MECHANICAL TRANSMISSION OF GROUNDNUT BUD NECROSIS ORTHOTOSPOVIRUS, AN INDUCER OF BUD NECROSIS/ SPOTTED WILT DISEASE IN TOMATO

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Abstract

Tomato, an important vegetable crop is being prone to many viral disease, among which *Groundnut bud necrosis virus* (GBNV) causes huge yield loss. The study conducted in 2021 across diverse tomato fields in Coimbatore District, Tamil Nadu, investigated the prevalence and symptomatology of GBNV. Disease occurrence varied from 9.67% to 30.33% across different villages. Controlled experiments were conducted using infected tomato leaves for controlled sap inoculation in a glasshouse, providing insights into GBNV's distribution and symptoms. Field observations unveiled a symptom progression caused by GBNV, starting from chlorotic ring spots on leaves and developing into necrotic ring spots. Young shoots exhibited necrosis and bud withering, stems and petioles displayed necrotic streaks, and fruits showed chlorotic rings. Early infections resulted in plant wilting and stunted growth, highlighting the virus's impact. A cowpea assay demonstrated GBNV's efficient transmission (100%) and induced chlorotic and necrotic spots on leaves within 4 and 8 days post-inoculation, respectively. These findings aligned with prior research, confirming the virus's virulence and systemic spread. Pathogenicity was successfully established in tomato plants, meeting Koch's postulates. Inoculated plants exhibited symptoms akin to natural infections, including necrotic rings on leaves and stem necrotic streaks.

Symptom progression over time and systemic effects further supported GBNV's virulence.

Key words: Tomato, GBNV, Cowpea, local lesion, pathogenicity

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Introduction

Tomato (*Solanum lycopersicum* L.) is an important vegetable crop known for its nutritional value, including lycopene, vitamins A and C, minerals, and organic acids. It is the second most widely grown vegetable worldwide, with India being the second-largest producer after China. In India, tomato cultivation is prominent in states such as Tamil Nadu, Andhra Pradesh, Karnataka, Madhya Pradesh, Gujarat, Odisha, and West Bengal. However, tomatoes are vulnerable to more than 200 diseases caused by various pathogenic fungi, bacteria, viruses, and nematodes. Viral diseases, including *Tobacco mosaic virus* (TMV), *Tomato mosaic virus* (ToMV), *Tomato yellow leaf curl virus* (TYLCV), *Tomato spotted wilt virus* (TSWV), *Pepino mosaic virus* (PepMV), *Cucumber mosaic virus* (CMV), and *Tomato torrado virus* (ToTV), pose a significant threat to tomato crops worldwide. Among these viruses, bud necrosis/spotted wilt (Tospovirus), leaf curl (Begomovirus), and mosaic (Tobamovirus) have been observed to cause substantial yield losses. *Groundnut bud necrosis orthotospovirus* (GBNV) is an important tospovirus that affects both tomatoes and peanuts (Akhter *et al.*,2012). GBNV belongs to serogroup IV within the Tospovirus genus and is prevalent in several countries, including India, China, Bangladesh, Indonesia, Iran, Nepal, Pakistan, Sri Lanka, Thailand, and Vietnam (Hanssen *et al.*,2010). In India, GBNV has been reported in various states, with disease incidence ranging from 19% to 34% in tomato crops. The virus can infect multiple plant families, with major hosts including *Arachis hypogaea*, *Solanum lycopersicum*, and *Solanum tuberosum.* In tomato, bud necrosis disease can result in significant yield reductions, ranging from 80% to 100% (Suganyadevi *et al.*2018,). The disease is characterized by the development of bronze or purple-colored leaves, necrosis in buds and petioles, and the formation of concentric rings on fruits that subsequently turn into necrotic patches. The severity of the disease is influenced by factors such as the plant's age, geographical location, and season, with greater impact observed under conditions of high ambient temperatures and during rainy/post-rainy seasons. This virus is transmitted by thrips (Thysanoptera – *Thrips palmi*) in a circulative and propagative manner (Rabeena *et al.*,2019; Tamilnayagan *et al.*,2019). GBNV research provides insights into virus-host interactions, transmission mechanisms, and vector biology, offering valuable knowledge to develop sustainable solutions for managing its effects on agricultural productivity and food security.

The local lesion assay serves as a vital tool for studying virus behavior in plants, involving the targeted introduction of a virus into a defined area of a plant to observe its response. Cowpea plants are commonly chosen for such assays due to their sensitivity to a wide range of plant viruses (Basavaraj *et al.*,2018). To maintain GBNV within cowpea plants, healthy plants are initially infected through methods like mechanical inoculation or grafting with infected tissue (Salazar *et al.*,2010). Careful observation identifies plants consistently displaying characteristic GBNV symptoms, which are then propagated through regular sub-culturing onto new plants while maintaining strict isolation measures to prevent cross-contamination. This orchestrated approach facilitates the sustained presence and multiplication of GBNV, enabling researchers to gain insights into viral behavior, symptom development, and potential management strategies, ultimately contributing to the understanding and protection of agricultural systems.

