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# Research Article
# Serological, Molecular Characterization and Diagnostic Methods of Groundnut Bud Necrosis Virus Infecting Onion (*Allium cepa* L.) in South India

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## Abstract

**Background and Objective:** Groundnut Bud Necrosis Virus (GBNV) (family Bunyaviridae , genus *Tospovirus*) is an emerging plant viral disease. The GBNV was a very broad host range infecting many economically important crops throughout in India. So the aim of this study is to survey, screening, identification of GBNV infecting onion to know the genetic diversity and compare the sensitivity limit of ELISA, RT-PCR and IC-RT-PCR in GBNV infected onion samples. **Materials and Methods:** The straw colored, mosaic and necrotic lesions exhibiting by young leaves of onion plants were collected from different locations in South India. The disease samples (No. of samples = 73) were initially screened by DAC-ELISA by using the GBNV coat protein polyclonal antibodies. Total RNA was isolated from the positive ELISA samples and amplified with GBNV coat protein gene specific primers. Comparison of the sensitivity limit of ELISA, RT-PCR and IC-RT-PCR in GBNV infected onion samples. **Results:** In DAC-ELISA, 50 (68.49%) samples were confirmed as GBNV infected from collected onion samples (n = 73) in the field. In RT-PCR, 61 samples (83.56%) were confirmed by RT-PCR method and 68 samples (93.15%) were confirmed by IC-RT-PCR based on the coat protein gene of GBNV. The sequence analysis revealed that the coat protein gene shared 93-100 and 95-100% sequence identity with GBNV at the nucleotide and amino acid levels, respectively. The sensitivity was compared in DAC-ELISA, RT-PCR and IC-RT-PCR showed $10^{-3}$, $10^{-5}$ and $10^{-6}$ dilutions, respectively and not observed in healthy onion samples. **Conclusion:** The IC-RT-PCR was found to be more sensitive than RT-PCR and ELISA for the detection of GBNV.

Key words: Onion, cloning, GBNV, ELISA, RT-PCR, molecular characterization, IC-RT-PCR, sequence identity

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

## INTRODUCTION

Onion (*Allium cepa* L.) a member of Liliaceae is one of the most important vegetable crops. India is the second largest producer of onion with the production of 194.02 lakhs MT in the area of 12.04 lakhs ha[1]. It has several medicinal uses, including treatment for diabetes, fever, jaundice, spleen enlargement etc.[2]. The compounds such as flavonoids and phenolics in onion had properties such as anti-cancer, anti-cholesterol, anti-inflammatory and antioxidant properties.

Several viruses belong to the genera of *Allexivirus*, *Carlavirus*, *Potyvirus* and *Tospovirus* are infects the onion crop and cause severe economic losses[3]. Among them tospoviruses diseases causes great yield losses in a wide range of economically important plant species throughout the world[4]. Only serogroup VI (Iris yellow spot virus) of *Tospovirus* was infected to onion crop has been reported[5]. Now, in India the serogroup IV, groundnut bud necrosis disease also a member of *Tospovirus* was first observed in onion in the Kadapa district of Andhra Pradesh during 2011[6]. The groundnut bud necrosis virus has a wide host range infecting ornamental and vegetable crops and leads to severe yield losses[7]. It is transmitted in a persistent manner by thrips (*Thrips palmi*)[8]. The GBNV know to affect onion inducing symptoms like straw colored, mosaic, necrotic lesions on the young leaves. Necrosis starts at the apical portion of young leaves and flower stalks, eventually leading to flower abortion and plant death.

The objective of this study is to confirm the suspicions of GBNV infections of onion growing on the major areas of Andhra Pradesh, Tamil Nadu and Karnataka states in South India on the basis of immuno and nucleo-diagnosis.

## MATERIALS AND METHODS

**Virus isolates and maintenance:** The Groundnut Bud Necrosis Virus (GBNV) suspecting onion samples were collected from different places in Andhra Pradesh, Tamil Nadu and Karnataka states in South India. Naturally affected onion samples (n = 73) showing straw colored, mosaic and necrotic lesions were observed on the young leaves.

