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RESEARCH ARTICLE :

A serological report on groundnut bud necrosis virus (GBNV) and tobacco streak virus (TSV) infecting groundnut and parthenium in Guntur and Prakasam districts of A.P.

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SUMMARY : Samples showing symptoms ranging from chlorosis to necrosis, collected from different fields during the survey in Guntur and Prakasam districts during *Rabi* 2013-14 alone cannot determine the presence of disease and its casual organism. To consolidate, these samples were subjected to a serological test DAC-ELISA. Out of 38 groundnut and parthenium samples of Guntur district tested, 22 samples were positive for GBNV while two were positive to TSV. Out of 24 samples of Prakasam district, 13 were positive to GBNV, two were positive to TSV alone and nine samples were positive to both viruses (mixed infections).

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KEY WORDS:
GBND, PSND, TSV, GBNV, DAC-ELISA

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BACKGROUND AND OBJECTIVES

In India, major groundnut growing areas are Gujarat, Andhra Pradesh, Tamil Nadu, Karnataka, Rajasthan, Maharashtra, Madhya Pradesh, Orissa and Uttar Pradesh. Andhra Pradesh has the second largest area under groundnut with 1.31 M ha area with a production of 0.84 M t that recorded a productivity of 641 kg ha^{-1} during 2011-2012 (Directorate of Economics and statistics, Government of India, 2013). Groundnut bud necrosis disease was first recorded in India in 1949.GBND causing virus was earlier reported as *Tomato Spotted Wilt Virus* (TSWV) but based on serological studies it is now referred to as *Groundnut Bud Necrosis Virus* (GBNV), a distinct genus classified under the family Bunyaviridae, transmitted by *Thrips palmi* Karny. (Reddy *et al*., 1995). Adults were able to transmit the virus that was acquired only during larval stage (Black, 1954). Peanut stem necrosis disease epidemics were recorded in Anantapur district of Andhra Pradesh, India during 2000. Initially PSND was suspected as GBND caused by GBNV, but later it was found to be caused by

a distinct virus known as *Tobacco Streak Virus* (TSV) of the genus *Ilarvirus*, family Bromoviridae (Reddy *et al*., 2002). Thus, the disease in peanut was named as peanut stem necrosis disease (PSND) transmitted by*Frankliniella schultzei*, *Scirtothrips dorsalis* and *Megalurothrips usitatus*in passive manner. The virus gains entry into the host through the infected parthenium pollen, carried by the thrips when dislodged on leaf surface while feeding (Lavakumar *et al.*, 2008). Symptoms in the field alone cannot specify the presence of respective diseases and their viruses. Hence, occurrence of both the diseases and its viruses in Guntur and Prakasam districts were supported by serological study (Direct Antigen Coating Enzyme Linked Immunosorbent Assay (DAC-ELISA).

RESOURCES AND METHODS

Groundnut plants exhibiting chlorotic or necrotic lesions in Guntur and Prakasam districts were collected in polythene bags and the collected samples were kept in cool conditions until virus assay was conducted. Present investigations were carried out in molecular biology laboratory in the Department of Plant Pathology, Agriculture Research Station, Kadhari, A.P. DAC-ELISA as described by Hobbs *et al*. (1987) was followed for detection of *Groundnut Bud Necrosis Virus* (GBNV) and *Tobacco Streak Virus* (TSV) in all test samples. ELISA was performed in 96 well polysterene microtitre plates using antisera of GBNV (1:5000) and TSV (1:10000). Coating buffer and healthy samples were used as negative control whereas infected sample was used as positive control.

Procedure for DAC-ELISA :

– Suspected young leaflets showing primary symptoms of GBND and PSND were extracted in coating buffer at 1:50 with mortar and pestle.

– Extract was collected into the 2 ml eppendorf tube and centrifuged at 10000 rpm for 15 min. Supernatant of the centrifuged sample was collected in a new eppendorf tube without disturbing the pellet.

– Healthy plant extract and buffer samples were loaded in first two wells of ELISA plates followed by suspected samples. In the last left over wells positive controls were added and incubated the loaded plates for 1 h 30 min at 37 ^{0}C.

– Contents of the incubated ELISA plates were emptied and three washings were given with PBS-T, taking five min for each wash.

– Healthy leaf tissue of groundnut was macerated in PBS-TPO and centrifuged at 10000 rpm for 10 min, the supernatant was collected and again centrifuged at 10000 rpm for 10 min.