Materials and methods

Survey and sample collection

A field survey was conducted in different locations of Coimbatore district, Tamil Nadu during 2022 to document the bud necrosis disease in tomato. Totally 60 plants were observed in each field at different stage of the crop and the percent disease incidence was calculated using the formula (Reddy *et al.*, 2008).

$$\textbf{Percent disease incidence} = \frac{\textbf{Number of infected plants}}{\textbf{Total number of plants observed}} \times \mathbf{100}$$

The tomato plants exhibiting symptoms of GBNV *viz.*, bronzing of leaves, chlorotic and necrotic lesions in the stem were collected from the field. About four to five symptomatic leaves were collected per plant. The samples were stored at -80°C and used for further studies.

Local lesion assay

The infected samples collected from the field was maintained in cowpea plants (*Vigna ungiculata* cv. CO 7) through mechanical inoculation, since cowpea leaves produce characteristic local lesion symptoms within 3-4 days after inoculation (Manjunatha *et al.*, 2010). The seeds of cowpea cv. CO 7 were raised in PL480 glasshouse, Department of Plant Pathology, TNAU, Coimbatore under insect-proof condition. The sap inoculation was performed in six days old cowpea plants (two leaf stage) with 0.1M phosphate buffer (pH 7.2) and 600 mesh size of carborundum powder as abrasive (Hull.,2009). The buffer was prepared with 0.1% potassium dihydrogen phosphate (KH_2Po_4), 0.1% Disodium hydrogen phosphate (Na_2HPo_4) amended with β-mercaptoethanol. Two minutes after inoculation the plants were washed with sterile water and the plants are incubated at 28-30˚C under insect-proof cage.

Pathogenicity test

One-month old healthy tomato plants were selected for the experiment. The purified GBNV obtained from cowpea plants through local lesion assay was mechanically inoculated onto the tomato plants. The virus-containing sap was gently rubbed onto the leaves using a sterilized cotton swab, ensuring uniform distribution of the virus. Inoculated tomato plants were carefully monitored under controlled greenhouse conditions. Symptom development was assessed over an incubation period of 12 days, and characteristic GBNV symptoms such as necrotic streaks, rings, and bud necrosis were recorded. Tomato plants exhibiting symptoms consistent with GBNV infection were sampled for re-isolation of the virus. Leaf tissue displaying necrotic lesions was collected, and the virus was re-isolated using previously established protocols. The re-isolated GBNV was subjected to ELISA to confirm its identity with the original GBNV isolate.

Result and discussion

Survey and sample collection

The investigation was conducted across diverse tomato fields within Coimbatore District, Tamil Nadu in 2021. Disease prevalence exhibited variation, ranging from 9.67% in Karadimadai village to a peak of 30.33% in Vandikaranur village, as indicated in Table 1. This disease manifested at various growth stages, spanning from early development to maturity. Leaves bearing symptoms such as chlorotic and necrotic ring spots were procured from the fields and employed for controlled sap inoculation experiments within a controlled glasshouse environment. This comprehensive survey offered valuable insights into the inherent distribution and symptomatology of GBNV in tomato plants.

The field survey observations unveiled a sequential progression of symptoms caused by GBNV. The initial infection led to the emergence of chlorotic ring spots on the leaves, which subsequently transformed into necrotic ring spots. Prominent infection on young shoots triggered necrosis and withering of the buds. Stems and petioles exhibited necrotic streaks, while fruits displayed the appearance of chlorotic rings. Notably, infections occurring during the early growth phases of the crop resulted in the overall wilting and stunted growth of the entire plant (Plate 1). This outcome provides crucial insights into the dynamic symptom development and distribution pattern of GBNV within the context of tomato plants.

Local lesion assay on cowpea

The virus sample collected from the field was successfully maintained through sap inoculation on cowpea (cv. CO7) plants. Cowpea plants at the two-leaf stage (7 days old) were used for inoculation. The virus transmission efficiency in cowpea was found to be 100%, indicating a highly effective spread of the virus within the plant. Upon inoculation, characteristic symptoms of viral infection became evident in the cowpea plants. Within 4 dpi, the leaves exhibited chlorotic spots, which were followed by the development of necrotic ring spots by 8 dpi (Figure.1). These observations are consistent with previous studies on the pathogenicity of GBNV (Vanthana *et al.*, 2019; Manjunatha *et al.,* 2010). Furthermore, systemic infection was observed in the virus-inoculated cowpea plants, leading to the crinkling of the top leaves. This systemic response is a common manifestation of viral infections, indicating the virus's ability to spread throughout the plant. The results align with the findings of Manjunatha *et al*. (2010), who reported that GBNV inoculation using a 0.05M phosphate buffer (pH= 7.0) containing 0.02M mercaptoethanol induced local lesions within 4-5 days in cowpea plants. This rapid onset of symptoms underscores the virulence of GBNV. In line with Vanthana *et al*. (2019), our study confirms the development of chlorotic ring spots that transformed into necrotic spots within 4-5 dpi in cowpea plants. These consistent symptom patterns emphasize the reproducibility of GBNV's effects on cowpea, highlighting its potential threat to crop production. The observed symptoms, including chlorotic and necrotic spots, as well as systemic crinkling of leaves, underscore the virulence and rapid progression of GBNV infection in cowpea. These findings contribute to the understanding of GBNV's interactions with cowpea plants and the production of local lesions.