**Enzyme linked immunosorbent assay (ELISA):** The onion samples collected from the different places in South India subjected to direct antigen coating-ELISA (DAC-ELISA)[9] using specific polyclonal GBNV antiserum. The suspected leaf samples were ground in carbonate buffer (pH 9.6) at 1:10 dilution (w/v) and crude leaf extracts were used as antigens. The healthy leaf tissue extract was used as a control. Each sample extract of 100 µL was loaded into wells of ELISA plates (Nunc MaxiSorb Denmark) and incubated at 37°C for 60 min. The antigen coated plates were washed 3 times with PBS-T buffer. The plates were then blocked with blocking buffer (PBS-TPO with 5% skimmed milk powder) and incubated at 37°C for another 60 min. The plates were then washed with PBS-T as described above. The polyclonal antisera of GBNV was used as primary antibodies at 1:5000 (v/v) dilutions in antibody buffer (PBS-TPO-0.15 M NaCl in 0.1 M phosphate buffer pH 7.4, 0.05% tween 20, 2% polyvinyl pyrrolidine, 0.2% ovalbumin), incubated for 60 min at 37°C and washed 3 times with PBS-T as above. Goat antirabbit-ALP conjugate (Sigma, Germany) at 1:10000 (v/v) dilution in antibody buffer was added and incubated at 37°C for 60 min. Para-nitrophenyl phosphate (PNPP) (Sigma, Germany) was used as a substrate at 5 mg/10 mL of substrate buffer (Diethanolamine buffer, pH 9.8). Absorbance values were recorded in an ELISA plate reader (Bio-Rad USA) at 405 nm after 15-30 min of substrate addition. Antigen buffer control was included along with leaf antigen samples. The reactions were terminated using 3 N NaOH (50 µL well$^{-1}$). Positive samples with A405 values were twice or more greater than twice the value of healthy onion samples were considered as virus.

**Maintenance of GBNV pure culture:** The GBNV-ELISA positive samples were maintained in *Vigna unguiculata* (cv-C 152) cultivar as a local lesion assay host for further studies.

**Isolation of total RNA:** Total RNA from 100 mg of healthy and GBNV infected onion leaf samples was isolated using RNeasy plant Minikit according to the manufacturer's instructions (Qiagen USA) (https://www.qiagen.com/fi/shop/a-z-list/rneasy-mini-kit).

**cDNA strand synthesis:** The first strand cDNA synthesis was carried out by using 10 pmol of the GBNV-CP-reverse primer and M-MuLV-reverse transcriptase (Fermentas USA) according to the manufacturer's protocol. Six microliters of the isolated total RNA was denatured at 55-60°C for 10 min and then subjected to Reverse Transcription (RT) (Fermentas, USA) step by adding sequence specific GBNV CP-reverse primer and nuclease free water up to 12.5 µL in an RNase-free 0.2 mL PCR tubes and incubated the mix at 65°C for 5 min and chilled on ice. To the mix, 4 µL 5x reaction buffer, 2 µL 10 mM dNTP mix, 20 U ribonuclease inhibitor and 200 U of M-MuLV-reverse transcriptase (Fermentas USA) were added and incubated for

Table 1: Survey and samples collections of Groundnut Bud Necrosis Virus (GBNV) infecting onion samples

| Sample and place | No. of samples | ELISA | RT-PCR | IC-RT-PCR |
|---|---|---|---|---|
| Onion-Andhra Pradesh | 28 | 19 | 24 | 27 |
| Onion-Tamil Nadu | 22 | 14 | 17 | 20 |
| Onion-Karnataka | 23 | 17 | 20 | 21 |
| Total | 73 | 50 (68.49%) | 61 (83.56%) | 68 (93.15%) |

1 h at 42°C. The reaction was terminated by heating the reaction mix for 10 min at 70°C.

**Polymerase Chain Reaction (PCR) amplification:** The reverse transcribed cDNA (crude cDNA, 2 µL) was then subjected to PCR in a 25 µL reaction volume involving 2.5 µL of 10×PCR buffer, 2.5 mM $MgCl_2$, 0.2 mM dNTP mix (Fermentas USA), 10 pmol of each GBNV-CP-forward and reverse primers and 1 U of Taq DNA polymerase (Fermentas, USA) and the PCR mix was subjected to thermal cycling conditions of 94°C for 5 min followed by 35 cycles of 94°C for 30 sec, 56°C for 1 min and 72°C for 1 min with a final extension of 72°C for 10 min in a thermal cycler (Eppendorff, Germany).

