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Investigation on Incidence, Thrip Species, Weed Hosts and '*N*' Gene Characterization of *Groundnut bud Necrosis Virus* Infecting Groundnut (*Arachis hypogaea* L)

K Sarath Babu, K Vemana, A K Patibanda, B Sreekanth and V Srinivasa Rao
Department of Plant Pathology, Agricultural College, Bapatla, A. P.

ABSTRACT

Peanut bud necrosis disease caused by *Groundnut bud necrosis virus* is a major constraint for groundnut production in south and south east Asia. Roving survey was conducted in 70 fields belonging to 70 villages spread over 12 groundnut growing districts in Andhra Pradesh, India for assessing the incidence of Peanut bud necrosis disease (PBND) during *kharif* and *rabi* 2017-18. PBND incidence was significantly higher in *rabi* 2017-18 (13.6%) compared to *kharif* 2017-18 (5.3%). DAC-ELISA results were positive for all suspected PBND infected groundnut plants with mean absorbance (A_{405}) values ranging from 0.56 to 1.1 in *kharif* 2017-18 and 0.07-1.2 in *rabi* 2017-18. Mean thrip population per terminal bud was four in both *kharif* and *rabi* 2017-18 seasons. During survey, GBNV symptoms and thrip damage was observed on alternate weed species. The representative groundnut GBNV isolate (GBNV-GN-BPIND) from Ananthapuramu district, when artificially sap inoculated on cowpea seedlings in glasshouse, typical GBNV symptoms were observed within 4-7 days of post-inoculation (DPI). The Nucleocapsid (*N*) protein gene of GBNV-GN-BPIND groundnut isolate was amplified by RT-PCR and it shared wide range of nucleotide identities (78.66-98.48 %) with other GBNV isolates reported from world. Further, phylogenetic analysis of GBNV nucleocapsid protein (*N*) gene region of present study isolates (GBNV-GN-BPIND) clustered with chilli, water melon, cowpea, groundnut-Bangalore, pea and french bean GBNV isolates and formed single clade.

Keywords: *GBNV, Groundnut, Nucleocapsid (N) protein gene, PBND, Survey.*

Groundnut is an annual oilseed legume crop grown in diverse environment conditions all over the world between 40°N and 40°S. India and China are the world's largest groundnut producers, accounting for 11 per cent and 38 per cent, respectively, of world production. In India, Andhra Pradesh (A.P.) groundnut is cultivated in 5.37 lakh ha area with 6.42 lakh t production (2019-20) (APEDA). The groundnut is susceptible to many biotic stresses caused by fungi, bacteria, viruses and nematodes. Among these pathogens, the viruses have a significant economic impact on groundnut production. Peanut bud necrosis disease (PBND) caused by *Groundnut bud necrosis virus* (GBNV) is an important disease of groundnut in south and southeast Asia. In India, PBND incidence on groundnut was first reported in 1968 (Reddy *et al*., 1968). Later in 1979, causal organism of the disease was confirmed as TSWV by haem agglutination test with TSWV antiserum (Ghanekar *et al*., 1979). Sreenivasulu *et al*. (1991) identified that antisera developed to detect TSWV failed to detect virus in PBND infected plants. Reddy *et al*. (1991) confirmed that the virus was distinct from *Tomato spotted wilt virus* (TSWV) and named as *Groundnut bud necrosis virus* (GBNV). PBND incidence is widely distributed in the main groundnut-growing regions of India *viz*., A.P., Tamil Nadu (T.N), Maharashtra, Gujarat, Rajasthan and western Uttar Pradesh and further, the disease is endemic in the states of A.P. and T.N. (Reddy *et al*., 1983). GBNV is transmitted by *Thrips palmi* in propagative manner (Vijayalakshmi *et al*., 1995). In India, the incidence of PBND account for 30 to 90 per cent of yield loss in groundnut (Ghanekar *et al*., 1979; Basu *et al*., 1995). GBNV belongs to the family *Bunyaviridae* and the genus *Tospovirus* having tripartite genome containing 9.0 kb large (L), 5.0 kb medium (M) and 3.0 kb small (S) membrane-bound RNA. Based on sequence homology of 'N' protein and serological cross reactivity genus *Tospovirus* consists of five serogroups. Serogroup I consists of TSWV (Haan *et al*., 1990), serogroup II consists of TSWV-B (Brazil isolate), *Groundnut ring spot virus* (GRSV) and *Tomato chlorotic spot virus* (TCSV) (De Avila *et al*., 1990), serogroup III consists of *Impatiens necrotic spot virus* (INSV) (Law *et al*., 1990). The serogroup IV consists of GBNV and *Watermelon silver mottle virus* (WSMV) (Adam *et al*., 1998) and sero group V consists of *Peanut yellow spot virus* (PYSV) (Satyanarayana *et al*., 1998). PBND is a serious constraint for groundnut production in India and the disease is endemic in the state of A.P. Hence, present study was focused on prevalence of

PBND in *kharif* and *rabi* seasons, thrip species existing in groundnut ecosystem and weed hosts that help in perpetuation of the disease. Further, nucleocapsid (N) protein gene of representative GBNV groundnut isolate from A.P. was sequenced and compared with other GBNV isolates from different hosts and locations in India and other countries.

