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Survey, serological and molecular detection of *Groundnut bud necrosis virus* (*Orthotospovirus arachinecrosis*) infecting blackgram (*Vigna mungo* (L.) Hepper) in major Blackgram growing districts of Andhra Pradesh

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Abstract
Groundnut bud necrosis virus (Orthotospovirus arachinecrosis) is a major thrips-transmitted constraint to blackgram production. A roving survey conducted during *rabi* 2025–2026 in five major blackgram-growing districts of Andhra Pradesh recorded disease incidence ranging from 10.0 to 45.0%, with the highest incidence in Guntur (45.0%). Characteristic symptoms were confirmed by mechanical sap transmission, DAC-ELISA and RT-PCR, which amplified an ~800 bp nucleocapsid (N) gene fragment. Sequence analysis of the Tirupati isolate (PZ766619) showed 99.64% identity with the Anantapur GBNV isolate and 98.44% with the *Peanut bud necrosis virus* Hyacinth bean isolate, confirming GBNV and providing authenticated inoculum for resistance screening and integrated disease management.

Keywords: Blackgram, *Groundnut bud necrosis virus*, DAC-ELISA, RT-PCR, nucleocapsid gene, survey

Introduction
Blackgram (*Vigna mungo* (L.) Hepper), commonly known as urd bean or mash bean, is one of the most important pulse crops cultivated in tropical and subtropical regions of Asia, particularly in India. It belongs to the family *Fabaceae* (subfamily *Papilionoideae*) and is believed to have originated in the Indian subcontinent. It is widely cultivated because of its high nutritional value. The seeds contain approximately 24-26 per cent protein, 55-60 per cent carbohydrates, 1.0-1.5 per cent fat, 3-5 per cent dietary fibre and appreciable amounts of essential minerals such as calcium, phosphorus, iron, magnesium and potassium (Jegadeesan *et al.,* 2021). India is the world's largest producer and consumer of blackgram, contributing more than 70 per cent of the global production (Singh *et al.,* 2019). According to the Government of India, blackgram production during 2024-25 was estimated at approximately 21.06 lakh tonnes (Third Advance Estimates), while Andhra Pradesh emerged as the leading blackgram producing state with an estimated production of about 3.62 lakh tonnes. Among the various biotic and abiotic factors limiting yield in blackgram, viral diseases such as *Yellow mosaic virus* (YMV), *Groundnut bud necrosis virus* (*Orthotospovirus arachinecrosis*) (GBNV), and *Urdbean leaf crinkle virus* (ULCV) are responsible for major production losses.

GBNV belonging to the family Tospoviridae has emerged as a serious threat, causing leaf and bud necrosis in blackgram (Divyamani *et al.,* 2024) [4] and is transmitted by *Thrips palmi* in a persistent propagative manner. (Rajasekhar *et al.,* 2024) and it was considered to be a major threat, causing 40% yield loss (Nene, 1972) The earliest symptoms of GBNV appear as chlorotic spots along the lateral veins of young leaves. As the disease progresses, leaves exhibit downward curling, twisting and distortion, followed by veinal necrosis. Infected leaves become brittle, and severe infection leads to petiole necrosis, terminal bud necrosis, stunting and premature defoliation. Reddish-brown necrotic streaks are commonly observed on the veins, petioles and stems, indicating systemic virus infection (Divyamani *et al.,* 2024) [4]. Comprehensive studies integrating field survey with serological and molecular confirmation of GBNV are limited. Therefore, the present study was undertaken to assess the incidence and distribution of GBNV in major blackgram-growing districts of Andhra Pradesh, confirm the virus through DAC-ELISA and RT-PCR, and molecularly characterize the GBNV isolate based on the nucleocapsid (N) gene sequence.