**Immunocapture reverse transcription polymerase chain reaction (IC-RT-PCR):** The ELISA positive and healthy onion samples were used for immunocapture (IC) and further used for RT-PCR amplification. The GBNV antiserum (1:500) was diluted in coating buffer (0.05 M carbonate buffer pH 9.6) and the PCR tubes were coated with 50 µL of antiserum, incubated for 1 h at room temperature. Leaf tissue, 100 mg was homogenized in 1 mL of extraction buffer (500 mM tris-HCl pH 8.3, 10 mM $Na_2SO_3$, 3 mM $NaNO_3$, 140 mM NaCl, 2% PVP, 0.05% tween 20) and centrifuged at 5000xg for 10 min. The tubes were washed 3 times with PBS-tween (pH 7.4) with a 3 min interval between each wash. Then, 50 µL of sample supernatants were added to the PCR tubes and incubated for 1 h at room temperature. The tubes were washed three times with wash buffer and finally with sterile DEPC water.

**Comparative sensitivities of ELISA, RT-PCR and IC-RT-PCR:** To compare the sensitivity limit of ELISA, RT-PCR and IC-RT-PCR. Dilutions $10^{-1}$ to $10^{-6}$ of GBNV infected onion leaf sap were prepared by grinding buffer and total RNA up to $10^{-6}$ dilutions. Antibody coating and washing steps were performed as described earlier.

**Cloning of RT-PCR products and genome sequencing analysis:** The amplified PCR product was eluted by QIAquick gel extraction kit (Qiagen) and cloned into pTZ57R/T vector (Fermentas, USA) according to the manufacturer's instructions. The resulting ligation products were transformed into *Escherichia coli* strain DH5α cells. Recombinant clones were identified by restriction endonuclease digestion and PCR. The resulted positive clones sequenced at Eurofins Genomics India Pvt., Ltd., Bangalore. Multiple sequence alignments were generated using CLUSTAL W[10]. Sequence phylograms were constructed using TREEVIEW software (bootstrap analysis with 1000 replicates)[11]. The coat protein genes of other known tospoviruses were collected from GenBank[12]. Both nucleotide and amino acid sequences of coat protein gene of different *Tospovirus* species were compared and the corresponding phylogenetic trees were generated.

The GBNV infected onion leaf samples were collected in and around areas of Andhra Pradesh, Tamil Nadu and Karnataka states in South India and detected by ELISA, RT-PCR and IC-RT-PCR (Table 1).

## RESULTS AND DISCUSSION

The groundnut bud necrosis virus infected onion plants showed straw colored, mosaic and necrotic lesions on the young leaves were observed. The necrosis starts with the apical portion of young leaves, flower stalks and finally its results in flower abortion and the death of the plant. These infected samples were collected from different places in Andhra Pradesh (Vizag, Guntur, Kadapa), Tamil Nadu (Coimbatore, Tiruchirapalli, Perambalur) and Karnataka (Dharwad) states in South India. Based on above symptomatology, the GBNV infections were confirmed by direct antigen coating (DAC)-ELISA[9] using GBNV polyclonal antibodies. The GBNV was easily sap transmitted to cowpea (Cv-c-152), both localized and systemic symptoms were observed on cowpea plants. After 5-6 days inoculation, chlorotic lesions were initially observed on cowpea leaves, which later turned into necrotic spots, followed by veinal necrosis. These leaves turned into pale yellow in color before senescence. Emerging new leaves showed systemic symptoms which consisted of mild mosaic, chlorotic ring spots and necrotic spots. The virus-affected cowpea plants reacted with GBNV polyclonal antiserum directed against the coat protein of GBNV.

