Indian J. Virol. Vol. 12, No. 1, pp 79-82 (January 1996)
Short Communication

IDENTIFICATION OF BUD BLIGHT OF SOYBEAN (*Glycine max* L. MERR.) THROUGH ELISA AND INFECTIVITY ASSAY

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SUMMARY

In DAC-ELISA, the virus from soybean bud blight infected plants reacted strongly with the antisera of bud necrosis virus (BNV) of groundnut. Of 19 soybean samples tested, 14 samples were positive for BNV alone. However, one sample was positive for BNV, cowpea mild mottle virus (CMMV) and peanut stripe virus (PStV) in repeated DAC-ELISA tests. The results of dot immunobinding assay also showed positive reaction with the antisera of BNV at 10^{-1} and 10^{-2} dilution. Mechanical inoculation on cowpea cv. C-152 and groundnut cv. JL 24 exhibited necrotic ring spots and chlorotic spots respectively after 6 days of inoculation. These symptoms were also typical to BNV indicating the association of BNV in causing bud blight of soybean in India.

Soybean (*Glycine max* L. Merr.) is the most important oilseed crop cultivated during monsoon season in different parts of Madhya Pradesh. It is vulnerable to attack by number of viral diseases, of which bud blight caused by tobacco ring spot virus (TRSV) is one of the important diseases reported for the first time in India from Kangra Valley in Himachal Pradesh (Gupta, 1976). It was subsequently reported from different parts of the country (Thakur *et al.*, 1993) but suddenly, it assumed near epiphytotic proportions in Malwa region of Madhya Pradesh during kharif, 1987 (Srivastava *et al.*, 1987). Based on symptomatology, physical properties, seed transmission and serology, bud blight was known to be caused by TRSV (Anonymous, 1992-93, Gupta, 1976, Srivastava *et al.*, 1987 and Thombre *et al.*, 1989). Later studies however, indicated no virus like agent to be associated with bud blight of soybean (Singh *et al.*, 1991). Nevertheless, some others indicated the involvement of tomato spotted wilt virus (TSWV) (Reddy *et al.*, 1983) or bud necrosis virus (BNV) (personal communication). To answer these questions, an attempt was made to identify the causal agent based on serological and biological tests.

Soybean plants exhibiting minute chlorotic spots on young trifoliate leaves and bud blight symptoms on terminal buds were collected from research Farm, Indira Gandhi Agricultural University, Raipur. These plants were then taken to the Legume Virology Unit, ICRISAT, Patancheru (A.P.) for detection of associated virus through following techniques.

Indirect enzyme linked immnuosorbent assay (ELISA)

Direct antigen coating (DAC) ELISA was employed (Hobbs *et al.*, 1987). Nineteen samples of soybean exhibiting bud blight symptoms were tested alongwith BNV infected groundnut leaves (glass house sample) as a known positive control and healthy leaves of soybean as negative control. All soybean samples were extracted using carbonate buffer (0.01 M, pH 9.6) with DIECA as antioxidant. Crude antisera of BNV, cowpea mild mottle virus (CMMV) and peanut stripe virus (PStV) were used at a 1:1000 dilution. All antisera were cross absorbed with healthy soybean leaf extract (in antigen buffer at 50 g/litre)

for 1h at 37°C. Rabbit Fc-specific IgG's were conjugated to penicillinase (Hindustan Antibiotics Ltd., Pimpri, India) using the glutaraldehyde method (Sudarsana and Reddy, 1989) and employed at 1:10,000 dilution. Thereafter, bromothymol blue (0.15 mg/ml of d.w. containing 0.01 M NaOH) having benzyl penicillin (0.5mg/ml of bromothymol blue) and adjusted to pH 7.2 was used as a substrate buffer. Absorbence values were taken at 620 nm with a Titertek Multiscan MCC ELISA reader 1h after adding the substrate. A sample was considered infected when its mean absorbence value were greater than that of mean (-) 2 SD of the mean healthy controls.