Materials and Methods
1. Roving survey for the incidence of *Groundnut bud necrosis virus* (*Orthotospovirus arachinecrosis*) (GBNV) in blackgram from the major blackgram growing districts of Andhra Pradesh during *rabi,* 2025-2026.
Bud necrosis disease of infected blackgram plant tissues (leaves, petioles and stems) were collected from major blackgram growing districts of Andhra Pradesh, Guntur, Krishna, Tirupati, Kurnool, and YSR Kadapa during the year *rabi,* 2025-2026 to assess the incidence of GBNV. Young leaves of blackgram infected with necrosis disease exhibiting clear symptoms like veinal and petiole necrosis, chlorotic spots, necrosis of bud and plants with stunted growth were collected and maintained on cowpea (Pusa komal*)* through artificial sap inoculation (Divyamani *et al.*,2024) [4]. The samples were brought to the laboratory in labelled air tight polythene bags, confirmation of the presence of GBNV from infected samples from survey and artificial sap inoculated cowpea samples was carried out by DAC-ELISA and RT-PCR (Thein *et al*., 2003; Kundu *et al*., 2015) [7]

$$\text{Percent Disease Incidence} = \frac{\text{Number of diseased plants}}{\text{Total no. of plants observed}} \times 100$$

$$\text{Thrips damage (\%)} = \frac{\text{Number of thrips damaged leaves}}{\text{Total no. of leaves}} \times 100$$

Data collected during the survey included district, mandal, village, geographic coordinates, stage of crop, variety, thrips damage (%), symptoms and disease incidence (%) and other viral diseases.

2. Sap Inoculation and Pathogenicity Test

Symptomatic blackgram plants exhibiting typical GBNV necrosis symptoms collected during the survey were used as the inoculum source for virus establishment on cowpea (*Vigna unguiculata* cv. Pusa Komal) through mechanical sap inoculation. Healthy cowpea seedlings were raised under insect-proof glasshouse conditions and inoculated at the cotyledonary stage. Virus inoculum was prepared by homogenizing infected leaf tissue in chilled 0.01 M potassium phosphate buffer (pH 7.0) containing 0.1% β-mercaptoethanol. Cotyledonary leaves were dusted with celite (diatomaceous earth) before inoculation, after which the sap was gently rubbed onto the leaf surface. Inoculated leaves were rinsed with sterile distilled water, and plants were maintained under protected conditions for symptom development.

3. Detection of GBNV by direct antigen coating–enzyme-linked immunosorbent assay (DAC-ELISA)

Symptomatic blackgram leaf samples collected during the roving survey and artificially sap inoculated cowpea samples were tested for the presence of GBNV using the DAC-ELISA following the standard protocol. Leaf tissues were homogenized in coating buffer and incubated in ELISA plates. After blocking, GBNV-specific polyclonal antiserum from ICRISAT, Hyderabad was added, followed by alkaline phosphatase-conjugated secondary antibody. The substrate *p*-nitrophenyl phosphate (pNPP) was then added, and absorbance was recorded at 405 nm using an ELISA microplate reader. Samples exhibiting optical density (OD) values greater than twice that of the healthy control were considered positive for GBNV infection.

4. Detection of GBNV by reverse transcription-polymerase chain reaction (RT-PCR)

4.1 RNA Isolation and cDNA Synthesis

Total RNA was extracted from GBNV-infected cowpea (*Vigna unguiculata* cv Pusa komal) leaves using the GeneJET RNA Purification Kit (Thermo Scientific, USA) following the manufacturer's protocol. RNA quality and quantity were assessed using a NanoDrop spectrophotometer, and samples with an A260/A280 ratio of 2.0–2.2 were used for cDNA synthesis. First-strand cDNA was synthesized from 1500 ng of total RNA using the RevertAid H Minus Reverse Transcriptase Kit (Thermo Scientific, USA) with random hexamer primers according to the manufacturer's instructions. The synthesized cDNA was subsequently used as the template for RT-PCR amplification of the nucleocapsid (N) gene of *Groundnut bud necrosis orthotospovirus* (GBNV).

