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RESEARCH ARTICLE

# Serological and molecular detection of Groundnut bud necrosis virus (GBNV) causing bud blight disease in tomato

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ABSTRACT

Tomato bud blight disease caused by Groundnut bud necrosis virus (GBNV) is a distinct member in the genus *Tospovirus* of the Bunyaviridae, and is the most economically important disease affecting tomato crop. Diagnosis of virus was done using two different methods, *i.e.,* Direct antigen coated-enzyme linked immunosorbent assay (DAC-ELISA) and reverse transcription-polymerase chain reaction (RT-PCR). Bud blight affected samples collected from Dharwad, Belgaum, Haveri, Bengaluru-rural and Kolar showed positive reaction with polyclonal antibodies of GBNV in DAC-ELISA and amplification of 831 bp GBNV-coat protein by RT-PCR with degenerate primers.

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## INTRODUCTION

The genus *Tospovirus* of the family *Bunyaviridae* composed of 19 species, and of them 14 have been identified from Asia (Pappu *et al*., 2009). GBNV is the most economically important virus affecting a variety of crops such as peanut, potato, tomato, soybean, urdbean, mungbean, cowpea and pea (Akram *et al*., 2004, Jain *et al*., 2007, Pappu *et al.,* 2009, Akram and Naimuddin, 2010).

In India, host range and serological studies indicated on tomato *Tospovirus* is considered as a strain of GBNV and designated as a GBNV – To and identified as the causal agent of the tomato bud blight disease (Umamaheswaran *et al.,* 2003). The disease was first observed in tomato variety marglobe in Nilgiri hills during 1964 with the ripening fruits exhibited spots with circular markings as concentric bands of red and yellow broken rings of about one cm in diameter (Todd *et al.,* 1975). The disease is characterized by bronze or purple coloured leaves, severe necrosis of buds, petioles, and pale yellow or red concentric rings turning into necrosis on fruits (Raja and Jain, 2006). In Karnataka this disease on tomato causes severe yield losses every year (Manjunatha, 2008). It is believed to be restricted to the Indian sub-continent. However, there are reports of GBNV from other parts of Asia (Thuan and Trung, 1996) and spread by polyphaguous insect vector, *Thrips palmi* (Umamaheshwaran *et al.,* 2003). An unusual disease of tomato characterized by leaf mottling and necrotic streaks on veins, shortened internodes, necrosis of terminal buds, and concentric rings on fruits was observed during surveys conducted from 2010 to 2011 in Godagari Upzila, Rajshahi district, Bangladesh (Akhter *et al.,* 2012).

## MATERIAL AND METHODS

Survey for bud blight disease was conducted during *Rabi* / summer season 2011-12. The infected samples were collected from farmers' fields in Dharwad, Haveri, Belgaum, Bengaluru - rural and Kolar districts of Karnataka and were taken for detection of GBNV by two different methods, i.e., Direct antigen coated-enzyme linked immuno sorbent assay (DAC-ELISA) and reverse transcription-polymerase chain reaction (RT-PCR).

**Source of samples :**

Five GBNV virus infected samples of the tomato plants

collected from Dharwad, Haveri, Belgaum, Bengaluru - rural and Kolar districts and one healthy sample, were tested through ELISA and RT-PCR detection. Healthy sample was maintained as control.

**Source of antiserum :**

For serological studies, polyclonal antisera of GBNV obtained from ICRISAT, Hyderabad was used for DAC-ELISA.

**DAC- ELISA detection :**

Direct antigen coating (DAC) indirect ELISA was performed as per the procedure of Hobbs *et al.* (1987). The antgen was extracted with coating buffer (100 mg of leaf sample/750 ìl buffer). It is important to mention that an antibody dilution of $10^{-1}$ was used. The ELISA absorbance at A405 nm was obtained after 20 min using an ELISA reader. Sample which gave double folds of the ELISA values of the healthy ( control) was considered as a positive as recommended by Clark and Adams (1977).

**Molecular detection GBNV by RT-PCR**

*Total RNA isolation:*

Five field samples of affected leaves of tomato were taken for RNA isolation. RNA from corresponding healthy sample was also extracted to be used as control. Total RNA was extracted using RNeasy Plant Mini Kit (Qiagen Inc., Chatsworth, CA, USA).