Total RNA was extracted from the infected onion tissue and subjected to RT-PCR using primers to the coat protein gene[13] resulted in an amplicon of the expected size (~830 bp) (Fig. 1). The identity of the above PCR product was cloned into

pTZ57R/T vector, confirmed by restriction digestion and sequencing. The selected clones (Onion-Vizag, Onion-Guntur, Onion-Kadapa, Onion-Coimbatore, Onion-Tiruchirapalli, Onion-Perambalur and Onion-Dharwad) were sequenced and the sequences deposited in NCBI GenBank (Accession No. JQ809456, JQ809454, JQ269832, JQ809453, JQ809457, JQ809455 and JX294486) respectively. The size of cloned GBNV coat protein gene was 831 bp and codes for 277 amino acids. The multiple sequence alignment was done to the present study isolates (BioEdit v 7.0.5) and other published GBNV sequences obtained from the national centre for biotechnology information (HQ199845, HQ324113, HQ324115, EF179100, FJ447359, EF532937, HM770020, AY184354, AY512650, DQ058078, AY882003, AF467289, AY529713, AF515821, AY512648, AY512651, AY512647, AY426317). The high coat protein sequence similarities were obtained 93-100 and 95-100% at the nucleotide and amino acid levels, respectively (Table 2). Further, phylogenetic analysis of GBNV coat protein region of present studied isolates (Onion-TN-Tiruchirapalli, Onion-TN-Perambalur, Onion-AP-Guntur, Onion-AP-Vizag, Onion-AP-Kadapa, Onion-KA-Dharwad and Onion-TN-Coimbatore) clustered with Taro-Nellore, Cotton-Tirupati, Peanut-Coimbatore, Peanut-Tirupati and Potato-Rajasthan as a separate clade (Clade-1). Tomato-Tirupati and Soybean-Delhi formed into a separate clade (Clade-2). Field bean and Tomato-Karnataka formed into a separate clade (Clade-3). Black gram-Hyderabad and Mungbean-Maharashtra formed into a separate clade (Clade-4). Carrot-Hyderabad and Peanut-Gadag formed into a separate clade (Clade-5). Chilli-Raipur isolate is formed into a separate clade (Clade-6). Jute-Tirupati isolate also formed into a separate clade (Clade-7). Cowpea-Coimbatore and Cotton-Delhi formed into a separate clade (Clade-8) (Fig. 2).

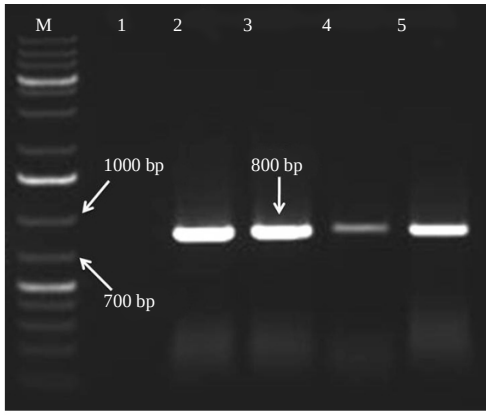


Fig. 1: Agarose gel electrophoresis of RT-PCR products, Lane M: 1 kb DNA ladder, Lane 1: Healthy onion leave sample, Lane 2-5: GBNV infected onion leave samples

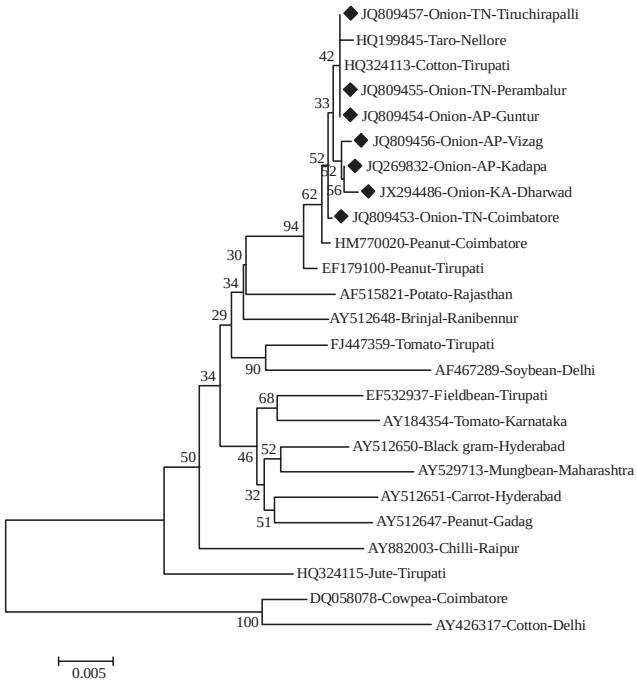


Fig. 2: Phylogenetic tree constructed using neighbor-joining method of MEGA version 4.1. The phylogenetic tree constructed based on the coat protein gene nucleotide sequences of GBNV isolates from onion with those of other reported GBNV isolates