4.2 RT-PCR Amplification of the GBNV Nucleocapsid (N) Gene

Molecular confirmation of GBNV was carried out by reverse transcription-polymerase chain reaction (RT-PCR) using GBNV-specific primers targeting the nucleocapsid (N) gene
(forward,5'ATCGATCATATGTCTAACGTCAAGCAACT C-3'; and reverse, 5'-
CTAGGGATCCTTACAATTCCAGCGAAGGACC-3')
PCR amplification was performed in a 25 µl reaction mixture containing 10× PCR buffer, $MgCl_2$, dNTPs, forward and reverse primers, Taq DNA polymerase, and 100 ng of cDNA template. Amplification was carried out in an Applied Biosystems ProFlex PCR System with an initial denaturation at 94°C for 5 min, followed by 35 cycles of denaturation at 94°C for 45 s, annealing at 49°C for 45 s, and extension at 72°C for 1 min, with a final extension at 72°C for 10 min. The amplified products were resolved on 1 % agarose gel stained with ethidium bromide and visualized using a gel documentation system to confirm the expected amplicon size.

4.3 Sequence Analysis

The purified RT-PCR amplicons of the GBNV nucleocapsid (N) gene were commercially sequenced (Eurofins Genomics India Pvt. Ltd., Bengaluru, India). The obtained nucleotide sequences were assembled, edited and aligned using BioEdit software (Hall, 1999). Sequence similarity was determined by comparing the generated sequence with available GBNV sequences in the NCBI GenBank database using the BLASTn program, and the sequence was submitted to GenBank to obtain an accession number.

Results and Discussion

1. Occurrence, Distribution and Serological and molecular detection of *Groundnut bud necrosis virus* (*Orthotospovirus arachinecrosis*) (GBNV)

A roving survey was conducted during *rabi* 2025-2026 in five major blackgram-growing districts of Andhra Pradesh, namely Guntur, Tirupati, YSR Kadapa, Krishna and Kurnool, to assess the occurrence and distribution of GBNV. Disease incidence, thrips damage, crop growth stage and symptom expression were recorded from representative villages. Disease incidence varied from 10.0 to 45.0%, with the highest mean incidence recorded in Guntur (45.0%), followed by Tirupati (41.0%), YSR Kadapa (35.0%), Kurnool (14.0%) and Krishna (10.0%). The highest disease incidence (45.0%) was observed in LBG-623 at Lam village, Guntur, during the flowering stage and was associated with the highest thrips damage (60.0%). In Tirupati, maximum disease incidence (41.0%) was recorded in LBG-623, whereas the highest thrips damage (40.5%) was observed in TBG-104. YSR Kadapa recorded a maximum disease incidence of 35.0% in LBG-645 with 45.0% thrips damage. In contrast, Krishna (10.0–20.5%) and Kurnool (14.0–19.0%) exhibited comparatively lower disease incidence and thrips infestation (Table 1, Fig. 1).

Table 1: Incidence of Groundnut bud necrosis virus (Orthotospovirus arachinecrosis) (GBNV) in major black gram growing districts of Andhra Pradesh. *Rabi*,2025-2026