MATERIALS AND METHODS

Survey

During *kharif* and *rabi* 2017-18 roving survey was conducted in 12 groundnut growing districts of A.P., *viz*., Ananthapuramu, YSR Kadapa, Chittoor, Kurnool, Sri Potti Sriramulu Nellore, Prakasham, Guntur, Krishna, West Godavari, Visakhapatnam, Vizianagaram, Srikakulam, and assessed the per cent disease incidence of PBND. Suspected GBNV infected samples were collected from all surveyed locations (Table1 and 2). In each field five spots were chosen randomly by walking across the field and, were counted number of GBNV infected plants and total number of plants per square meter. Further, the per cent disease incidence was determined using the following formula.

Per cent disease incidence (%) =

$$\frac{\text{Number of infected plants}}{\text{Total number of plants}} \times 100$$

Suspected GBNV plant samples exhibiting characteristic symptoms of chlorotic spots, chlorotic and necrotic ring spots on leaves, oak leaf pattern spots on leaf , chlorosis of plant, axillary shoot formation, twisted leaves, terminal bud necrosis, stunted growth and malformed leaves *etc*. were collected and tested by direct antigen coating enzyme linked immunosorbent assay (DAC-ELISA) using GBNV specific antisera (Clark and Joseph, 1984) supplied by International Crops Research Institute for the Semi-Arid Tropics (ICRISAT). The Optical density (OD) value of the test sample showing more than two folds than the OD values of the control sample (negative control) was considered as positive (Rodoni *et al*., 1999).

During survey, thrip population was recorded from terminal bud on randomly selected ten plants of one-meter row from each field. Further thrips were collected in Acetic acid-glycerol-alcohol solution (AGA) (a mixture of 10 parts of 60% ethanol with one part of glycerine and one part of acetic acid) from each field and were got identified at National Bureau of Agricultural Insect Resources (NBAIR), Bangalore.

During the survey, information on name of the variety and suspected weeds showing symptoms of GBNV thrip damage found in and around the field were also recorded.

Isolation and maintenance of GBNV culture

Plants showing typical GBNV symptoms were collected from naturally infected plants at Bathalapalli village of Ananthapuramu district and virus was maintained on cowpea (*Vigna unguiculata* cv. PUSA KOMAL) by following standard sap inoculation method in glasshouse at 25± 2^0C. The standard sap extract was prepared for inoculation by grinding samples in a chilled mortar and pestle using phosphate buffer (0.05 M, pH 7.2) @ 1:5 (w/v) containing 0.1% mercaptoethanol. A small quantity of fine celite (diatomaceous earth) was dusted on surface of seven-day old cowpea seedlings before inoculation. The inoculum was rub-inoculated on the upper surface of young leaves with the help of pestle in one direction. The inoculated leaves were washed after 3 min with distilled water to remove excess inoculum and celite powder. The inoculated plants were maintained in an insect proof greenhouse for about three weeks to observe development of symptoms. To maintain purity of the virus, single chlorotic lesions were used for transmiting to healthy cowpea seedlings. Further, GBNV culture was mass multiplied on cowpea and re-inoculated on groundnut healthy seedlings and plants showing typical symptoms of GBNV were used for molecular studies.

Molecular characterization of 'N' gene of GBNV

GBNV-Bathalapalli (GBNV-GN-BPIND) groundnut isolate from A.P. was selected for molecular characterization of nucleocapsid (*N*) protein gene using specific primers and compared with already reported GBNV isolates from India and other countries. RNA was isolated using 100 mg of GBNV infected and healthy leaves of groundnut by RNeasy Plant Mini Kit (Qiagen Inc., Valencia, CA) according to the manufacturer's instructions. The first strand cDNA synthesis was carried out using revert aid first strand cDNA synthesis kit (Thermo fisher) according to the manufacturer's protocol. The reverse transcribed cDNA was then subjected to PCR in a 50 µl reaction volume containing 5 µl of 10 X PCR buffer, 3 µl 25 mM $MgCl_2$, 2 µl of cDNA template (50-60 ng), 1 µl each of 10 µM for ward ("5ATGTCTAACGT YAAGCARYTCACCN-3") and reverse ("5TTAC AATTCCAGCGAAGKRCYN-3") primer, 0.5ìl of Taq DNA polymerase (5U/µl), 1ìl of 10 mM dNTPs, and the final volume was made up to 50 µl by adding RNase free water. The PCR mix was subjected to thermal cycling conditions at 94 °C for 5 min followed by 35 cycles at 94 °C for 45 sec, 50 °C for 45 sec and

72 °C for 1 min with a final extension of 72°C for 30 min in a thermal cycler (Eppendorff, Germany). PCR product thus obtained was run on 1.5 per cent agarose gel for 1 h at 70 volts. The amplified PCR product was gel eluted by QIAquick gel extraction kit (Qiagen) according to the manufacturer's instructions. The gel eluted PCR products sequenced bidirectionally at Eurofins Genomics India Pvt. Ltd., Bangalore. The resulted sequences assembled using bioedit (version 7.0.5.3). To know the homology with other reported GBNV isolates, N gene (792 bp) sequence of present study GBNV isolate was compared using BLASTN program from the NCBI website (www.ncbi.nlm.nih.gov). After confirmation in NCBI, the partial sequence was deposited in GenBank. For further analysis, twenty-six reported GBNV-N gene sequences available in database were downloaded from NCBI website (Table 3). Multiple sequence alignments were generated using MUSCLE algorithm of MEGAX (Version 10.1.1). Nucleotide sequences of 'N' gene of different GBNV isolates were compared and the corresponding phylogenetic tree was generated with 1000 bootstrap replicates following neighbor-joining phylogeny of MEGAX (Sudhir *et al*., 2018).