– Antiserum was diluted with PBS-TPO in 1:10000 (1µl antiserum in 10 ml PBS- TPO) for TSV where as for GBNV 1:5000 (1 µl antiserum in 5 ml PBS- TPO). Diluted antisera were cross absorbed with healthy groundnut tissue extracted in PBS-TPO to avoid non-specific reactions and 200 µl of antisera were dispensed into each well of microtitre plates. The plates were incubated for 30 min at 37^{0}C and were washed in three changes of PBS-T, taking five min for each wash.

– 200 µl of cross reaction solution was loaded into each well of microtitre ELISA plate and incubated for 1h 30 min at 37 ^{0}C and the plates were washed in three changes of PBS-T, taking five min for each wash.

– Required quantity of enzyme conjugated secondary antibody (IgG) was added into the PBS-TPO and the solution was stirred for 15-20 min. 200 µl of secondary antibody was added into the wells of microtitre ELISA plate and was incubated for 1h 30 min at 37 ^{0}C and plates were washed in three changes of PBS-T, taking five min for each wash.

– 200 µl substrate solution was dispensed into the wells of microtitre ELISA plate in the dark room and was incubated for 1hr at 37 ^{0}C. The reaction was terminated by adding 100 µl of 3 M NaOH solution to each well. Optical density (O.D) value was recorded for each plate with the help of ELISA reader at 405 nm.

In all the ELISA experiments conducted, the values were accepted as positive when the O.D value was two to three times greater than the mean values of healthy sample (Hobbs *et al*., 1987).

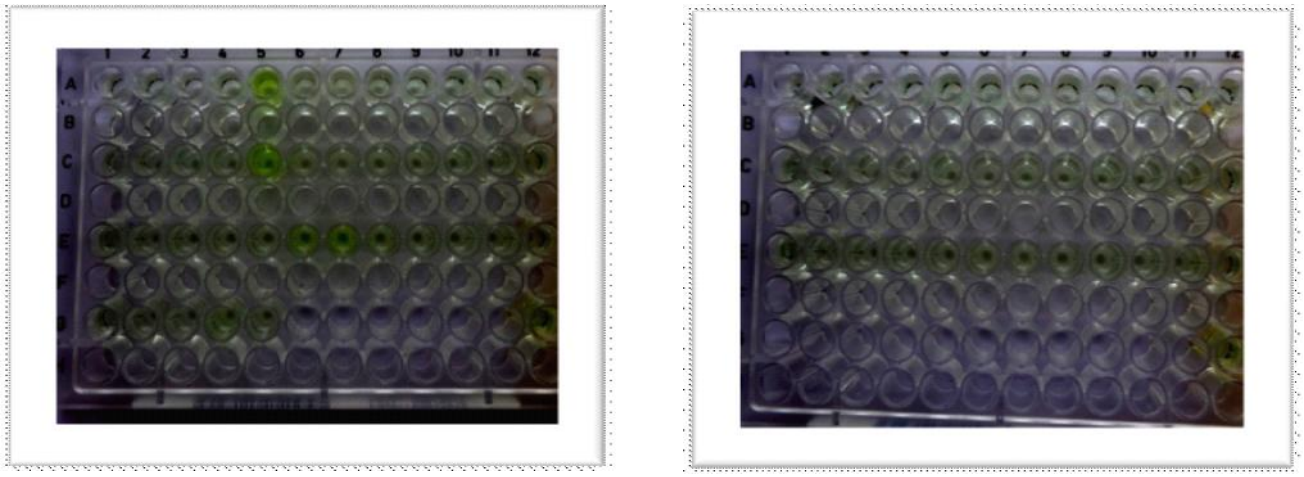
OBSERVATIONS AND ANALYSIS

Out of 38 groundnut and parthenium samples of Guntur district tested, 22 samples were positive for GBNV while two were positive to TSV (Table 1 and Fig 1). Out of 24 samples of Prakasam district, 13 were positive to GBNV, two were positive to TSV alone and nine samples were positive to both viruses (mixed infections) (Table 2 and Fig 2).

