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Serological and Molecular Detection of Peanut Bud Necrosis Virus (PBNV) causing Bud Necrosis Disease in Groundnut

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Groundnut bud necrosis disease caused by *Peanut bud necrosis virus* (PBNV) is a distinct member in the genus *Tospovirus* of the *Bunyaviridae*, and is the most economically important disease affecting groundnut crop. Bud necrosis infected groundnut plant samples were collected from ten groundnut growing districts of Karnataka were subjected to Direct antigen coated-enzyme linked immunosorbent assay (DAC-ELISA) and reverse transcription-polymerase chain reaction (RT-PCR) to detect GBNV. All the samples showed positive reactions with polyclonal antibodies of GBNV in DAC-ELISA and amplification of 831 bp GBNV coat protein gene by RT-PCR with degenerate primers.

Key words: Groundnut, GBNV-CP, DAC-ELISA, RT-PCR, Detection, Bud necrosis.

Groundnut bud necrosis virus (GBNV) is one of the most economically important virus diseases of groundnut crop. In India, bud necrosis disease caused by *Groundnut Bud Necrosis Virus* (GBNV) or *Peanut Bud Necrosis Virus* (PBNV) has become one of the major diseases. Bud necrosis disease in groundnut has been described in India since 1962 with seven different names: groundnut mosaic, groundnut rosette, bunchy top, chlorosis, ring mottle, bud blight and ring mosaic (Reddy, 1988) commonly called as bud necrosis disease. In Karnataka, the disease was first reported from Dharwar (Siddaramaiah *et al.*, 1977).

Until 1990, in India GBND was reported to be caused by *Tomato spotted wilt virus* (TSWV) (Reddy *et al.*, 1991). The advancements in the serological and molecular detection techniques for plant viruses, revealed the existence of several distinct Tospoviruses (German *et al.*, 1992). In 1992, the virus causing PBND was identified as a distinct Tospovirus and named as *Peanut bud necrosis virus* (PBNV) (Reddy *et al*., 1995).

In groundnut bud necrosis disease initially starts as mild chlorotic mottle or spots, later develop into necrotic and chlorotic rings and streaks. The primary symptoms are seen on only young quadrifoliate leaves as chlorotic and necrotic spots appear leading to sudden necrosis of bud with or without chlorotic or necrotic rings on leaves. Secondary symptoms are stunting, auxiliary shoot proliferation and malformation of leaflets. Necrosis of terminal bud is a characteristic symptom. Early infected plants show stunted and bushy appearance. Seeds from diseased plants are small, shrivelled, mottled and discoloured, however late infected plants produce normal sized seeds but the testa on such seeds often mottled and cracked.

Because of diseased plants express different kinds of symptoms such as mild mosaic, mottling, chlorotic and necrotic ring spots etc. It is difficult to identify disease in early stage of

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infection under field condition. Therefore, in the present investigation on serological and RT-PCR based techniques were employed for the detection of GBNV in groundnut.

MATERIALS AND METHODS

The GBNV infected groundnut plant samples showing typical symptoms were collected from farmers' fields of major groundnut growing ten districts of Karnataka during *kharif* and rabi/ summer seasons 2012-13 and subjected for detection of GBNV by Direct antigen coated-enzyme linked immunosorbent assay (DAC-ELISA) and reverse transcription-polymerase chain reaction (RT-PCR) methods.

Serological detection

Ten GBNV infected samples of the groundnut plants were collected from each districts i.e., Dharwad, Haveri, Belgaum, Bagalkot, Raichur, Gadag, Koppal, Bellary Chitradurga, Tumkur and healthy sample was tested as control. For serological studies polyclonal antiserum of GBNV was obtained from ICRISAT, Hyderabad (AP). Direct antigen coating (DAC) ELISA was performed as per the procedure of Hobbs *et al.* (1987). The antigen was extracted from 100 mg of sample per 1 ml of coating buffer and an antibody dilution of 10^{-3} was used. The ELISA absorbance at 405 nm were obtained after 20 min using an ELISA plate reader (Compaq Imaging System). Sample which gave double folds of the ELISA values of the healthy (control) were considered as a positive (Clark and Adams, 1977).

Molecular detection

Ten field samples of infected groundnut plants were used for RNA isolation. RNA from corresponding healthy plant sample used as control. Total RNA was extracted using RNeasy Plant Mini Kit (Qiagen Inc., Chatsworth, CA, USA). cDNA was synthesized from mRNA through reverse transcriptase in a 20 ¼l reaction mixture containing 5 ng of total RNA isolated from the infected and non infected samples of groundnut. 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' TTACAMTTCCARMG AAGKRCHAG 3' 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 1 min of denaturation at 94ºC, 1 min annealing at 52ºC, 1 min for extension at 72ºC, followed by one cycle of final extension for 10 min at 72ºC. RT-PCR amplified products were analyzed in 1percent agarose gel stained with ethidium bromide, electrophoresis at 60V for 1 h.