RESULTS AND DISCUSSION

Roving survey

Roving survey was conducted for assessing the incidence of peanut bud necrosis disease (PBND) in 12 districts of A.P. during *kharif* and *rabi* 2017-18 (Table 1 and 2). Major groundnut cultivars Kadiri-6 (K-6) and TAG-24 were mostly cultivated in districts surveyed. Of the 70 fields, spread over twelve districts of A.P. surveyed, 66 fields were found with infection of GBNV revealing the wide spread occurrence of the disease in A.P. Mean PBND incidence ranged from 2.6-12.3 per cent in different districts of A.P during *kharif 2017-18*. The maximum PBND incidence of 16.0 % was recorded in Machilipatnam (Krishna district) while nil incidence was recorded in Kothapatnam (Prakasham), Mulakalacheruvu (Chittoor district), Pulivendula (YSR Kadapa district) and Aluru (Kurnool district). Maximum mean thrip population (7 thrips/ terminal bud) was recorded at Ananthapuramu, followed by Krishna district (6 thrips/ terminal bud) and the minimum thrip population (3 thrips/top bud) was recorded from Chittoor, Sri Potti Sriramulu Nellore, Srikakulam, West Godavari, and YSR Kadapa districts.

Mean PBND incidence ranged from 5.8 to 18.4 per cent in different districts of A.P. during *rabi 2017-18*. Maximum PBND incidence of 20.8 per cent was recorded in Chinnamusturu (Ananthapuramu district) and Mallavolu (Krishna district) while nil incidence was recorded in Dattirajeru (Vizianagaram district) and Veldurti (Kurnool district). Similarly from other parts of India, incidence of PBND accounted for 30 to 90 per cent of yield loss in groundnut (Ghanekar *et al*., 1979; Basu *et al*., 1995). Pande and Rao (2000) reported, 4 to 25 per cent incidence of PBND in Chittoor, 10 to 25 per cent disease incidence in Kadapa, 3 to 18 per cent in Ananthapuramu, 6 -15 per cent disease incidence in Kurnool districts of Andhra Pradesh during *kharif* 1999. Gopal *et al. (*2011) reported 4.3 to13.3 % PBND incidence in Kurnool districts of A.P.

Roving survey revealed that, PBND incidence to be significantly higher in *rabi* 2017-18 (13.6%) compared to *kharif* 2017-18 (5.3%) in groundnut growing districts of A.P. However, several researchers earlier reported significantly lower incidence of GBNV in groundnut and other crops during *rabi* season (Reddy *et al*., 1983; Sreenivasulu, 1994 and Gopal *et al*., 2011). Alternate wetting and drying coupled with high temperature might have favoured for buildup of thrips and subsequently increased PBND during *rabi* 2017-18. Further, continuous rains could be the reason for low occurrence of PBND during *kharif* 2017 as it facilitates washing off effect on thrips.

Maximum thrip population (5 thrips/terminal bud) was recorded from Anathapuramu, Krishna, Nellore districts and minimum thrip population (2 thrips/ terminal bud) was recorded from Vizianagaram district during *rabi* 2017-18. Three predominant thrip species were found and identified as *Scirtothrips dorsalis*, *Thrips palmi, Thrips simplex* as per the NBAIR report.

DAC-ELISA results were positive for all suspected PBND infected groundnut plants with mean absorbance ($A_{405;}$1h) values ranging from 0.56 to 1.1(*kharif* 2017-18) and 0.07-1.2 (*rabi* 2017-18) when compared to 0.2 O.D. in healthy control (Table 1 and 2). During survey weed species such as *Acanthospermum hispidum, Achyranthus aspera, Ageratum conyzoides, Calotropis gigantea, Commelina benghalensis, Corchorus trilocularis, Parthenium hysterophorus, Physalis minima* and *Vigna triloba,* were observed with GBNV and thrip damage symptoms in and around the fields (Fig 1; Table 1 and 2) in both the seasons. Presence of these alternate weed flora might be the reason for higher disease occurrence in some locations *viz*., Machilipatnam (16.0 %), Bathalapalli (12.5%) during *kharif* 2017-18 and Mallavolu, Chinnamusturu (20.8%) during *rabi* 2017-18. Reddy *et al*. (1983) reported that weed hosts *A. conyzoides* and *A. hispidum* to be important reservoirs of GBNV in India. Vemana *et al*. (2015) reported GBNV on *Parthenium* and confirmed it as reservoir host for GBNV and poses a severe threat to groundnut crop. Gopal *et al. (*2011)

Table 1. Incidence of PBND in groundnut growing districts of Andhra Pradesh in *kharif* 2017-18