Table 2: Sequence identities of present study isolates at amino acid (above the diagonal) and nucleotide (below the diagonal) levels, respectively with other reported GBNV isolates

| Sequences | JQ809456 | JQ809454 | JQ269832 | JQ809453 | JQ809457 | JQ809455 | JX294486 | HQ199845 | HQ324113 | HQ324115 | EF179100 | FJ447359 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| JQ809456 | 100.0 | 99.7 | 99.6 | 99.8 | 99.7 | 99.7 | 99.7 | 99.6 | 99.7 | 97.3 | 99.5 | 98.1 |
| JQ809454 | 99.6 | 100.0 | 99.6 | 99.8 | 100.0 | 100.0 | 99.7 | 99.8 | 100.0 | 97.5 | 99.5 | 98.1 |
| JQ269832 | 99.2 | 98.9 | 100.0 | 99.5 | 99.6 | 99.6 | 99.6 | 99.5 | 99.6 | 97.2 | 99.1 | 97.8 |
| JQ809453 | 99.6 | 100.0 | 98.9 | 100.0 | 99.8 | 99.8 | 99.6 | 99.7 | 99.8 | 97.4 | 99.6 | 98.3 |
| JQ809457 | 99.6 | 100.0 | 98.9 | 100.0 | 100.0 | 100.0 | 99.7 | 99.8 | 100.0 | 97.5 | 99.5 | 98.1 |
| JQ809455 | 99.6 | 100.0 | 98.9 | 100.0 | 100.0 | 100.0 | 99.7 | 99.8 | 100.0 | 97.5 | 99.5 | 98.1 |
| JX294486 | 99.6 | 99.2 | 98.9 | 99.2 | 99.2 | 99.2 | 100.0 | 99.6 | 99.7 | 97.3 | 99.2 | 97.9 |
| HQ199845 | 99.2 | 99.6 | 98.5 | 99.6 | 99.6 | 99.6 | 98.9 | 100.0 | 99.8 | 97.4 | 99.3 | 98.0 |
| HQ324113 | 99.6 | 100.0 | 98.9 | 100.0 | 100.0 | 100.0 | 99.2 | 99.6 | 100.0 | 97.5 | 99.5 | 98.1 |
| HQ324115 | 99.2 | 99.6 | 98.5 | 99.6 | 99.6 | 99.6 | 98.9 | 99.2 | 99.6 | 100.0 | 97.3 | 97.2 |
| EF179100 | 99.2 | 99.6 | 98.5 | 99.6 | 99.6 | 99.6 | 98.9 | 99.2 | 99.6 | 100.0 | 100.0 | 98.4 |
| FJ447359 | 99.2 | 99.6 | 98.5 | 99.6 | 99.6 | 99.6 | 98.9 | 99.2 | 99.6 | 100.0 | 100.0 | 100.0 |
| EF532937 | 97.8 | 98.1 | 97.4 | 98.1 | 98.1 | 98.1 | 97.4 | 97.8 | 98.1 | 98.5 | 98.5 | 98.5 |
| HM770020 | 99.6 | 100.0 | 98.9 | 100.0 | 100.0 | 100.0 | 99.2 | 99.6 | 100.0 | 99.6 | 99.6 | 99.6 |
| AY184354 | 97.8 | 98.1 | 97.1 | 98.1 | 98.1 | 98.1 | 97.4 | 97.8 | 98.1 | 98.5 | 98.5 | 98.5 |
| AY512650 | 97.8 | 98.1 | 97.1 | 98.1 | 98.1 | 98.1 | 97.4 | 97.8 | 98.1 | 98.5 | 98.5 | 98.5 |
| DQ058078 | 95.6 | 96.0 | 94.9 | 96.0 | 96.0 | 96.0 | 95.2 | 95.6 | 96.0 | 96.3 | 96.3 | 96.3 |
| AY882003 | 99.2 | 99.6 | 98.5 | 99.6 | 99.6 | 99.6 | 98.9 | 99.2 | 99.6 | 100.0 | 100.0 | 100.0 |
| AF467289 | 98.1 | 98.5 | 97.4 | 98.5 | 98.5 | 98.5 | 97.8 | 98.1 | 98.5 | 98.9 | 98.9 | 98.9 |
| AY529713 | 98.1 | 98.5 | 97.4 | 98.5 | 98.5 | 98.5 | 97.8 | 98.1 | 98.5 | 98.9 | 98.9 | 98.9 |
| AF515821 | 98.9 | 99.2 | 98.1 | 99.2 | 99.2 | 99.2 | 98.5 | 98.9 | 99.2 | 99.6 | 99.6 | 99.6 |
| AY512648 | 98.9 | 99.2 | 98.1 | 99.2 | 99.2 | 99.2 | 98.5 | 98.9 | 99.2 | 99.6 | 99.6 | 99.6 |
| AY512651 | 98.5 | 98.9 | 97.8 | 98.9 | 98.9 | 98.9 | 98.1 | 98.5 | 98.9 | 99.2 | 99.2 | 99.2 |
| AY512647 | 97.8 | 98.1 | 97.1 | 98.1 | 98.1 | 98.1 | 97.4 | 97.8 | 98.1 | 98.5 | 98.5 | 98.5 |
| AY426317 | 95.2 | 95.6 | 94.5 | 95.6 | 95.6 | 95.6 | 94.9 | 95.2 | 95.6 | 96.0 | 96.0 | 96.0 |