RESULTS AND DISCUSSION

DAC-ELISA was employed by using GBNV antiserum to detect the presence of GBNV in different field samples. 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 varied from sample to sample, which may be due to variation in the concentration of the virus in the bud necrosis diseased plant samples.

Serological (ELISA) (Clark and Adams, 1977; Hobbs *et al.,* 1987; Adam *et al.,* 1993**)** and molecular (RT-PCR) (Jain *et al.,* 1998; Reddy *et al.,* 2008; Akram and Naimuddin, 2010) techniques have been used to detect GBNV infecting different crops.

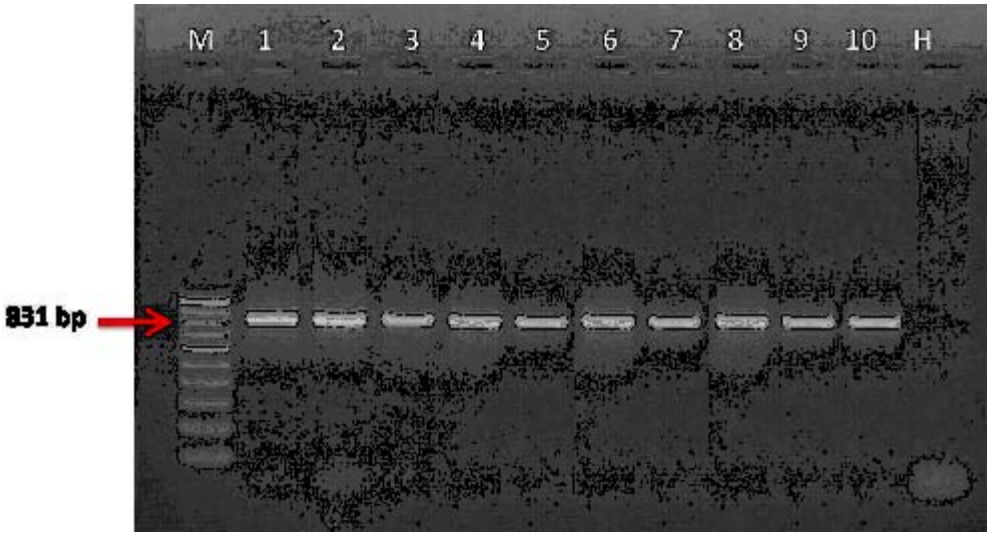
The serological based detection of GBNV virus suggested the association of an isolate of GBNV with bud necrosis disease of groundnut. From DAC- ELISA the absorbance values ranged form 1.907 to 2.604. The maximum absorbance value was in Bagalkot sample followed by Dharwad, Belgaum, Raichur districts (Table 1). The results are in confirmation with earlier findings through ELISA in groundnut (Reddy *et al*., 2008), tomato (Manjunatha, 2008; Balol and Patil, 2014), *Tomato spotted wilt virus* (TSWV) on groundnut (Dang *et al.,* 2009).

All ten field samples showed positive results in RT-PCR. Analysis in agarose gel electrophoresis revealed the presence of amplicons of ~831bp corresponding to *CP* genes of GBNV (Fig.1). Various researches have detected GBNV based in RT-PCR on various crops i.e., on groundnut (Gupta and Shukla, 2011; Chander Rao *et al*., 2012), tomato (Manjunatha, 2008; Balol and Patil, 2014; Raja and Jain, 2006; Akhter *et al.*, 2012), mungbean (Thien *et al*., 2003) and pea (Akram and Naimuddin, 2010) and in potato stem necrosis

Table 1. Serological detection of GBNV in groundnut bud necrosis samples

S. No.	Groundnut leaf samples District	Village/ Place	DAC-ELISA Absorbance values at 405 nm *	Result
1	Dharwad	UAS, Dharwad	2.526	+
2	Haveri	Yettinahalli	1.907	+
3	Belgaum	Tadakoda	2.472	+
4	Bagalkot	Hulageri	2.604	+
5	Raichur	Devadurga	2.239	+
6	Gadag	Lakkundi	2.444	+
7	Koppal	Hanumasagara	2.588	+
8	Bellary	Hosahalli	2.751	+
9	Chitradurga	Rayapura	2.531	+
10	Tumkur	Gundarlahalli	2.490	+
11	Healthy	-	0.713	-
12	Buffer	-	0.281	-

* Average of three replications.



Lane M: 100 bp Marker, 1= Dharwad , 2= Haveri, 3= Belgaum, 4= Bagalkot , 5= Raichur, 6=Gadag, 7=Koppal, 8=Bellary, 9=Chitradurga, 10=Tumkur and H= Healthy sample.

Fig.1. Molecular detection of GBNV in groundnut bud necrosis disease samples

caused by GBNV (Kaushal *et al.,* 2010). From the present study and it is concluded that groundnut bud necrosis disease caused by GBNV is more prevalent in groundnut production in different areas of Karnataka.