S. No.	District	Mandal (Village)	GPS coordinates	GBNV	Mean incidence	Symptoms	GBNV-DAC-ELISA-(A_{405} nm;1 h)	Thrip population		Weeds in and around field
				% per m^2	(%)			per top bud leaves	Mean population	
1	Srikakulam	Ponduru (Kesuvadasupuram)	18°10.597'N 083°47.466'E	8.6	5.4	CR, SG, ML	0.74	3	3	Parthenium hysterophorus, Ageratum conyzoides, Commelina benghalensis Vigna triloba,
		Laveru (Gumadam)	18°10.900'N 083°47.078'E	3.4		BN, SG	0.61	4		
		Santhabommali (Vallabarayapadu)	18°07.475'N 083°23.883'E	4.3		SG, BN	0.92	3		
2	Vizianagaram	Vizianagaram (Gajularega)	18°07.482'N 083°23.884'E	7.1	5.4	ASP, SG	0.72	7	4	Achyranthus aspera, Parthenium hysterophorus, Commelina benghalensis
		Dattirajeru (Gadasam)	18°20.369'N 082°24.840'E	3.7		BN, ML	0.56	3		
3	Visakhapatnam	Hukumpeta (Marripalem)	17°52.728'N 082°20.667'E	7.4	8	CR, BN, OLP	0.82	4	4	Ageratum conyzoides, Corchorus trilocularis, Parthenium hysterophorus Acanthospermum hispidum, Corchorus trilocularis
		Chintapalli (Asarada)	17°54.728'N 082°24.666'E	8.0		BN, SG	0.66	6		
		Chintapalli	17°56.710'N 082°24.662'E	8.6		ASP, BN	0.78	3		
4	West Godavari	Nallajerla (Dubacherla)	16°55.860'N 081°20.996'E	7.2	7.2	BN, SG, OLP	0.86	3	3	Physalis minima, Vigna triloba
5	Krishna	Vunguturu (Avitipalli)	14°26.285'E 079°58.288'N	8.6	12.3	CR, BN, ML	0.79	7	6	Parthenium hysterophorus, Ageratum conyzoides, Vigna triloba, Pysalis minima
		Machilipatnam (Kothapatnam)	16°13.064'E 081°11.434'N	16.0		BN, SG, DP	0.91	6		
6	Sri Potti Sriramulu Nellore District	Muthukuru (Buchareddy palem)	14°16.579'E 080°04.481'N	8.3	7.8	BN, SG, ML	1.2	4	3	Parthenium hysterophorus, Vigna triloba, Physalis minima.
		Kavali (Thummalapenta)	14°53.610'E 080°03.788'N	7.4		ASP, BN, DP	0.82	3		
7	Prakasham	Kothpatnam	15°26.460'N 080°10.255'E	0.0	5.1	NIL	0.12	3	3	Parthenium hysterophorus, Ageratum conyzoides,Physalis minima, Calotropis gigantea
		Kothpatnam (Aluru)	15°26.617'N 080°10.193'E	4.1		CR, BN, SG	0.71	5		
		Vetapalem (Akkaipalem)	17°48'24.103'N 80°21'33.08'E	8.6		ASP, BN, ML	1.12	4		
		Chirala (Prasadanagaram)	15°49'37.59'N 80°22'46.22'E	7.8		BN, ML, SG	0.82	2		

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8	Guntur	Bapatla	15°57'12.88'N 80°32'25.56'E	8.69	7.1	BN, ML, SG	0.88	4	4	Ageratum conyzoides, Commelina benghalensis, Parthenium hysterophorus, Achyranthus aspera, Vigna triloba,
		Karlapalem	15°56'42.202'N 80°32'47.352'E	4.34		BN, ML, ASP	0.75	3		
		Karlapalem (Yazali)	15°58'13.869'N 80°34'40.232'E	8.69		BN, DP	0.72	5		
9	Ananthapuram	Kadiri	14°6'34.187'N 78°8'42.503"E	8.6	7.3	BN, CR, ML	0.71	7	7	Parthenium hysterophorus, Ageratum conyzoides, Achyranthus aspera, Vigna triloba, Acanthospermum hispidum, Corchorus trilocularis
		Bathalapalli (Nallaboinapalli)	14°26'8.915"N 77°54'7.21"E	12.5		BN, CR, ML	0.67	8		
		Uravakonda (Chinnamusturu)	15°26'.617'N 080°10.193'E	4.1		BN, CR, SG	0.51	6		
		Vajrakarur	15°02'62.58"N 77°38'60.99'E	3.8		SG, ML	0.13	7		
		Bathalapalli (Sanjeevapuram)	14°32'27.16'N 77°44'18.04"E	4.1		CR, ML, BN	0.61	6		
		Vajrakarur (kadamalakunta)	15°01'62.48"N 77°38'60.90"E	3.6		CR, BN	0.52	7		
10	Chittoor	Mulakalacheruvu	13.78905'N 78.27806 'E	0	4.2	NIL	0.15	4	3	Parthenium hysterophorus, Ageratum conyzoides, Achyranthus aspera,Physalis minima
		Madanapalle	13.64752'N 78.46525'E	4		BN, CR, SG	0.82	3		
		S.V. Agriculture college, Tirupathi	13.6247'N 79.3714'E	8.6		BN, SG, OLP	0.95	2		
11	YSR Kadapa	Pulivendula	14.39253'N 78.2199'E	0	2.6	NIL	0.07	1	3	Parthenium hysterophorus, Vigna triloba, Physalis minima
		Gandi	14.3107'N 78.4922'E	4		BN, CR, SG	0.65	3		
		Vempalli	14.35353'N 78.46113'E	3.8		BN, SG	0.32	4		
12	Kurnool	Bethamcherla	15.59226'N 78.17602'E	4.1	3	CR, BN, SG	0.94	5	5	Parthenium hysterophorus, Commelina benghalensis, Vigna triloba.
		Allagadda	15.68783'N 78.41737'E	4		OLP, BN	0.12	7		
		Krishnagiri	15.55153'N 77.84649'E	4.3		CR, BN, ML	0.84	4		
		Aluru	15.50401'N 80.13231'E	0		NIL	0.22	2		