S. No	District	Mandal	Village	Co-ordinates	Stage of crop	Variety	Symptoms	Thrips damage (%)	Disease incidence of GBNV (%)	Other viral symptoms
1	Tirupati	Tirupati	Peruru	N 13° 36' 51" E 79° 19' 26"	Vegetative	TBG-104	M.C	45.0	40.5	L.C
			RARS, Tirupati	N 13° 31' 34" E 79° 22' 25"	Vegetative	LBG-623	B.N, M.C	30.5	41.0	L.C
		Pichhatur	Ramapuram	N 13° 21' 43" E 79° 46' 20"	Pre flowering	LBG-903	B.N, M.C,	8.50	15.3	L.C
			Pannuru	N 13° 21' 33" E 79° 46' 21"	Pre flowering	LBG-934	P.N, M.C	9.0	13.2	L.C
2	YSR Kadapa	Kadapa	Utukuru	N 14° 26' 11" E 78° 48' 27"	Pre flowering	LBG-645	V.N, P. N	45.0	35.0	L.C
			Kottapalle	N 14° 31' 04" E 78° 37' 24"	Pre flowering	TBG-129	M.C	33.0	22.5	L.C
		Kamalapuram	Dadireddy palli	N 14° 31' 51" E 78° 37' 17"	Pre flowering	T9	M.C, V. N	26.0	30.5	-
			Thollagangapalle	N 14° 32' 08" E 78° 45' 24"	Flowering	TBG-129	V.N	25.0	20.0	-
3	Krishna	Pamarru	Kothapet	N 16° 19' 35" E 80° 56' 56"	Pre flowering	LBG-752	V.N, P. N	11.0	10.0	-
			Komaravolu	N 16° 22' 30" E 80° 59' 00"	Pre flowering	LBG-782	M.C, V.N, P. N	10.5	12.0	-
		Vuyyuru	Vuyyuru	N 16° 23' 08" E 80° 49' 33"	Vegetative	G9	M.C, V. N	9.0	13.0	L.C
			Chinaogirala	N 16° 22' 34" E 80° 49' 14"	Pre flowering	Thuttu muntha	M.C, V.N, P. N	7.5	20.5	L.C
4	Guntur	Tadikonda	Lam	N 16° 21' 42" E 80° 25' 52"	flowering	LBG-623	V.N, P. N	60.0	45.0	Y.M. V
			Mothadaka	N 16° 28' 26" E 80° 24' 19"	Pre flowering	LBG-782	M.C, V.N, P. N	45.0	31.5	Y.M. V, L.C
		Pedakakani	Pedakakani	N 16° 20' 06" E 80° 29' 42"	Vegetative	LBG-685	V.N, M.C	15.1	22.5	Y.M. V
			Takkellapadu	N 16° 18' 40" E 80° 29' 28"	Pre flowering	LBG-683	M.C, V.N, P. N	18.5	30.5	Y.M. V
5	Kurnool	Krishnagiri	Krishnagiri	N 15° 33' 31" E 77° 48' 53"	Pre flowering	PU-31	M.C	7.2	15.0	-
			Chityala	N 15° 28' 47" E 77° 46' 52"	Pod formation	TAU-1	P. N	6.0	19.0	-
		Aluru	Aluru	N 15° 24' 39" E 77° 14' 07"	Pre flowering	TAU-1	M.C	4.5	18.0	-
			Molagavalli	N 15° 22' 37" E 77° 18' 52"	Vegetative	PU-31	V.N, P. N	7.8	14.0	L.C

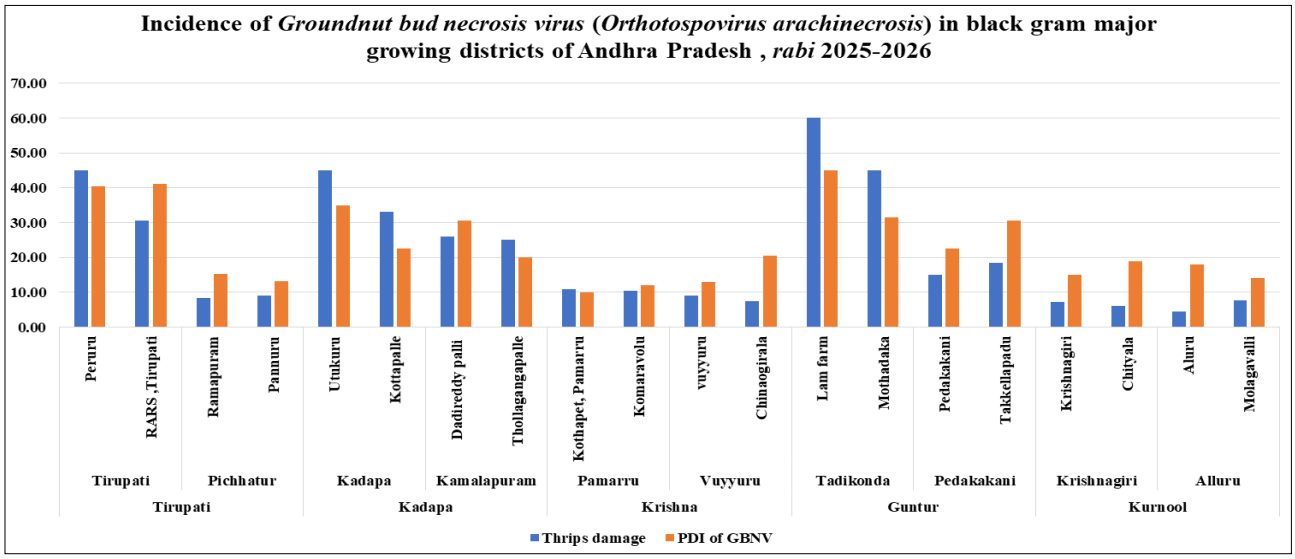

Fig 1: Graphical representation of incidence of Groundnut bud necrosis virus (Orthotospovirus arachinecrosis) of black gram in major growing districts of Andhra Pradesh, rabi 2025-2026