| Sequences | EF532937 | HM770020 | AY184354 | AY512650 | DQ058078 | AY882003 | AF467289 | AY529713 | AF515821 | AY512648 | AY512651 | AY512647 | AY426317 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| JQ809456 | 97.3 | 99.7 | 97.3 | 97.7 | 94.2 | 97.2 | 97.1 | 96.9 | 98.1 | 98.3 | 97.4 | 97.4 | 93.1 |
| JQ809454 | 97.5 | 99.7 | 97.5 | 97.7 | 94.4 | 97.2 | 97.3 | 97.2 | 98.4 | 98.3 | 97.4 | 97.4 | 93.3 |
| JQ269832 | 97.3 | 99.3 | 97.2 | 97.3 | 94.1 | 96.8 | 96.9 | 96.8 | 98.0 | 97.9 | 97.1 | 97.1 | 93.0 |
| JQ809453 | 97.4 | 99.8 | 97.4 | 97.8 | 94.3 | 97.3 | 97.2 | 97.1 | 98.3 | 98.4 | 97.5 | 97.5 | 93.2 |
| JQ809457 | 97.5 | 99.7 | 97.5 | 97.7 | 94.4 | 97.2 | 97.3 | 97.2 | 98.4 | 98.3 | 97.4 | 97.4 | 93.3 |
| JQ809455 | 97.5 | 99.7 | 97.5 | 97.7 | 94.4 | 97.2 | 97.3 | 97.2 | 98.4 | 98.3 | 97.4 | 97.4 | 93.3 |
| JX294486 | 97.3 | 99.5 | 97.3 | 97.4 | 94.2 | 96.9 | 97.1 | 96.9 | 98.1 | 98.0 | 97.2 | 97.2 | 93.1 |
| HQ199845 | 97.4 | 99.6 | 97.4 | 97.5 | 94.3 | 97.1 | 97.2 | 97.1 | 98.3 | 98.1 | 97.3 | 97.3 | 93.2 |
| HQ324113 | 97.5 | 99.7 | 97.5 | 97.7 | 94.4 | 97.2 | 97.3 | 97.2 | 98.4 | 98.3 | 97.4 | 97.4 | 93.3 |
| HQ324115 | 96.8 | 97.3 | 96.8 | 96.9 | 94.8 | 97.1 | 96.6 | 96.2 | 97.2 | 97.1 | 96.6 | 96.7 | 93.9 |
| EF179100 | 97.5 | 99.7 | 97.5 | 98.0 | 94.4 | 97.7 | 97.3 | 97.4 | 98.4 | 98.7 | 97.9 | 97.9 | 93.3 |
| FJ447359 | 97.7 | 98.1 | 97.7 | 98.0 | 94.5 | 97.2 | 97.9 | 97.1 | 98.0 | 98.1 | 97.7 | 97.8 | 93.5 |