* DAC-ELISA: Direct Antigen coated enzyme linked immunosorbent assay; Positive control O.D value: 1.4; Negative control (Healthy) O.D value: 0.2

* CR=Chlorotic rings, OLP= Oak leaf pattern, BN=Bud necrosis, SG= Stunted growth, ML=Malformed leaves, ASP= Auxiliary shoot proliferation, DP= Deformed pods, NIL =No symptoms

Table 2. Incidence of PBND in groundnut growing districts of Andhra Pradesh in *rabi* 2017-18

S. No.	District	Mandal (village)	GPS coordinates	GBNV % perm^2	Mean incidence	Observed symptoms	GBNV-DAC-(A_{405} nm;1 h)	Thrip population		Weeds in and around field
								Per top bud	Mean	
1	Srikakulam	Kotabommali (Pedda bammidi)	18.44632 N 84.11448 E	15.4	13.5	CR, BN, ML	0.84	4	3	Acanthospermum hispidum, Ageratum conyzoides Parthenium hysterophorus, Vigna triloba,
		Polaki (Koduru)	18.42494'N 84.18834'E	13.0		BN, CR, SG	0.82	2		
		Ranasthalam (Ranasthalam)	18.20242'N 83.69068'E	12.0		CR, ML, BN	0.68	3		
2	Vizianagaram	Lakkavarapukota (Marlapalli)	18.01681'N 83.22137'E	11.5	5.8	ASP, BN	0.61	4	2	Commelina benghalensis, Parthenium hysterophorus, Corchorus trilocularis
		Dattirajeru (Guchimi)	18.69854'N 83.38493'E	0.0		CR, BN, ASP	0.07	0		
3	Visakhapatnam	K.Kothapadu (Gondipalem)	17.88989'N 83.04605'E	12.0	12	CR, BN, ML	0.72	3	3	Parthenium hysterophorus, Acanthospermum hispidum
4	West Godavari	Nallajerla (Dubacherla)	16.92537'N 81.36086'E	16.2	14.3	BN, ASP	0.6	5	4	Ageratum conyzoides, Parthenium hysterophorus, Corchorus trilocularis
		Jeelugumilli	17.20564'N 81.24782'E	12.0		SG, ML	0.51	2		
5	Krishna	Guduru (Mallavolu)	16.17526'N 81.05111'E	20.8	18.4	BN, SG, OLP	0.82	6	5	Ageratum conyzoides, Calotropis gigantea, Parthenium hysterophorus
		Machilipatnam (Chilakalapudi)	16.2082'N 81.17611'E	16.0		CR, BN, SG, ASP	0.88	5		
6	Sri Potti Sriramulu Nellore District	Muthukuru (Eepuru bit)	14.26925'N 80.0479'E	17.9	18.4	BN, CR, SG	0.71	3	5	Acanthospermum hispidum, Pysalis minima, Parthenium hysterophorus, Vigna triloba
		Kavali (Thummalapenta	14.90207'N 80.06606'E	20.0		CR, BN, DP	0.61	5		
		Vutukuru	16.5998'N 80.19671'E	17.4		BN, OLP, SG	0.54	6		
7	Prakasham	Kothpatnam (Aluru)	15.44442'N 80.13938'E	16.0	14.8	BN, SG, OLP	0.83	4	4	Ageratum conyzoides, Parthenium hysterophorus,
		Vetapalem (Kothapeta)	15.85373'N 80.4099'E	12.5		BN, CR, SG	0.75	5		
		Ulavapadu (Karedu)	15.18881'N 80.04936'E	16.0		SG, ML, ASP	0.74	4		