Symptomatic plants consistently exhibited mild chlorosis, chlorotic spots, veinal necrosis, petiole necrosis, leaf crinkle and bud necrosis, with symptom severity varying among cultivars and crop growth stages. Severe symptoms were predominantly observed in susceptible cultivars LBG-623 and LBG-645. A strong positive association between thrips damage and disease incidence indicated the important role of thrips in GBNV epidemiology.

The present findings are in agreement with Suganyadevi *et al*. (2018), who reported similar symptomatology; Rajasekhar *et al.* (2021) [12], who documented higher disease incidence in Guntur and lower incidence in Krishna; and Divyamani *et al*. (2024) [4], who confirmed the association of GBNV with the bud necrosis disease complex of blackgram. The survey confirmed the widespread occurrence of GBNV in the major blackgram-growing regions of Andhra Pradesh and highlighted the influence of vector abundance, cultivar susceptibility and agro-climatic conditions on disease distribution, emphasizing the need for continuous surveillance and integrated thrips management.

2. Bio assay

GBNV symptomatic blackgram samples collected during survey were used for mechanical sap inoculation studies on cowpea (Pusa Komal) as diagnostic host for GBNV infection, the inoculated plants were observed for symptoms development. Local lesions that developed 4-5 days post inoculation were used for further inoculation by taking single lesions and subsequently maintained pure virus cultures on cowpea for artificial screening of blackgram genotypes under protected glasshouse conditions.

3. Confirmation of *Groundnut bud necrosis virus* (*Orthotospovirus arachinecrosis*) (GBNV) in field surveyed blackgram and artificially sap inoculated cowpea samples by DAC-ELISA

Symptomatic blackgram leaf samples collected during the roving survey and artificially sap inoculated cowpea samples were subjected to DAC-ELISA for the serological confirmation of GBNV. Field samples exhibiting characteristic symptoms such as chlorotic spots, veinal necrosis, petiole necrosis and bud necrosis were collected from blackgram plants. Similarly, sap-inoculated cowpea plants showing chlorotic spots, necrotic spots, veinal chlorosis, marginal chlorosis and mixed chlorotic and necrotic lesions were also tested for GBNV infection (Fig 2). The presence of GBNV was confirmed based on the absorbance values recorded at 405 nm (A_{405}). Samples exhibiting optical density (OD) values greater than twice those of the healthy control were considered positive for GBNV infection. The DAC-ELISA results confirmed the presence of GBNV in both the field surveyed blackgram samples and the artificially sap-inoculated cowpea samples (Table 2).