| EF532937 | 100.0 | 97.3 | 98.1 | 98.1 | 94.7 | 96.6 | 96.8 | 97.4 | 97.7 | 97.8 | 97.8 | 97.9 | 93.8 |
| HM770020 | 98.1 | 100.0 | 97.3 | 97.8 | 94.2 | 97.4 | 97.1 | 97.2 | 98.1 | 98.5 | 97.7 | 97.7 | 93.1 |
| AY184354 | 98.1 | 98.1 | 100.0 | 98.0 | 94.5 | 96.6 | 96.8 | 97.5 | 97.7 | 97.5 | 97.7 | 97.8 | 93.7 |
| AY512650 | 98.5 | 98.1 | 98.1 | 100.0 | 94.5 | 97.1 | 96.9 | 98.1 | 97.8 | 98.0 | 98.3 | 98.4 | 93.7 |
| DQ058078 | 96.0 | 96 | 95.6 | 96.0 | 100.0 | 94.2 | 93.9 | 94.5 | 94.8 | 94.4 | 94.7 | 94.3 | 98.0 |
| AY882003 | 98.5 | 99.6 | 98.5 | 98.5 | 96.3 | 100.0 | 96.3 | 96.7 | 97.2 | 97.8 | 97.1 | 96.9 | 93.3 |
| AF467289 | 97.4 | 98.5 | 97.4 | 97.4 | 95.2 | 98.9 | 100.0 | 96.5 | 97.2 | 97.1 | 96.6 | 96.7 | 92.9 |
| AY529713 | 98.9 | 98.5 | 98.5 | 98.9 | 96.3 | 98.9 | 97.8 | 100.0 | 97.3 | 97.7 | 97.4 | 97.5 | 93.5 |
| AF515821 | 98.1 | 99.2 | 98.1 | 98.5 | 96.0 | 99.6 | 98.5 | 98.5 | 100.0 | 98.4 | 97.4 | 97.8 | 93.5 |
| AY512648 | 98.1 | 99.2 | 98.1 | 98.1 | 96.0 | 99.6 | 98.5 | 98.5 | 99.2 | 100.0 | 97.8 | 97.9 | 93.6 |
| AY512651 | 99.2 | 98.9 | 98.9 | 99.2 | 96.7 | 99.2 | 98.1 | 99.6 | 98.9 | 98.9 | 100.0 | 98.1 | 94.1 |
| AY512647 | 98.5 | 98.1 | 98.1 | 98.5 | 96.0 | 98.5 | 97.4 | 98.9 | 98.1 | 98.1 | 99.2 | 100.0 | 93.5 |
| AY426317 | 95.6 | 95.6 | 95.2 | 95.6 | 99.2 | 96.0 | 94.9 | 96.0 | 95.6 | 95.6 | 96.3 | 95.6 | 100.0 |