Dot Immunobinding assay (DIBA)

Soybean sample (c) exhibiting bud blight symptoms was tested with BNV infected groundnut leaves (b) as known positive and healthy groundnut leaves (a) as negative controls. These samples were extracted using carbonate buffer (pH 9.6) at 10^{-1}, 10^{-2} and 10^{-3} dilution. The extract (5 µl) was then immobilised onto a nitrocellulose membrane (Schleicher and Schull BA 83, 0.45 µm pore size) using micropipette. It was airdried (15 min.) and blocked for 1h by putting in 5 ml of blocking solution (bovine serum albumin 5%). It was cross adsorbed with the healthy leaf extract of groundnut containing BNV antiserum at 1:1000 dilution. The membrane was then immobilised in 5 ml of BNV antiserum (groundnut) for 45 min. Trapped antibodies were probed with anti-EC horse-radish peroxidase (Sigma, USA) conjugate prepared by periodate oxidation method (Barbara and Clark, 1982) and used at 1:500 dilution. The membrane was finally put into the solution of substrate buffer. The substrate, 3, 3', 5, 5'-tetramethyl benzidine combined with an enhancer was obtained from Kirkegaard and Perry Laboratories USA. The substrate on reaction with the enzyme changed the colour of dot to bluish.

Infectivity assay

Four soybean samples showing strong positive reaction in DAC-ELISA, referred to be separate isolates, were used for biological assay. They were individually extracted (usually with a pre chilled mortar and pestle) using chilled 0.05 M phosphate buffer, pH 7.0. The extract at a dilution of 10^{-1} was used for inoculation. It was kept chilled (4°C) till inoculated. The assay plants prior to inoculation were dusted with fine carborundum (400 mesh) powder so as to form a thin layer of abrasive on the surface. Each isolate was inoculated on four plants each of cowpea cv. C-152 and groundnut cv. JL 24 which were the diagnostic and systemic assay hosts, respectively for BNV. These plants were then kept in glasshouse. They were observed carefully for production of symptoms.

In DAC-ELISA, the virus from soybean bud blight infected plants reacted strongly with the antisera of BNV from groundnut. Of 19 samples tested, 14 samples were positive for BNV alone (Table 1). However, one sample was positive for BNV, CMMV and PStV in repeated DAC-ELISA tests. Crude antisera of CMMV and PStV used against 19 samples of soybean bud blight failed to detect the virus excepting one sample. The results of DIBA also showed positive reaction with the antisera of BNV at 10-1 and 10-2 dilution as against no reaction observed in control at any of the dilutions. Infectivity assay on cowpea and groundnut exhibited chlorotic rings/nectrotic ring spot and chlorotic spots respectively after six days of inoculation by two isolates only. Remaining two isolates did not exhibit any symptoms. The symptoms produced by two soybean isolates

Table 1: Detection of virus in bud blight infected samples of soybean by DAC-ELISA

Bud blight samples	Antisera*		
	BNV	CMMV	PStV
1	0.436	1.151	1.341
2	0.278	1.201	1.228
3	0.744	1.157	1.234
4	0.134	1.158	1.235
5	1.127	1.210	1.222
6	0.112	1.190	1.228
7	0.263	1.180	1.315
8	0.310	1.170	1.310
9	0.433	1.171	1.331
10	0.184	1.112	1.310
11	0.579	1.210	1.312
12	0.840	1.182	1.282
13	0.171	1.230	1.232
14	0.077	1.210	1.282
15	0.540	1.210	1.312
16	0.082	1.111	1.310
17	1.382	1.171	1.317
18	0.075	1.213	1.293
19	0.240	0.368	0.510
Neg. control (Healthy soybean leaves)	1.405	1.236	1.372
+ve control (BNV infected groundnut leaves)	0.092	0.082	0.078
Buffer	1.932	1.929	1.938

*BNV Bud Necrosis Virus
CMMV Cowpea Mild Mottle Virus
PStV Peanut Stripe Virus

were typical of BNV on these hosts. The results of serological and biological testing indicated clearly that the bud blight of soybean is caused by BNV of groundnut. BNV is also known to cause bud necrosis disease of several economically important crops viz. mungbean and urdbean (Nene, 1972), tomato, cowpea (Ghanekar *et al.*, 1979b), peanut (Reddy *et al.*, 1983) etc., in India. The recent findings have shown that the TSWV reported from India is serologically different from that of TSWV isolates and impatience necrotic sport virus reported from Netherlands, Australia, Greece and USA (Reddy *et al.*, 1992).

ACKNOWLEDGEMENTS

The first author is thankful to the Training and Fellowship Division, ICRISAT, Patancheru (A.P.) for financial assistance.

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