In ELISA highest OD values were recorded in young leaves showing mixed infection and chlorotic concentric

Table 1: Reaction of suspected samples of Guntur district to GBNV and TSV antiserum in DAC-ELISA

Sr.No.	Sample description	OD values			
		GBNV	Reaction	TSV	Reaction
1.	Groundnut leaves exhibiting necrotic symptoms	0.04		0.03	
2.	Groundnut leaves exhibiting chlorotic and necrotic symptoms	0.79	+	0.09	
3.	Groundnut leaves exhibiting necrotic symptoms on middle leaf	0.11	+	0.07	
4.	Groundnut leaves exhibiting veinal chlorosis	0.21	+	0.04	
5.	Groundnut leaves exhibiting necrotic symptoms	0.11	+	0.06	
6.	Groundnut leaves having necrotic regions, axillary bud proliferation	0.07		0.13	+
7.	Groundnut leaves exhibiting mosaic symptoms	0.08	+	0.06	
8.	Groundnut leaves exhibiting necrotic symptoms	0.09	+	0.06	
9.	Groundnut leaves showing necrosis at middle of the leaf	0.09	+	0.06	
10.	Groundnut leaves exhibiting necrotic symptoms	0.01		0.02	
11.	Groundnut leaves exhibiting necrotic symptoms	0.04		0.02	
12.	Groundnut leaves exhibiting typical symptoms of GBNV	0.06		0.03	
13.	Groundnut leaves showing middle chlorosis and necrosis	0.06		0.004	
14.	Groundnut leaves exhibiting mixed infection curves	1.15	+	0.06	
15.	Groundnut leaves exhibiting chlorotic symptoms	0.10	+	0.03	
16.	Groundnut leaves exhibiting necrotic symptoms	0.07		0.04	
17.	Groundnut leaves exhibiting veinal chlorosis	0.07		0.04	
18.	Groundnut leaves exhibiting necrotic symptoms and oak leaf pattern	0.08	+	0.05	
19.	Groundnut leaves exhibiting necrotic symptoms	0.07		0.04	
20.	Parthenium pollen	0.09	+	0.05	
21.	Groundnut leaves exhibiting chlorotic and necrotic rings	0.10	+	0.06	
22.	Groundnut leaves exhibiting chlorotic symptoms	0.07		0.05	
23.	Groundnut leaves exhibiting chlorosis on both sides of edges of the leaf	0.08	+	0.03	
24.	Parthenium pollen in Bapatla	0.08	+	0.04	
25.	Groundnut leaves exhibiting necrotic symptoms	0.06		0.04	
26.	Groundnut leaves exhibiting necrotic symptoms	0.09	+	0.05	
27.	Groundnut leaves exhibiting necrotic symptoms	0.30	+	0.07	
28.	Groundnut leaves exhibiting necrotic symptoms	0.42	+	0.05	
29.	Groundnut leaves exhibiting necrotic symptoms	0.16	+	0.06	
30.	Groundnut leaves exhibiting necrotic symptoms	0.09	+	0.07	
31.	Groundnut leaves exhibiting necrotic symptoms	0.09	+	0.08	
32.	Groundnut leaves exhibiting necrotic symptoms	0.11	+	0.09	
33.	Parthenium pollen in Bapatla	0.06		0.17	+
34.	Groundnut leaves exhibiting necrotic symptoms	0.07		-0.10	
35.	Groundnut leaves exhibiting necrotic symptoms	0.07		0.11	
36.	Groundnut leaves exhibiting necrotic symptoms	0.07		-0.11	
37.	Groundnut leaves exhibiting necrotic symptoms	0.17	+	-0.10	
38.	Groundnut leaves exhibiting necrotic symptoms	0.07		-0.09	
	Positive control	0.43		0.43	
	Negative control (Healthy)	0.04		0.05	
	Buffer control	0.13		0.13	