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REFERENCES

1. Adam, G., Yeh, S.D., Reddy, D.V.R. and Green, S.K., Serological comparison of Tospovirus isolates from Taiwan and India with Impatiens necrotic spot virus and different tomato spotted wilt virus isolates. *Arch. Virol.* 1993;**130**: 237-250.
2. Akhter, M. S.,Holkar, S. K.,Akanda, A. M.,Mandal, B.,Jain, R. K., First report of *Groundnut bud necrosis virus* in tomato in Bangladesh. *Plant Dis.* 2012; **96** (6): 917-918.

3. Akram, M. and Naimuddin, First report of *Groundnut bud necrosis virus* infecting pea (*Pisum sativum*) in India. *New Dis. Rep.* 2010; [doi:10.5197/j.20440588. 2010.021.010].
4. Balol G.B. and Patil, M. S., Biological charecterization and detection of *Groundnut bud necrosis virus* (GBNV) in different parts of tomato. *J. Pure and Applied Microbiol.* 2014; **8**(1): 749-752.
5. Chnder Rao, S., Bhatnagar-Mathur,P., Kumar, P. L., Reddy, A. S., and Sharma, K. K., Pathogen-derived resistance using a viral nucleocapsid gene confers only partial non-durable protection in peanut against peanut bud necrosis virus. Arch. Virol. 2012; DOI 10.1007/s00705-012-1483-8
6. Clark, M.F. and Adam, A.N., Characteristics of the microplate method of enzyme-linked immunosorbent assay for detection of plant viruses. *J. Gen. Virol.*1977;**34**: 475-483.
7. Dang, P. M., Rowland, D. L. and Faircloth, W. H., Comparison of ELISA and RT-PCR assays for the detection of *Tomato spotted wilt virus* in peanut. *PeanutSci*., 2009;**36**: 133-137.
8. German, T. L., Ullman, D. E., and Moyer, J. W., Tospoviruses: diagnosis, molecular biology, phylogeny and vector relationships. *Annu. Rev. Phytopathol.*1992;**30**: 315-348.
9. Gupta, U.P. and Shukla, L. K., Groundnut necrosis disease caused by a strain of *Groundnut bud necrosis virus. Indian J. Research*, 2011, **5:**46-50.
10. Hobbs, H.A., Reddy, D.V.R., Rajeshwari, R. and Reddy, A.S., Use of direct antigen coating and protein A coating ELISA procedures for detection of three peanut viruses. *Plant Dis.*,1987;**71:** 747-749.
11. Jain, R. K., Pappu, S. S., Pappu, H. R. And Culbbreath, A. K., Molecular diagnosis of *Tomato spotted wilt* tospovirus infection of peanut and other greenhouse crops. *Plant Dis.*1998; **82**(8):900-904.
12. Kaushal, N., Bhatnagar, A., Tiwari, J. K., Kumar, D., Kaundal, P., Pandey, S. K. and Garg, I. D., Print capture RT-PCR to detect *Groundnut bud necrosis virus* cause of potato stem necrosis disease. *Potato J*., 2010; **37** (3 - 4): 117-120.
13. Manjunatha, L., Studies on bud blight disease of tomato caused by groundnut bud necrosis virus.M.Sc. Thesis, University of Agricultural Sciences, Dharwad (India), 2008.
14. Raja, P. and Jain R. K., Molecular diagnosis of groundnut bud necrosis virus causing bud blight of tomato. *Indian Phytopath.*2006; **59**(3): 359-362.
15. Reddy, A.B., Krishna Reddy, M., Salil Jalali, Patil, M.S. and Usharani, Detection of a *tospovirus* infecting tomato *(Solanum lycopersicum). Indian J. Virol.*2008; **19**(1): 32-35.
16. Reddy, D. V. R., Virus disease in "groundnut" Ed. Reddy, P. S., Indian Council of Agricultural Research Publication, New Delhi. 1988; pp: 508-525.
17. Reddy, D. V. R., Satyanarayan, T., Buiel, A. A. M., Dwivedi, S. L., Reddy, A. S., Ratna, A. S., Lakhmi, V., Rao, R. G. V., Naidu, R. A. and Wightman, J. A., Peanut bud necrosis disease an overview. Recent studies on peanut bud necrosis disease: *Proc. Meet. ICRISAT* Asia Center, Andhra Pradesh, India, 20 Mar 1995.
18. Reddy, D. V. R., Sudarshana, A. S., Ratna, A. S., Reddy, A. S. and Amin, P. W., The occurrence of yellow spot virus, a member of *tomato spotted wilt virus* group on peanut (*Arachis hypogaea* L.) in India. In; *In virus thrips plant interaction of tomato spotted wilt virus* Ed. Hsu, H. T. and Lawson, R. H. *Proc. USDA workshop, US Dep. Agric., Agric. Res. Ser.* USA, 1991; pp: 77-88.
19. Siddaramaiah, A. L., Krishnaprasad, K. L. and Jayamariah, S., Bud necrosis diseases of groundnut in Karnataka. *Kissan world*, 1977;**4** : 42-44.
20. Thien H. X., Bhat, A. I. and Jain, R. K., Mungbean necrosis disease caused by a strain of *Groundnut Bud Necrosis Virus. Omonrice*, 2003;**11**: 110-116