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8	Guntur	Bapatla (Bapatla east)	15.89574'N 80.50494'E	19.2	13.2	BN, SG, ML	0.92	3	4	Ageratum conyzoides, Commelina benghalensis, Parthenium hysterophorus, Physalis minima.
		cherukupalle ho arumbaka (Dhindi)	15.90837'N 80.77463'E	12.5		CR, BN, DP	0.76	4		
		Karlapalem (Yazali)	15.97097'N 80.56328'E	18		CR, OLP, BN	0.56	4		
9	Anantapuram	Kadiri	14.1101'N 78.14727'E	20	17.7	CR, BN, DP	0.86	6	5	Ageratum conyzoides, Achyranthus aspera, Corchorus trilocularis, Vigna triloba, Acanthospermum hispidum, Physalis minima, Parthenium hysterophorus.
		Bathalapalli (Musturu)	14.51568,N 77.77506'E	17.4		CR, BN, ML	0.75	7		
		Uravakonda (Chinnamusturu)	14.89752'N 77.27571'E	20.8		CR, BN, ASP	0.72	6		
		N.P.kunta	14.06268'N 78.41026'E	16		BN, ML	0.71	0		
		Mudigubba (Malakavemula)	14.27162 N 78.05553'E	19.2		BN, CR, DP	0.67	7		
		Nallamada (Gopepalli)	14.12846'N 78.0285'E	12.5		SG, ASP	0.51	5		
10	Chittoor	Nindra (Arur)	13.3605'N 79.70434'E	14.3	11.1	CR, SG, ASP	0.65	2	3	Ageratum conyzoides, Achyranthus aspera, Parthenium hysterophorus.
		Madanapalle (vempalli)	13.53857'N 78.46525'E	8.7		BN, CR, ASP	0.61	3		
		B.Kothakota (Bayyappagaripalli)	13.67085'N 78.37385'E	10.3		BN, ASP	0.53	4		
11	YSR Kadapa	Chakrayapeta	14.39253'N 78.2199'E	11.5	11.6	BN, ML, ASP	0.71	3	3	Parthenium hysterophorus, Vigna triloba, Pysalis minima, Calotropis gigantea, Commelina benghalensis
		Kamalapuram (Peddacheppali)	14.3107'N 78.4922'E	15.4		CR, BN, ASP	0.63	4		
		Vempalli	14.37753'N 78.44691 'E	8		BN, ASP	0.51	2		
12	Kurnool	Pyapalli (Gudipadu)	15.18522'N 77.77696'E	12	9.1	CR, BN	0.46	3	3	Acanthospermum hispidum, Achyranthus aspera, Vigna triloba, Commelina benghalensis, Parthenium hysterophorus
		Tuggali (Yeddula doddi)	15.35605'N 77.5943'E	15.4		ASP, SG	0.67	5		
		Veldurti (Ramallakota)	15.46363'N 78.14175'E	0		CR, ASP, SG	0.12	2		

* DAC-ELISA: Direct Antigen coated enzyme linked immunosorbent assay; Positive control O.D value: 1.4; Negative control (Healthy) O.D value: 0.2

* CR=Chlorotic rings, OLP= Oak leaf pattern, BN=Bud necrosis, SG= Stunted growth, ML=Malformed leaves, ASP= Auxiliary shoot proliferation, DP= Deformed pods, NIL =No symptoms

Table 3. List of accession numbers for nucleocapsid (NP) protein gene of GBNV isolates used for comparison

S.No.	Accession no	Location	Host	Isolate code used
1	JX198661	Bangalore, Karnataka India	*Arachis hypogaea* (groundnut)	GBNV-GN-BRIND
2	EF179100	Tirupati, India.	*Arachis hypogaea* (groundnut)	GBNV-GN-TPTIND
3	HM770022	Thiruvallur, Tamil Nadu ,India	*Arachis hypogaea* (groundnut)	GBNV-GN-TNIND
4	HM770021	Nellore, Andhra Pradesh,India	*Arachis hypogaea* (groundnut)	GBNV-GN-NLRIND
5	HM131489	Kurnool, Andhra Pradesh, India	*Arachis hypogaea* (groundnut)	GBNV-GN-KRLIND
6	JX524443	Kolkata ,West Bengal,India.	*Arachis hypogaea* (groundnut)	GBNV-GN-WBIND
7	JF968416	Kadapa, Andhra Pradesh, India	*Arachis hypogaea* (groundnut)	GBNV-GN-KDPIND
8	AY882004	Pune, Maharashtra, India	*Dolichus lablab* (Fieldbean)	GBNV-FB-IND
9	EF179099	Tirupati, India	*Vigna unguiculata* (cowpea)	GBNV-CW-IND
10	JX524452	Raipur, chattisgarh ,India	*Vigna radiata* (mungbean)	GBNV-MB-IND
11	KX244331	India	*Parthenium hysterophorus*	GBNV-PH-IND
12	JX524449	Coimbatore, TN, India	*Vigna mungo* (urdbean)	GBNV-UB-IND
13	JF281104	Shahjahanpur, Uttar Pradesh ,India	*Pisum sativum* (pea)	GBNV-PS-IND
14	JN662492	India	*Phaseolus vulgaris* (french bean)	GBNV-PV-IND
15	EU373796	India	*Citrullus lanatus* (watermelon)	GBNV-WM-IND
16	JQ269832	Kadapa, Andhra Pradesh, India	*Allium cepa* (onion)	GBNV-ON-IND
17	JX511966	Pantnagar, Uttarakhand	*Glycine max* (soy bean)	GBNV-SB-IND
18	JX524556	India	*Abelmoschus esculentus* (okra)	GBNV-OK-IND
19	MN735676	India	*Cucumis sativus* (cucumber)	GBNV-CS-IND
20	KY798417	Varanasi, Uttar Pradesh	*Momordica charantia* (bittergourd)	GBNV-MC-IND
21	JQ406582	India	*Vigna umbellate* (rice bean)	GBNV-RB-IND
22	FJ997641	India	*Capscicum annum* (chili)	GBNV-CH-IND
23	AY618561	India	*Solanum lycopersicum* (tomato)	GBNV-TO-IND
24	KX757228	Iran	*Rudbeckia sp* .(coneflowers)	GBNV-CF-IR
25	HM122221	China	*Piper nigrum* (pepper)	GBNV-PN-CH
26	AF134400	Thailand	Solanum lycopersicum(tomato)	GBNV-TO-TH
27	MW014341	India	*Arachis hypogaea* (groundnut)	GBNV-GN-BPIND