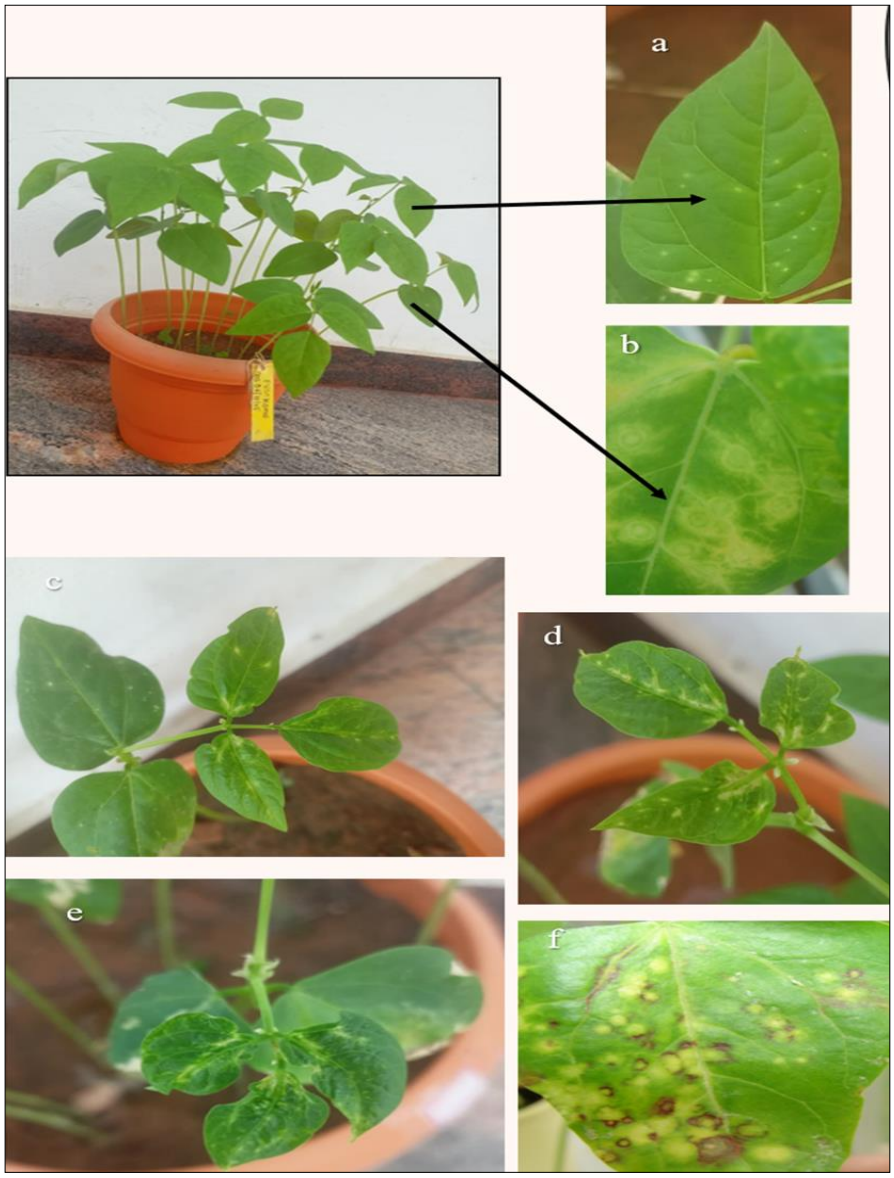

Fig 2: Visual symptoms of artificial sap inoculated cowpea showing a) Chlorotic spots b) Chlorotic concentric rings c) Mid rib chlorosis d) Veinal chlorosis e) Marginal chlorosis f) Mixed symptoms of chlorotic and necrotic spots

Table 2: Serological confirmation of Groundnut bud necrosis virus (Orthotospovirus arachinecrosis) (GBNV) in symptomatic blackgram samples using ELISA

S. No	Infected samples	OD Values at 405 nm		Reaction
		1 h	2 h	
1	Blackgram veinal necrosis	2.5	3.1	Positive
2	Blackgram petiole necrosis	2.7	3.4	Positive
3	Blackgram bud necrosis	2.7	3.3	Positive
4	Blackgram petiole necrosis	2.5	3.2	Positive
5	Blackgram small leaves with chlorotic patch at mid rib region	2.2	2.9	Positive
6	Blackgram small leaves with necrotic patch at mid rib region	2.9	3.1	Positive
7	Blackgram old leaves with chlorotic spots at mid rib region	2.4	3.0	Positive
8	Blackgram old leaves necrotic patch on mid rib region	2.8	3.1	Positive
9	Blackgram small leaves with chlorotic spots on leaves	2.7	2.9	Positive
10	Blackgram small leaves with necrotic spots on leaves	3.0	3.3	Positive
11	Blackgram mixed infection of chlorotic and necrotic spots	2.4	2.8	Positive
12	Chlorotic symptoms of cowpea	2.0	3.1	Positive
13	Necrotic spots symptoms of cowpea	1.7	3.0	Positive
14	Veinal necrosis symptoms of cowpea	2.1	3.3	Positive
15	Veinal chlorosis symptoms of cowpea	2.4	3.0	Positive
16	Mixed infection chlorotic and necrotic spots	2.2	3.2	Positive
17	Healthy plant sample	0.5	1.2	Negative