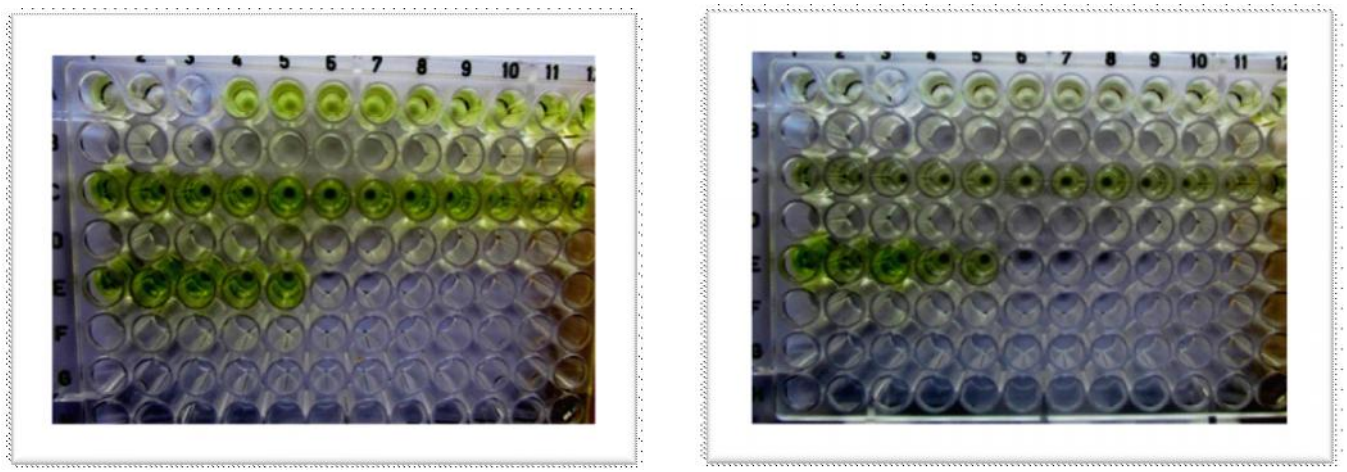


GBNV TSV

Fig. 1: Reaction of GBNV and TSV infected groundnut leaf samples of Guntur district in DAC ELISA

Table 2 : Reaction of suspected samples from Prakasam district to GBNV and TSV antiserum in DAC-ELISA

Sr. No.	Sample description	OD values			
		GBNV	Reaction	TSV	Reaction
1.	Groundnut leaves exhibits chlorotic spots	0.46	+	0.03	-
2.	Groundnut leaves exhibits fully chlorotic spots	0.54	+	0.06	-
3.	Groundnut leaves exhibits GBNV rings	0.42	+	0.06	-
4.	Groundnut GBNV	0.52	+	0.08	-
5.	Groundnut GBNV rings	0.76	+	0.05	-
6.	Groundnut leaves exhibits veinal chlorosis	0.47	+	0.07	-
7.	Groundnut GBNV	0.49	+	0.09	-
8.	Parthenium pollen	0.12	-	0.11	+
9.	Groundnut fully chlorotic and light necrotic spots	0.62	+	0.14	+
10.	Groundnut leaves exhibits okra leaf pattern	0.77	+	0.06	-
11.	Groundnut leaves exhibits GBNV spots	0.80	+	0.05	-
12.	Groundnut leaves necrotic at the upper edge of leaf and curl	0.51	+	0.04	-
13.	Fully chlorotic leaves of groundnut	0.57	+	0.05	-
14.	Necrotic with GBNV rings	1.28	+	0.08	-
15.	GBNV symptoms in ground nut	0.73	+	0.04	-
16.	Parthenium pollen	0.14	-	0.12	+
17.	Chlorotic and necrotic at midrib region of leaves groundnut	0.97	+	0.15	+
18.	GBNV at upper edge of groundnut leaf	1.14	+	0.11	+
19.	Groundnut leaves exhibits fully chlorotic rings	0.85	+	0.14	+
20.	Groundnut leaves exhibits light necrotic spots	0.77	+	0.10	+
21.	Chlorotic midrib region of leaves groundnut	0.56	+	0.13	+
22.	Chlorotic and necrotic at midrib region of leaves groundnut	1.06	+	0.78	+
23.	GBNV at upper edge of groundnut leaf	0.87	+	1.12	+
24.	Groundnut leaves exhibits fully chlorotic rings	1.18	+	1.04	+
	Positive control	1.20		0.94	
	Negative control (Healthy)	0.09		0.05	
	Buffer control	0.17		0.17	



GBNV TSV

Fig. 2: Reaction of GBNV and TSV infected groundnut leaf samples of Prakasam district in DAC ELISA

rings. This may be due to the presence of high concentration of virus in young tissue and availability of infected parthenium pollen. The significance of infected pollen abundance in spread of disease was reported by Reddy *et al*. (2002). These results are in agreement with the reports of Bhat *et al*. (2001); Prasadarao *et al*. (2003) and Prasadarao *et al*. (2009). Some of old leaf samples have showed low OD values in ELISA, which may be due to low virus concentration as reported by Sadeghi *et al* (2000).

Conclusion:

Serological tests samples collected from surveyed field have revealed that GBNV was predominant than TSV in both the districts. The ELISA results indicated that the leaf area showing chlorotic / necrotic lesions either alone or in combination were positive to GBNV, TSV or both and strengthen the symptomatic expressions of both the diseases.

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