetable crops has been studied by Kunkaliker *et al.* (2011) and they reported [as](#) 91.3% to 100% similarities. Our present isolates found 97.8% to 100% identity at the amino acid level and 97.1% to 100% identity at the nucleotide level. Nucleotide sequences of the NP gene of GBNV isolates including four tomato isolates (present study) infecting different hosts in different agro-ecological zones across the India were recorded with 97% - 100%. There were no variations in sequences of GBNV isolates either based on geographical locations or with hosts.

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Table 1. Sources and Genbank accession numbers of Nucleocapsid protein gene isolates obtained from NCBI

Accession numbers	Virus	Isolate	Source
MK032858	GBNV	Coimbatore, Tamil Nadu	Tomato
MK032859	GBNV	Krishnagiri, Tamil Nadu	Tomato
MK032860	GBNV	Dharmapuri, Tamil Nadu	Tomato
MK032861	GBNV	Madurai, Tamil Nadu	Tomato
AY618561	PBNV	Coimbatore, Tamil Nadu	Tomato
EU373768	GBNV	Jalna, Maharastra	Tomato
AY510132	PBNV	Jalna, Maharastra	Tomato
AY512648	PBNV	Rainbennur, Karnataka	Brinjal
AY173043	PBNV	Banglore, Karnataka	Tomato
AY882000	PBNV	Arvikendra, Pune	Tomato
AY510133	PBNV	Kurnool, Andhrapradesh	Tomato
AQ844886	PBNV	Jalna, Maharastra	Brinjal
EU373768	GBNV	Jalna, Maharastra	Tomato
EF179100	PBNV	Jalna, Maharastra	Peanut
EU373791	GBNV	Jalna, Maharastra	Tomato
AY727923	GBNV	Pune, Maharastra	Tomato
AY618563	PBNV	Gulburga, Karnataka	Tomato
KX244337	GBNV	Banglore, Karnataka	<i>Solanum nigrum</i>
KR017710	TSV	USA	<i>Dahlia pinnata</i>

Table 2. Per cent homology of aminoacid (below diagonally) and nucleotide sequences (above diagonally) identities of the GBNV nucleocapsid protein gene of different isolates

Seq ->	MK 032 858 (CB E)	MK 032 859 (KG I)	MK 032 860 (DM P)	MK 032 861 (MD U)	AY 618 561	EU 373 768	AY 510 132	AY 512 648	AY 173 043	AY 882 000	AY 510 133	GQ 844 886	KX 244 337
MK 032 858 (CB E)	100	99.1 0	100	97.4 0	100	100	99. 60	99. 60	99. 60	100	99. 60	99.6 0	100
MK 032 859 (KG I)	99.6 0	100	99.1	97.1 0	99. 6	99. 60	99. 20	99. 20	99. 20	99. 60	100	99.2 0	99. 60
MK 032 860 (DM P)	100	99.6	100	97.4	100	100	99. 6	99. 60	99. 60	100	99. 60	99.6 0	100
MK 032 861 (MD U)	98.1 0	97.8 0	98.1 0	100	98. 10	98. 10	98. 50	97. 80	97. 80	98. 10	97. 80	97.8 0	98. 10
AY6 185 61	100	99.1 0	100	97.4 0	100	99. 30	99. 30	99. 20	99. 20	99. 20	99. 10	99.0 0	98. 70

EU3	99.3	99	99.3	97.3	100	100	99.	99.	99.	99.	99.	99.1	98.
737	0		0	0			20	10	10	30	00	0	60
68													
AY5	99.3	99.0	99.3	98.0	99.	99.	100	99.	99.	99.	99.	98.9	98.
101	0	0	0	0	60	60		30	10	10	00	0	40
32													
AY5	99.2	99.3	99.2	97.4	99.	99.	99.	100	99.	99.	99.	98.7	98.
126	0	0	0	0	60	60	20		00	00	30	0	30
48													
AY1	99.2	98.9	99.2	97.2	99.	99.	99.	99.	100	99.	98.	98.7	98.
730	0	0	0	0	60	60	20	20		00	90	0	30
43													
AY8	99.2	98.9	99.2	97.2	100	100	99.	99.	99.	100	98.	99.2	98.
820	0	0	0	0	.00	.00	60	60	60		90	0	50
AY5	99.1	100.	99.1	97.1	99.	99.	99.	99.	99.	99.	100	98.6	98.
101	0	00	0	0	60	60	20	20	20	60		0	40
33													
GQ	99.0	98.6	99.0	97.1	99.	99.	99.	99.	99.	99.	99.	100	98.
844	0	0	0	0	60	60	20	20	20	60	20		50
886													
KX2	98.7	98.4	98.7	96.5	100	100	99.	99.	99.	100	99.	99.6	100
443	0	0	0	0	.00	.00	60	60	60	.00	60	0	
37													

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Fig. 1. Appearance of chlorotic and necrotic spots on tomato leaves

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Fig. 2: GBNV inoculated cowpea Cv. CO5 with chlorotic spots at 4 days after post inoculation