The serological confirmation of GBNV in symptomatic blackgram samples through DAC-ELISA in the present investigation is in agreement with the earlier reports. Prasad Rao *et al.* (2003) reported the successful detection of GBNV in symptomatic blackgram and mungbean samples collected from different pulse growing districts of Telangana using DAC-ELISA, confirming the widespread occurrence of the virus. Similarly, Sowmya Lakshmi and Manoj Kumar (2017) [8] identified GBNV as the predominant viral pathogen associated with necrosis disease in blackgram from Guntur district of Andhra Pradesh through DAC-ELISA, while a few samples were found to be infected with TSV. Further, Radha *et al.* (2018) [11] also confirmed the presence of GBNV in symptomatic groundnut samples like chlorotic spots, necrotic rings, stunting and wilting from YSR Kadapa district through DAC-ELISA. The agreement between the present findings and these earlier reports substantiates the reliability of The serological confirmation of GBNV in symptomatic blackgram samples through DAC-ELISA in the present investigation is in agreement with the earlier reports. Prasad Rao *et al.* (2003) reported the successful detection of GBNV in symptomatic blackgram and mungbean samples collected from different pulse growing districts of Telangana using DAC-ELISA, confirming the widespread occurrence of the virus. Similarly, Sowmya Lakshmi and Manoj Kumar (2017) [8] identified GBNV as the predominant viral pathogen associated with necrosis disease in blackgram from Guntur district of Andhra Pradesh through DAC-ELISA, while a few samples were found to be infected with TSV. Further, Radha *et al.* (2018) [11] also confirmed the presence of GBNV in symptomatic groundnut samples like chlorotic spots, necrotic rings, stunting and wilting from YSR Kadapa district through DAC-ELISA. The agreement between the present findings and these earlier reports substantiates the reliability of DAC-ELISA as a rapid and dependable serological technique for the confirmation of GBNV in symptomatic field samples.

4. Confirmation of presence of *Groundnut bud necrosis virus* (*Orthotospovirus arachinecrosis*) (GBNV) in artificially sap inoculated cowpea samples by RT-PCR.

Artificially sap inoculated cowpea symptomatic samples *viz.,* chlorotic spots, necrotic spots, veinal chlorosis, marginal chlorosis and mixed infection of chlorotic and necrotic spots of cowpea were subjected to molecular confirmation, for these set primers specific to GBNV and nucleocapsid protein (N) gene from GBNV Tirupati isolates were amplified and amplicons of expected size (~ 800 bp) observed (Fig 3). The nucleotide sequence of nucleocapsid protein of GBNV Tirupati isolate with accession number PZ766619 showing 99.64% identity with GBNV of Anantapur isolate (FJ355951.1), PBNV from Hyacinth bean isolate (AY882004.1) with 98.44 per cent.

These findings are in agreement with the reports of Jyothirmai Madhavi and Ahamed (2023), who observed high nucleotide (92.3-99.3%) and amino acid (93.4-100%) sequence identity among GBNV isolates infecting blackgram. The present GBNV isolate showed high nucleotide sequence similarity with the Anantapur isolate, indicating that the virus isolates are closely related. The amplification of the expected 800 bp fragment and the sequence similarity observed in the present study are in agreement with the findings of Divyamani *et al.* (2024) [4].