Pathogenicity test

In this study, we successfully demonstrated the pathogenicity of GBNV on tomato plants, confirming its ability to induce symptoms that closely resemble the field manifestations of the virus. The tomato plants inoculated with GBNV exhibited necrotic rings on leaves and necrotic streaks on stems, mirroring the symptoms observed in natural infections. This alignment between experimental and field symptoms validates the accuracy of our findings.

Our study followed the Koch postulates to establish the causal relationship between GBNV and the observed symptoms in tomato plants. The sap inoculation of GBNV on tomato plants resulted in the transmission of the virus, leading to the appearance of chlorotic ring spots at 6 dpi. Notably, necrotic streaks on the stem were observed at 21 dpi (Figure.2), further confirming the pathogenicity of the virus and satisfying Koch's postulates. These observations are consistent with previous research reports, reinforcing the robustness and reliability of our findings (Balol and Patil, 2014). In line with the progression of symptoms, the virus exhibited distinct temporal patterns on tomato plants. Chlorotic spots emerged on the leaves 6 dpi , and these developed into necrotic ring spots by 10 dpi. Moreover, a systemic infection was observed, characterized by the development of bud necrosis and necrotic streaks on the stem at 21 dpi. These systemic symptoms further substantiate the virulence of GBNV and its capability to spread throughout the plant. Our study's findings are in agreement with previous research, confirming the ability of GBNV to cause symptomatic infections in tomato plants.

In conclusion, our study adds to the growing body of evidence regarding GBNV's pathogenicity. By successfully transmitting the virus onto tomato plants and observing the development of characteristic symptoms, including necrotic rings on leaves and stem, we have reinforced the link between GBNV and the observed field symptoms. The progression of symptoms over time, as well as the successful transmission to cowpea plants, further validate our results and provide valuable insights into the behavior of GBNV in different host species.

Conclusion

In this study, the prevalence and symptomatology of GBNV were comprehensively investigated across tomato fields in Coimbatore District. The disease exhibited varying prevalence levels and manifested at different growth stages, causing a range of symptoms including chlorotic and necrotic ring spots. Controlled experiments validated the sequential progression of GBNV symptoms in tomato plants, offering insights into symptom dynamics. Sap inoculation experiments on cowpea plants confirmed GBNV's ability to induce characteristic symptoms, thereby reinforcing its pathogenicity. These results underscore the potential impact of GBNV on crop production and contribute to our understanding of its behavior within different host species.
Overall, this study sheds light on the distribution, symptomatology, and pathogenicity of GBNV, offering valuable insights for effective management strategies to mitigate its impact on agricultural systems**.**

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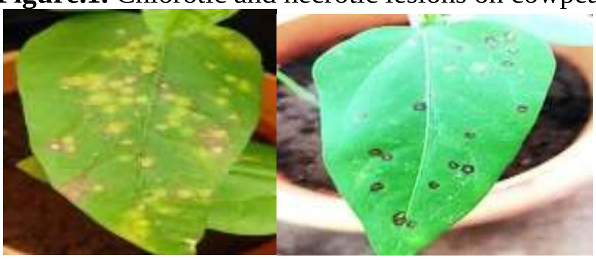
Table.1. *Groundnut bud necrosis virus* incidence in Coimbatore district, Tamil Nadu

Sl.No	Location (Coimbatore)	Variety	Stage of crop	Symptoms	Percent disease incidence
1.	Vandikaranur	Local variety	Vegetative stage	Chlorotic ring spots on leaves	30.33 %
2.	Madampetti	Shivam	Flowering stage	Bud necrosis	27.67 %
3.	Karadimadai	Local variety	Vegetative stage	Chlorotic ringspots	9.67 %
4.	Thenkarai	Shivam	First picking stage	Stem necrosis, bud necrosis	15.33 %
5.	Kuppanur	Shivam	Fruit formation stage	Necrotic ring spots and drying of leaves	21 %
6.	Narasipuram	Local variety	Vegetative stage	Bud necrosis	18.33
7.	Thondamuthur	Local variety	Flowering stage	Chlorotic ringspots	11.67
8.	Devarayapuram	Local variety	Vegetative stage	Stem necrosis, bud necrosis	26.67
9.	Sadivayal	Shivam	Fruit formation stage	Necrotic ring spots and drying of leaves	33.33
10.	TNAU Orchard	CO-3	Vegetative stage	Necrotic ring spots on leaves	12.67 %

Figure.1. Chlorotic and necrotic lesions on cowpea



a) Chlorotic ring spots on 4 days after inoculation,
b) Necrotic ring spots on 8 days after inoculation

Figure.2. Necrotic spots on tomato leaves and necrotic streaks on tomato stem

Symptoms on tomato plants: a) chlorotic and necrotic spots on leaf at 7-10 dpi, (c) Sunken necrotic streaks on stem and leaf petioles at 14–15 dpi