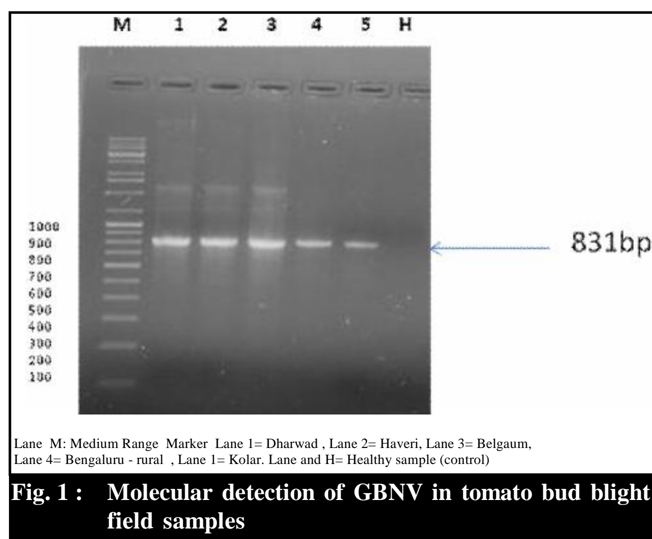
*RT-PCR for amplification of CP genes:*

cDNA was synthesized from mRNA through reverse transcriptase in a 20 ìl reaction mixture containing 5 ng of total RNA isolated from the infected and non infected samples of tomato. M-MuLV RT-Kit from Bangalore Genei was used for RT-PCR . The degenerate primers pair GBNV-CP.F5' ATGTCTAMCGTYAAGCAVCTHAMCG 3' and GBNV-CP.R5' TTACAMTTCCARMGAAGKRCHAG 3'was used for amplification of *CP* gene of GBNV. Amplification was performed in an automated Thermocycler (JH, BIO , Germany) programmed for one cycle 5 min as initial denaturation at 94ºC and 35 cycles involving 30 S of denaturation at 94ºC, 1 min annealing at 52ºC, 2 min for extension at 72ºC, followed by one cycle of final extension for 10 min at 72ºC. RT-PCR amplified products were analyzed by electrophoresis in 1per cent agarose gel at 60V for 1 h and staining with ethidium bromide.

## RESULTS AND DISCUSSION

DAC-ELISA was employed by using GBNV antiserum (polyclonal) to detect the presence of GBNV in different field samples (Table 1 and Fig. 1) . The GBNV was detected by DAC-ELISA in all the samples tested utilizing crude sap of the samples using coating buffer. However, the value of absorbance was varied with sample to sample, which may be due to variation in the concentration of the virus in the samples.

Out of five diseased field samples, all five (Table 1 & Fig.1) gave positive results in RT-PCR. RT-PCR products obtained with the primer pair GBNV-CP.F / GBNV-CP.R in agarose gel electrophoresis revealed presence of amplicons of ~ 831bp corresponding to *CP* genes of GBNV.




Lane M: Medium Range Marker Lane 1= Dharwad , Lane 2= Haveri, Lane 3= Belgaum, Lane 4= Bengaluru - rural , Lane 1= Kolar. Lane and H= Healthy sample (control)

**Fig. 1 : Molecular detection of GBNV in tomato bud blight field samples**

**Table 1 : Serological detection of GBNV in bud blight infected tomato samples**

| Tomato samples | DAC-ELISA detection | |
|---|---|---|
| | Absorbance value at 405 nm * | Result |
| Dharwad | 1.15 | + |
| Haveri | 0.959 | + |
| Belgaum | 0.946 | + |
| Bengaluru - rural | 0.850 | + |
| Kolar | 1.015 | + |
| Healthy leaf sample (control) | 0.279 | – |
| Buffer control | 0.205 | – |

* Average of four replications

Serological (ELISA) (Clark and Adams, 1977 and Hobbs *et al.,* 1987; Adam *et al.,* 1993) and molecular (RT-PCR) (Jain *et al.,* 1998; Reddy *et al.,* 2008; Akram and Naimuddin, 2012) techniques have been used to detect GBNV infecting different crops.

The serological and PCR based detection of GBNV virus suggested the association of an isolate of GBNV with bud blight disease of tomato. The results are in confirmation with earlier findings of the association of the GBNV with samples collected from Kerala (Umamaheshwaran *et al.,* 2003), Dharwad (Manjunatha , 2008), and from Pune and Rahuri (Maharashtra), Coimbatore (Tamil Nadu) and Kanpur (UP) (Raja and Jain, 2006). From the present study and previous review it can be concluded that bud blight of tomato caused by GBNV is prevalent in different parts of India, including Karnataka.

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