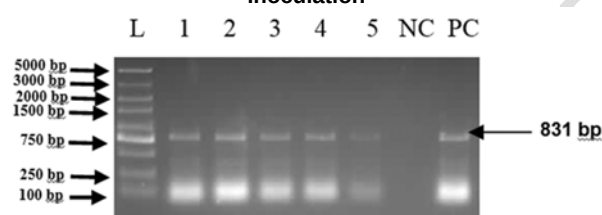


Fig. 3. RT-PCR analysis for nucleocapsid protein gene of GBNV isolates with the primer pair, GK PBNV CP F (5'-ATGTCTAACGTYAAGCAGCTC-3') and GK PBNV CP R (5'-TTACAATTCCAGCGAAG GAC-3') corresponding to the nucleocapsid protein gene of GBNV. DNA fragments of approximately 831 base pairs specific to the *N* gene of GBNV were amplified from the RNAs extracted from GBNV infected cowpea (Lane, 1-5), while no amplification was observed in RNA extracted from cowpea sample inoculated with healthy tomato (Lane, NC). L- 100bp Ladder; 1. Dharmapuri sample; 2. Coimbatore sample; 3. Krishnagiri sample; 4. Salem sample 5. Madurai sample; PC- Positive control (GBNV infected groundnut leaf)

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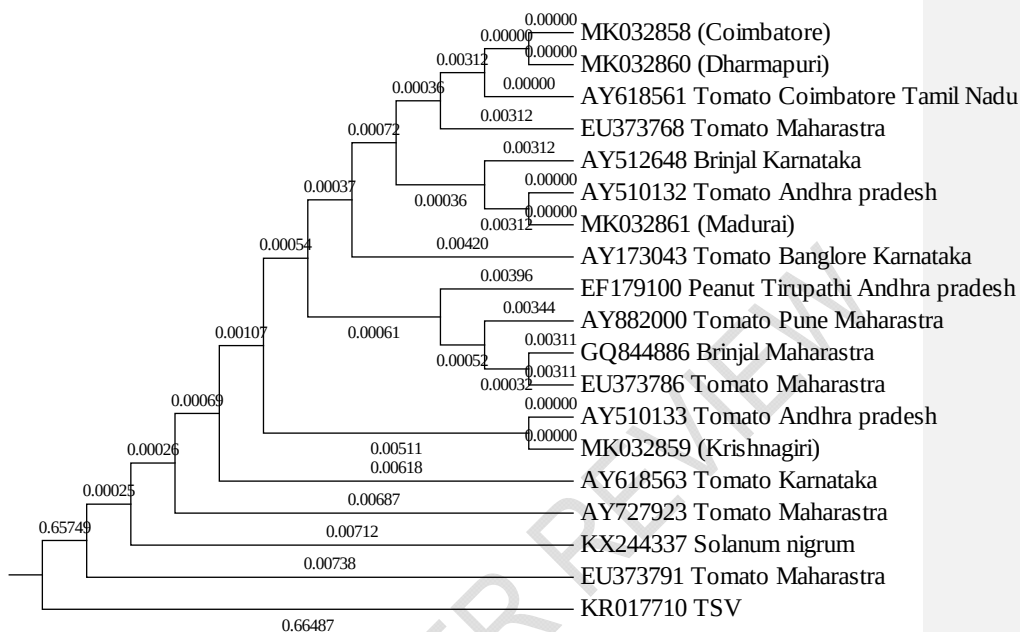


Fig. 4. Phylogenetic tree constructed using MEGA version 4.0 illustrating phylogenetic relationships based on the multiple alignments of the replicase gene sequences of different isolates of GBNV.

4. CONCLUSION

Nucleocapsid protein gene of GBNV isolates of Tamil Nadu compared with different crops and different agro-ecological zones in India have no much variation in nucleotide and aminoacid levels.

REFERENCES

- Batuman O, Miyao G, Kuo YW, Chen LF, Davis RM, Gilbertson, RL. An outbreak of a necrosis disease of tomato in California in 2008 was caused by a new ilarvirus species related to *Parietaria mottle virus*. Plant Disease. 2009;93:546.
- Bhat AI, Jain RK, Varma A, Lal, SK. 2002. Nucleocapsid protein gene sequence studies suggest that soybean bud blight is caused by a strain of *Groundnut bud necrosis virus*. Current Science. 2002;82:1389-1392.
- Brittlebank CC. Tomato diseases. Journal of Agriculture. 1919;17: 231-235
- De Avila AC, De Haan P, Kormelink R, Resende R de O, Goldbach RW and Peters D. Classification of tospoviruses based on the phylogeny of nucleoprotein gene sequences. Journal of General Virology. 1993;74:153-159.

246 Dong JH, Cheng X, Yin Y, Fang Q, Ding M, Li T, Zhang L, Su X, McBeath JH, Zhang Z.
247 Characterization of *Tomato zonate spot virus*, a new tospovirus in China. Archives of
248 Virology. 2008;**153**: 855–864.

249 Duffus JE, Liu HY, Wisler GC. Tomato infectious chlorosis virus – a new clostero-like virus
250 transmitted by *Trialeurodes vaporariorum*. European Journal of Plant Pathology.
251 1996;102: 219–226.