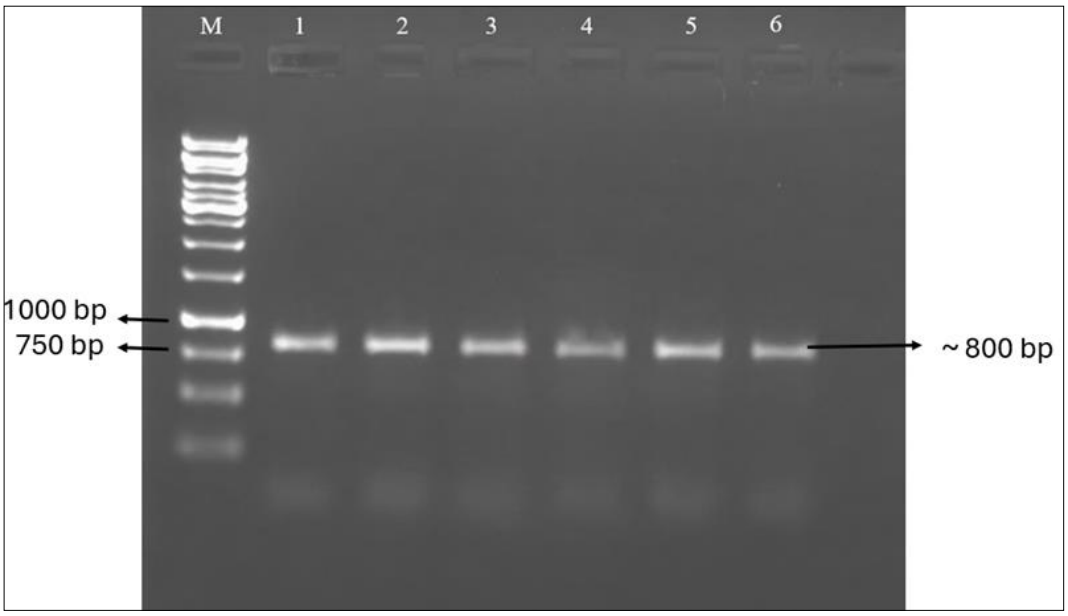

Fig 3: Confirmation of RT-PCR products with GBNV-CP in infected all artificial sap inoculated cowpea samples using GBNV specific primers in 1% agarose gel.
Lane M: marker, Lane 1 to 6: RT-PCR products with GBNV-CP in infected cowpea samples.

Conclusion

A comprehensive roving survey conducted during *Rabi* 2025-2026 in the major blackgram-growing districts of Andhra Pradesh (Tirupati, YSR Kadapa, Kurnool, Krishna and Guntur) revealed the occurrence of *Groundnut bud necrosis orthotospovirus* (GBNV), with variation in disease incidence among districts. The highest mean disease incidence was recorded in Guntur (45.0%), followed by Tirupati (41.0%), YSR Kadapa (35.0%), Kurnool (19.0%) and Krishna (10.0%). Disease incidence ranged from 22.5–45.0% in Guntur, 13.2–41.0% in Tirupati, 20.0–35.0% in YSR Kadapa, 10.0–20.5% in Krishna and 14.0–19.0% in Kurnool. The highest disease incidence (45.0%) was observed in LBG-623 at Lam, Guntur, and was associated with the highest thrips damage (60.0%), indicating a strong relationship between vector abundance and disease incidence under favourable environmental conditions and continuous cultivation of susceptible cultivars. Infected plants exhibited characteristic symptoms including mild chlorosis, chlorotic spots, veinal necrosis, petiole necrosis, bud necrosis, leaf crinkle and severe necrosis, with symptom expression varying according to crop growth stage and cultivar susceptibility. Mechanical sap inoculation of symptomatic blackgram samples onto cowpea (*Vigna unguiculata* cv. Pusa Komal) produced characteristic local lesions within 4–5 days after inoculation, confirming the infectivity of the virus isolates. Serological detection by direct antigen coating–enzyme-linked immunosorbent assay (DAC-ELISA) confirmed GBNV in naturally infected blackgram samples and mechanically inoculated cowpea plants, with positive samples recording absorbance values greater than twice those of the healthy control at 405 nm. Further confirmation by reverse transcription polymerase chain reaction (RT-PCR) using nucleocapsid (N) gene-specific primers amplified the expected ~800 bp fragment. Sequence analysis of the Tirupati isolate (GenBank accession PZ766619) revealed 99.64% nucleotide identity with the GBNV Anantapur isolate (FJ355951) and 98.44% identity with the *Peanut bud necrosis virus* isolate infecting hyacinth bean (AY882004), confirming the identity of the virus. The combined application of field survey, biological assay, DAC-ELISA and RT-PCR enabled reliable detection and molecular confirmation of GBNV, while the establishment of virus cultures and molecularly characterized isolates provides valuable material for future studies on host resistance, epidemiology, virus characterization and integrated disease management in blackgram.

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