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SERODIAGNOSIS OF PEANUT BUD NECROSIS VIRUS OF GROUNDNUT OCCURRING IN NORTH- EASTERN KARNATAKA

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ABSTRACT

The groundnut samples with peanut bud necrosis were collected from different parts of north eastern Karnataka and subjected to DAC-ELISA technique using polyclonal antiserum of PBNV and TSWV. All samples reacted positively with PBNV, while gave negative reaction against TSWV antisera. The weed samples prevailing in groundnut ecosystem, subjected to DAC-ELISA against PBNV also gave negative reaction. ELISA for different symptomatic PBND groundnut plants revealed that the primary symptomatic plants contained higher virus concentration. Furthermore, PBNV infected leaves with primary symptoms had highest virus titre. The study revealed that the virus causing bud necrosis in groundnut and tomato in north eastern Karnataka was PBNV. There was no natural infection of PBNV in major weeds prevailing in groundnut fields.

Key Words : Peanut bud necrosis disease, groundnut, ELISA

Peanut bud necrosis disease (PBND) caused by peanut bud necrosis virus (PBNV) has became a major constraint for groundnut production (Reddy, 1988). The virus belongs to Tospovirus group under the family Bunyaviridae. In north eastern Karnataka, PBND occurs during both kharif and rabi/summer seasons, causing yield losses from 30 to 90 per cent (Patil, 1993). Though PBND can be diagnosed quickly by visual examination, tomato spotted wilt virus (TSWV) induces almost similar symptoms on groundnut. Even tobacco streak virus (TSV) produces similar symptoms. Hence, identification of virus on the basis of symptoms is unreliable. Present investigation was therefore undertaken to confirm identity of virus causing PBND of groundnut and the infection of PBNV in different weeds prevailing in groundnut field. For this purpose ELISA (Enzyme linked immuno-absorbent assay) was employed.

The investigations were carried out during 2010 at Mycotoxicology and Virology laboratory, ICRISAT, Patancheru, Hyderabad. The groundnut samples showing typical primary and secondary symptoms of PBND viz., chlorosis and necrosis of quadrifoliate leaves, bud necrosis, malformed leaflets, axillary shoot proliferation, stunting etc. were collected from Raichur, Koppal and Bellary districts of Karnataka. Ten isolates were collected from groundnut, along with a suspected PBNV sample from tomato and the weeds (*Amaranthus viridis, Tridax procumbance, Malvastrum coromendalinum, Euphorbia hirta, Euphorbia geniculata, Portulaca olaracea* and *Parthenium hysterophorus*). The samples collected from groundnut and tomato were inoculated on cowpea cv. Pusa Komal (C-152), a local lesion host for PBNV. Direct antigen coating enzyme linked immunosorbent assay (DAC-ELISA) procedure described by Hobbs et al. (1987) was performed with alkaline phosphate (ALP) system. The crude extracts prepared in carbonate buffer (1:50) were added to ELISA plates. Polyclonal antiserum of PBNV with 1:5000 dilution and TSWV with 1:1000 dilution were used. ALP-labelled anti rabbit IgG were added at a dilution of 1:500 and absorbance values at 405 nm were measured using 'Titertek

multiskan' ELISA reader after 30 min. of reaction. The readings were considered positive if they were five times more than the healthy samples.

The virus isolates of groundnut and an isolate from tomato, when inoculated on to cowpea (*Vigna unguiculata* cv. C-152), produced chlorotic ring spots on cotyledonary leaves within 4-6 days. Similar kind of symptoms were observed by Prasad Rao et al.(2007). The results indicated that the isolates collected from groundnut and tomato belonged to tospovirus group.The results of DAC-ELISA indicated that all 11 groundnut samples showing primary and secondary symptoms of bud necrosis, along with PBND groundnut sample from ICRISAT, Hyderabad reacted positively with PBNV polyclonal antiserum raised against PBNV nucleocapsid protein (Table 1). Further, the same groundnut samples recorded absorbance values ranging from 0.08 to 0.10 against TSWV antiserum indicating negative reaction. The tomato sample with necrotic symptoms on leaves after subjecting to DAC-ELISA, were found positive (2.23) for presence of PBNV nucleocapsid protein, while reacted negatively (0.09) with TSWV polyclonal antiserum (Plate 2). The results confirmed that the virus causing peanut bud necrosis disease in groundnut and bud necrosis in tomato in north eastern Karnataka is PBNV. Reddy et al. (1992) reported that the virus

Table 1 : Detection of peanut bud necrosis virus in groundnut and tomato samples through DAC-ELISA

Sl.No.	District and crop	Taluk/place	Absorbance value (A_{405nm})			
			PBNV antiserum		TSWV antiserum	
			A_{405nm}	Reaction	A_{405nm}	Reaction*
1	Raichur(Groundnut)	MARS, Raichur	2.63	+	0.09	-
		Raichur	2.80	+	0.09	-
		Manvi	2.30	+	0.08	-
		Deodurga	2.20	+	0.09	-
		Sindhanur	2.59	+	0.10	-
		Lingasgur	2.45	+	0.09	-
2	Bellary(Groundnut)	Bellary	2.75	+	0.09	-
		Siraguppa	2.62	+	0.08	-
3	Koppal(Groundnut)	Koppal	1.20	+	0.09	-
		Yalaburga	2.60	+	0.08	-
		Kushtagi	1.88	+	0.09	-
4	Hyderabad(Groundnut)	ICRISAT, Hyderabad	2.23	+	0.09	-
5	Raichur(Tomato)	MARS, Raichur	2.23	+	0.09	-
6	PBNV +ve control	2.53	+	0.09	-	
7	TSWV +ve control	-	-	2.62	+	
8	Healthy	0.10	-	0.10	-	
9	Buffer control	0.09	-	0.09	-	