*** GBNV-GN-BPIND-Isolate. Under study**

reported weed species *viz*., *C. benghalensis, A. conyzoides,* and *V. triloba* in most of the peanut growing areas that help in spread of the disease. Even though thrips population was observed in some of the surveyed locations, the disease incidence was found low and that could be due to absence of specific vector of GBNV, as Vijayalakshmi *et al*. (1995) reported *T. palmi* helps in transmission of GBNV in persistent propagative manner. In the present study, the PBND incidence varied among the surveyed districts. The change in the severity of disease incidence in different locations might be due to absence of vector and alternate weed population during susceptible stage of crop.

Symtomatology of GBNV and thrip infestation in groundnut and weed hosts:

Different type of symptoms such as chlorotic rings, oak leaf pattern on leaves, top bud necrosis, stunted plants with malformed leaves, axillary shoot proliferation, deformed pods and kernels were observed on groundnut during survey (Fig 1). Diverse GBNV symptoms were observed on weed hosts *viz*., chlorotic spots in *A. hispidum,* chlorosis in *P. hysterophorus*, bud necrosis in *C. trilocularis* (Fig1). While, Thrip damage was measured by whitish patches on upper surface of leaves, curling of young leaves and silvering of leaves on both groundnut and weed hosts (Fig1).

GBNV symptoms in artificially sap inoculated Cowpea

The representative groundnut GBNV isolate (GBNV-GN-BPIND) upon artificial sap inoculation on one-week old cowpea seedlings in glasshouse for further molecular characterization and exhibited typical GBNV symptoms on cowpea within 4-7 days of post inoculation (DPI). A series of typical symptoms observed on cowpea are chlorotic spots (4 DPI),

Fig.1 Symptoms of GBNV on groundnut crop and weed hosts under field conditions (a: Chlorotic rings on leaf, b: Oak leaf pattern spots c: Necrosis of individual buds d: Chlorosis of plant with axillary shoot proliferation e: Stunting of plant with leaf malformation f: Deformed pods g: Deformed kernels h: chlorotic spots in *Acanthospermum hispidum* i: Chlorotic symptoms on parthenium leaf j: Thrips injury in parthenium k: Thrips injury in *Physalis minima* j: Terminal bud necrosis in *Corchorus trilocularis).*

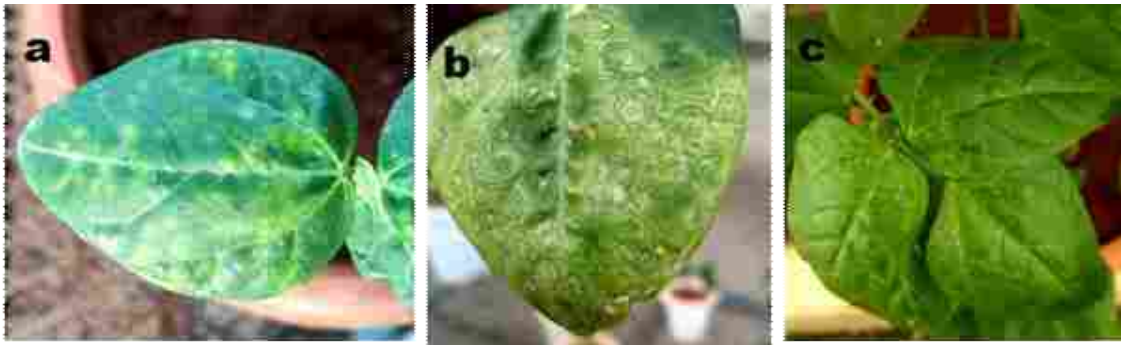

Fig 2. Symptoms of GBNV on cowpea (*Vigna unguiculata* cv. PUSA KOMAL) under glasshouse conditions (a: Chlorotic spots on leaf b: Chlorotic rings on leaf c: Small chlorotic spots on systemic leaf)

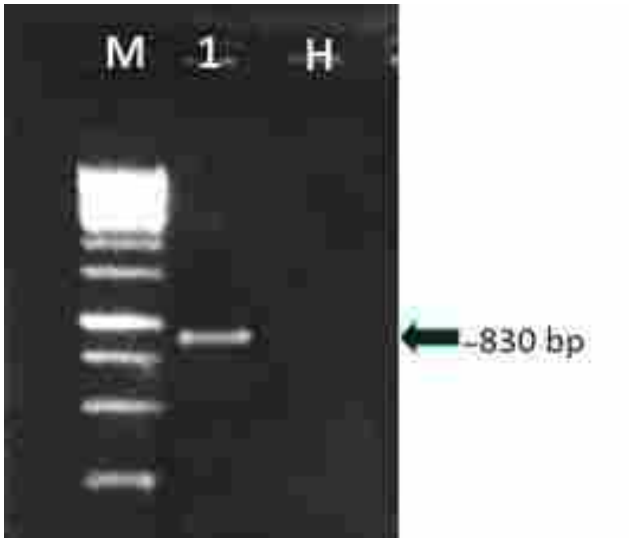

Fig 3. RT-PCR amplification of nucleocapsid (N) protein gene product of groundnut GBNV isolate (GBNV-GN-BPIND) maintained on cowpea (*Vigna unguiculata* cv. PUSA KOMAL) M =1 kb DNA ladder, Lane 1; GBNV on cowpea, Lane 2: healthy cowpea.

distinct chlorotic rings (7 DPI), small chlorotic and necrotic spots on systemic leaves (10-12 DPI) (Fig 2).