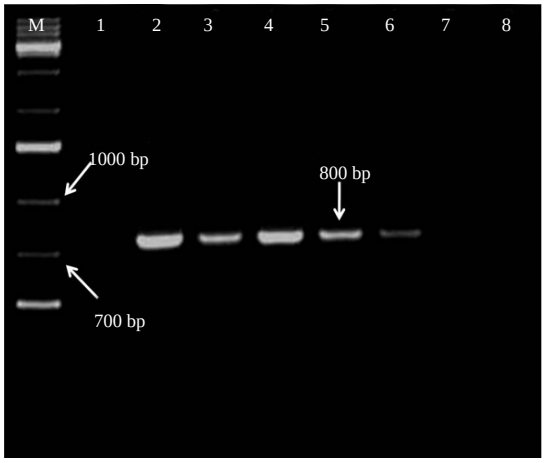


Fig. 3: RT-PCR analysis of GBNV-onion isolate associated with mosaic and necrosis disease at different dilutions of leaf extracts, Lane M: 1 kb DNA ladder, Lane 1: Healthy onion, Lane 2-8: $10^{-1}$, $10^{-2}$, $10^{-3}$, $10^{-4}$, $10^{-5}$ and $10^{-6}$ dilutions (v/v)

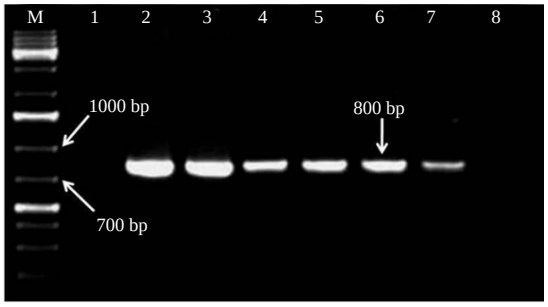


Fig. 4: IC-RT-PCR analysis of GBNV-onion isolate associated with mosaic and necrosis disease at different dilutions of leaf extracts Lane M: 1 kb DNA ladder, Lane 1: Healthy onion, Lane 2-8: $10^{-1}$, $10^{-2}$, $10^{-3}$, $10^{-4}$, $10^{-5}$ and $10^{-6}$ dilutions (v/v)

**ELISA, RT-PCR and IC-RT-PCR:** Among these collected onion samples (n = 73) from the field, 50 (68.49%) samples were confirmed as GBNV infected by direct antigen coating enzyme linked immunosorbent assay (DAC-ELISA) by using the GBNV specific antiserum, where as 61 samples (83.56%) were confirmed by RT-PCR method and 68 samples (93.15%) were confirmed by IC-RT-PCR based on the coat protein gene of GBNV.

**Comparison of ELISA, RT-PCR and IC-RT-PCR:** The relative sensitivities of DAC-ELISA, RT-PCR and IC-RT-PCR were compared by using serial dilutions of GBNV infected onion leaf sap (1:50 w/v) ($10^{-1}$ to $10^{-6}$). In ELISA, the GBNV was detected up to $10^{-3}$ dilutions by polyclonal antibodies, while GBNV was amplified in the RT-PCR up to $10^{-5}$ dilution (Fig. 3) but where as in IC-RT-PCR it was amplified up to $10^{-6}$ (Fig. 4). No amplification was observed in healthy onion samples.

Groundnut Bud Necrosis Virus (GBNV) is a broad host range and is an easily transmitted to Leguminosae, Solanaceae, Cucurbitaceae and Fabaceae members[14]. The virus properties, host range, transmission, virion morphology and antigenic relationships were initially used to identify in different tospoviruses including GBNV[15] and the classification based on these criteria is very misleading. Recently, the genetic sequences of several tospoviruses were either partially or fully sequenced and the genetic relatedness was also determined[16]. The coat protein gene of GBNV was compared with other several isolates from different crops in India[17-19]. The sequence analysis revealed that the *Tospovirus* isolates from different crops in India shared more than 95% sequence identity and thus, they have been identified as strains/isolates of GBNV. The symptomatology, transmission studies and serology were observed in groundnut[15,20]. Recently, GBNV infection was reported on jute in Chittoor district of Andhra Pradesh, India[21] and also taro in Nellore[22].

The general methods are ELISA, RT-PCR and IC-RT-PCR have been used for the detection of GBNV in different plant samples[18,23]. These variability studies of GBNV are not only useful in establishing differences among strains that infect the onion crop but will also aid in evolving transgenic plants with resistance to GBNV. The host-vector-virus relationship studies pertaining to GBNV infection in various crop species are also one area of study that needs attention. The GBNV in all the hosts is transmitted by thrips[8], detailed investigations on management of vector population and their role in transmission of disease at different time intervals are necessitated. The genetic relationships of various GBNV isolates infecting various crops in India is especially lacking and this study contributes in understanding the sequence diversity among various GBNV isolates infecting various crops in India.

## CONCLUSION

The variability studies of GBNV not only useful in establishing differences among the strains that infect different crops but also aid in evolving transgenic plants against to GBNV. The genetic relationships of various GBNV isolates infecting onion crop in South India is especially lacking and this study contributes in understanding the sequence diversity among GBNV isolates infecting onion crop in South India.

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