Table 2 : Detection of peanut bud necrosis virus in different symptomatic plants of groundnut

Sl.No.	Symptoms	Absorbance value(A_{405nm})	Reaction*
1	Chlorotic spots on leaves	2.63	+
2	Necrotic spots on leaves	2.50	+
3	Chlorotic and necrotic spots together	2.52	+
4	Necrotic bud	0.92	+
5	Malformed leaflets	1.60	+
6	Axillary shoot proliferation	2.20	+
7	Stunted plant	2.10	+
8	Positive control	3.04	+
9	Healthy	0.10	-
10	Buffer control	0.09	-

causing PBNV is serologically distinct from TSWV.

The absorbance values for weed samples ranged from 0.09 to 0.11 whereas, the PBNV positive sample recorded it as 3.04 (Table 3), which revealed that none of the weed samples were positive for the presence of PBNV. Thus there was no natural infection by PBNV in the collected weed samples. Although the virus show wide host range including tomato, chillies, potato, greengram, blackgram, cowpea and ornamentals (Reddy, 1991), absence of natural infection indicates that the weeds might be playing minor role as source of inoculums.

The results also revealed that all infected plant parts (Leaves, stem, roots, pegs and pods) were positive for the presence of PBNV nucleocapsid protein indicating systemic

Table 3 : Detection of peanut bud necrosis virus in major weed samples prevailing in groundnut ecosystem at MARS, Raichur

Sl.No.	Weed	Symptoms	Absorbance value (A_{405nm})	Reaction*
1	*Amaranthus viridis*	No symptoms	0.10	-
2	*Tridax procumbance*	No symptoms	0.09	-
3	*Malvastrum coromandalnum*	Yellowing	0.08	-
4	*Euphorbia hirta*	No symptoms	0.11	-
5	*Euphorbia geniculata*	No symptoms	0.09	-
6	*Portulaca oleracea*	No symptoms	0.09	-
7	*Parthenium hysterophorus*	No symptoms	0.08	-
8	PBNV +ve sample		3.04	+
9	Healthy sample		0.10	-
10	Buffer control		0.09	-

Plate 1. Chlorotic lesions of PBNV on local lesion cowpea cv. C-152.

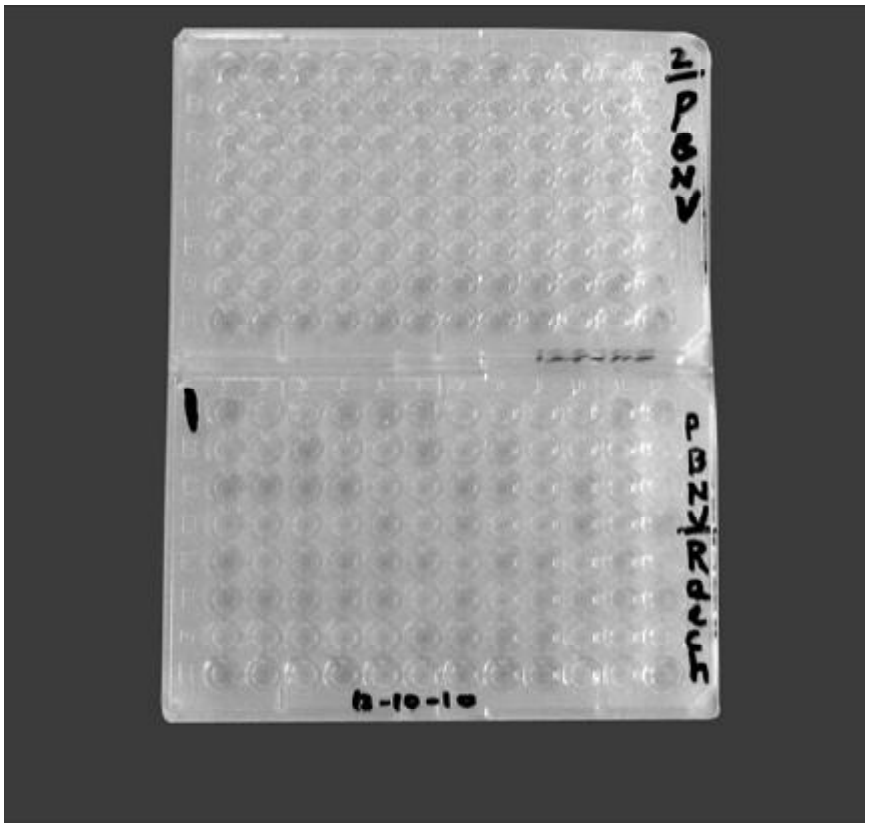

Plate 2. ELISA plate showing the reaction of different plant samples against peanut bud necrosis polyclonal antiserum.

infection. Among the different plant parts tested, the maximum virus titre was found in young symptomatic leaf, while lowest was in secondary root. Anjaneya Reddy et al. (2008) studied PBNV in different plant parts of tomato and reported highest virus titre in infected flower while lowest in ripened fruit. Young leaves got highest virus titre because of low inhibitory substances in them, than in other plant parts.

Thus the study revealed that the virus causing bud necrosis in groundnut and tomato is PBNV and there was no natural infection of PBNV in major weeds prevailing with groundnut.

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