Molecular characterization of GBNV-GN-BPIND isolate

Total RNA that was extracted from the GBNV inoculated cowpea seedlings when subjected to RT-PCR using nucleocapsid (N) protein gene specific primers resulted an amplicon of ~830 bp (Fig. 3). Gel eluted amplified product was sequenced bi directionally and deposited in NCBI Genbank (Accession No. MB014941). Partial nucleocapsid (N) protein gene of present study isolate (GBNV-GN-BPIND) shared high range of nucleotide identity (78.66-98.48 %) with other GBNV isolates reported from different crops and

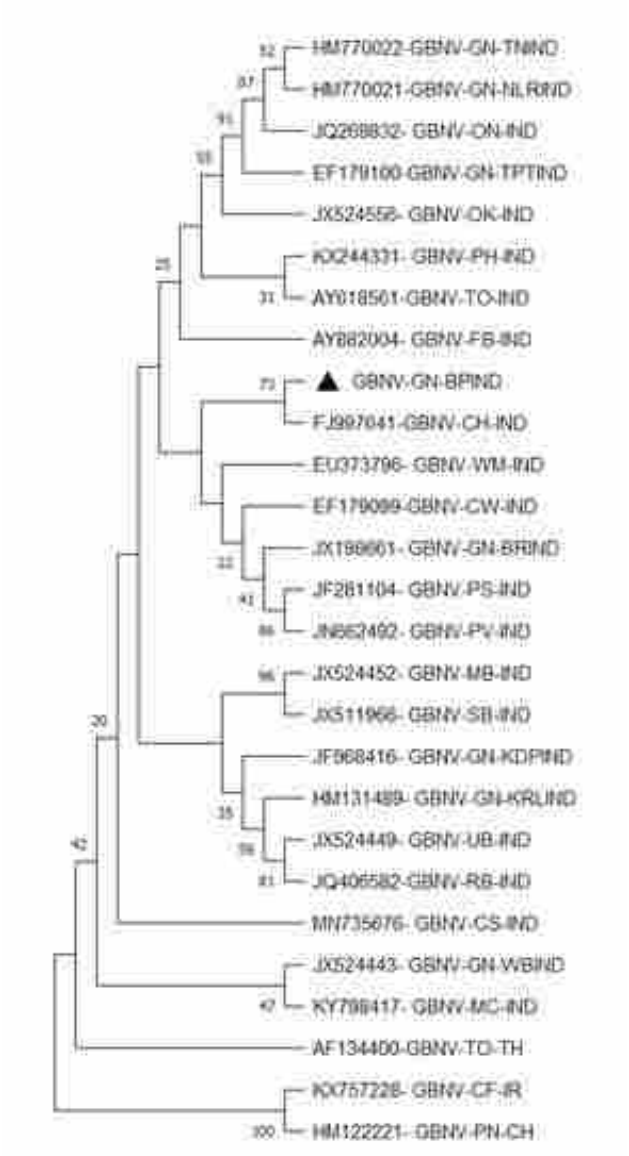

Fig 4. Phylogenetic relationship of GBNV-GN-BPIND isolate with other GBNV isolates reported from India and other countries. *The isolate of present study highlighted with triangle mark.

locations. GBNV-GN-BPIND isolate shared 96.67 to 97.73 of per cent nucleotide identity with groundnut isolates and 96.59 to 98.48 of per cent nucleotide identity with other crop GBNV isolates from India, which indicated that the nucleocapsid (N) protein gene sequences of present study isolate did not shown much variation with other GBNV isolates reported from India. Hence, the GBNV-GN-BPIND isolate should be regarded as the same strain of GBNV existing in India. Earlier studies also showed that GBNV Indian isolates have high conservation for their nucleotide sequence for 'N' gene (Suganyadevi *et al*., 2018). In phylogenetic analysis, GBNV-GN-BPIND isolates nucleocapsid (N) protein gene region clustered with chilli (FJ997641), water melon (EU373796), cowpea (EF179099), groundnut-Bangalore (JX198661), pea (JF281104) and french bean (JN662492) GBNV isolates and which formed single clade. All Indian GBNV isolates clustered separately with foreign GBNV isolates. GBNV isolates from Thailand formed separate clade and isolates from China and Iran clustered together and formed separate clade (Fig 4).

CONCLUSION

Survey results revealed the incidence of PBND to be relatively high in *rabi* compared to *kharif* season (2017-18) in A.P. The genetic relationships of various GBNV isolates infecting groundnut crop in India is especially lacking and this study contributes in understanding the sequence diversity among GBNV isolates infecting groundnut crop and other crops in India and other countries. The major thrip species identified should be tested for their transmission ability of GBNV. Further, epidemiology factors need to be studied for better understanding of disease development and spread in population.

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