252 Gallitelli. Properties of a tomato isolate of *Pelargonium zonate spot virus*. Annals of Applied
253 Biology. 1982;457-466p.

254 Hassani-Mehraban A, Saaijer J, Peters D, Goldbach R, Kormelink R. A new tomato–
255 infecting tospovirus in Iran. Phytopathology. 2005;95:852–858.

256 Jain RK, Pappu HR, Pappu SS, Reddy MK, Yani A. Watermelon bud necrosis tospovirus is a
257 distinct virus species belonging to serogroup IV. Archives of Virology. 1998;143:
258 1637-1644.

259 Krishnareddy M, Usha Rani RS, Anil Kumar K, Reddy K, Pappu, H. Capsicum chlorosis virus
260 (Genus Tospovirus) Infecting Chili Pepper (*Capsicum annuum*) in India. Plant
261 Disease. 2008;92: 1469-1469. 10.1094/PDIS-92-10-1469B.

262 Kunkalikar S, Sudarsana P, Rajagopalan P, Zehr UB, Naidu RA, Kankanallu, RS. First report
263 of *Capsicum chlorosis virus* in tomato in India. Plant Health Progress. 2007.doi:
264 10.1094/PHP-2007-1204-01-BR.

265 McMichael LA, Persley DM, Thomas JE. A new tospovirus serogroup IV species infecting
266 capsicum and tomato in Queensland, Australia. Australasian Plant Pathology.
267 2002;31:231–239.

268 Padidam M, Beachy RN, Fauquet CM. *Tomato leaf curl geminivirus* from India has a
269 bipartite genome and coat protein is not essential for infectivity. Journal of general
270 virology. 1995a;76:25- 35.

271 Pappu HR, Jones RA, Jain RK. Global status of tospovirus epidemics in diverse cropping
272 systems: successes achieved and challenges ahead. Virus Research.
273 2009;141:219–236.

274 Ravi KS, Kitkaru AS, Winter S. *Iris yellow spot virus* in onions: a new tospovirus record from
275 India. New Disease Reports. 2005;11: 21.

276 Renukadevi P, Nagendran K, Nakkeeran S, Karthikeyan G, Jawaharlal M. and Pappu, H.,
277 Malathi, V. G. 2015. First Report of *Tomato spotted wilt virus* Infection of
278 Chrysanthemum in India. Plant Disease., 99. 10.1094/PDIS-01-15-0126-PDN.

- Sambrook J, Fritsch ED, Maniatis T. Molecular Cloning: A Laboratory Manual, Cold Spring Harbor: Cold Spring Harbor Laboratory Press, Plainview, NY, 1989, 2nd Ed. p. 49-55. ISBN:7-03-002808-2/Q.372 (Chinese).
- Satyanarayana T, Gowda S, Lakshminarayana Reddy K, Mitchell SE, Dawson WO, Reddy, DVR. *Peanut yellow spot virus* is a member of a new serogroup of tospovirus genus based on small (S) RNA sequence and organisation. Archives of Virology. 1998;143: 353-364.
- Satyanarayana T, Mitchell SE, Reddy DVR, Kresovich S, Jarret R, Naidu RA, Gowda S, Demski JW. The complete nucleotide sequence and genome organization of the M RNA segment of *Peanut bud necrosis tospovirus* and comparison with other tospoviruses. Journal of General Virology. 1996a;77: 2347-2352.
- Subramanian KS, Narayanasamy P. Mechanical transmission of *Whitefly borne yellow mosaic virus* of *Lablab niger* Medikus. Current Science. 1973;47:92-99.
- Suganyadevi M, Manoranjitham SK, Senthil N, Raveendran M, Karthikeyan G. Prevalence of Bud Blight of Tomato Caused by Groundnut bud necrosis virus in Tamil Nadu. International journal of current microbiology and applied sciences. 2018;7(11). <https://doi.org/10.20546/ijcmas>
- Van der Vlugt RAA, Cuperus C, Vink J, Stijger ICMM, Lesemann DE, Verhoeven, Roenhorst JW. Identification and characterization of *Pepino mosaic potexvirus* in tomato. EPPO Bulletin. 2002;32(3): 503-508.
- Verbeek M, Dullemans AM, Heuvel JFJM, Maris PC, Vlugt RAA. Tomato marchitez virus, a new plant picorna-like virus from tomato related to *Tomato torrado virus*. Archives of Virology. 2008;153:127–134.
- Verbeek M, Dullemans AM, Van den Heuvel JFJM, Maris PC, Van der Vlugt RAA. Identification and characterisation of tomato torrado virus, a new plant picorna-like virus from tomato. Archives of Virology. 2007;152: 881–890
- Whitfield AE, Ullman DE, German TL. Tospovirus-thrips interactions. Annual Review of Phytopathology. 2005;43: 459–489
- Wisler GC, Li RH, Liu HY, Lowry DS, Duffus JE. *Tomato chlorosis virus*: a new whitefly-transmitted, phloem-limited, bipartite closterovirus of tomato. Phytopathology. 1998;88, 402–409.