Journal of Applied Horticulture, 9(2): 123-126, July-December, 2007

Occurrence and detection of sweet potato virus disease (SPVD) in West Bengal

B. Sinha and J. Tarafdar*

Department of Plant Pathology, Bidhan Chandra Krishi Viswa Vidyalaya, Mohanpur, Nadia-741252, West Bengal, E-mail: *bireswarsinha@gmail.com; *AICRP on Tuber Crops, Directorate of Research, BCKV, Kalyani, West Bengal. India. E-mail*: *jayanta.tcbckv@gmail.com*

Abstract

The natural occurrence of sweet potato virus disease (SPVD) in 26 Indian sweet potato cultivars was evaluated at Horticultural Experimental Field of the B.C.K.V. University, West Bengal during 2004-2005 seasons based on the possible symptoms and serology. The leaves from virus suspected plants were indexed for viruses by nitrocellulose membrane enzyme-linked immunosorbent assay (NCM-ELISA) and coat protein study. Disease incidence was highest in Pol-4-9 during 2004 (12.87%) and 2005 (25.19%). Results were confirmed in several seropositive plants with higher incidence and diversity of viruses**.** Sweet potato feathery mottle virus (SPFMV), sweet potato cauliflower mosaic like virus (SPCaLV), Sweet potato mild speck virus (SPMSV) and C-6 virus were detected serologically in single or mixed infections in many leaf samples of the cultivars. The frequency of C-6 virus was very high (73.07%) followed by SPCaLV (34.61%), SPFMV (26.92%) and SPMSV (23.07%). Attempt was made to characterize the virus coat protein of the partially purified virus from the leaves with most frequently observed symptoms. Protein analysis by sodium dodecyl sulfate polyacrylamide gel electrophoresis revealed a major protein band of 65 kDa, and 38 kDa which were assumed to be the viral coat proteins of associated virus. Minor protein bands of 24 kDa were also observed. The viral protein degraded upon storage at 4°C over time to yield a protein band of 22 kDa.

Key words: Sweet potato, viruses, symptoms, NCM-ELISA, coat protein

Introduction

Sweet potato (*Ipomoea batatas*) is considered to be the world's most important subsistence crop and is ranked seventh in global production. This crop is best suited to address the growing world concerns for food availability, nutrition and sustainable agricultural system. This crop is widely grown in tropical and sub-tropical regions with low input regimes. The crop is extremely important for developing countries, which produce 98% of the global production. Several biotic factors limit the productivity of this crop worldwide. Among these, viral diseases pose significant loss of the crop in terms of yield and quality of tuber as well as planting materials. Sweet potato virus disease (SPVD) is the main disease of this crop in Africa (Geddes, 1990) and elsewhere (Carey *et al.*, 1999). Despite the apparent broad meaning of the name, SPVD has become associated closely with symptoms caused by a combination of two viruses: Sweet potato chlorotic stunt virus (SPCSV) (Crinivirus; Closteroviridae) and Sweet potato feathery mottle virus (SPFMV) (Potyvirus; Potyviridae) (Gibson *et al.*, 1998; Karyeija *et al.,* 2000). In most cases these viruses occur as mixed infections and tend to be specific to members of the Convolvulaceae family (Moyer and Salazar, 1989). Moreover, it has been shown that these viral mixtures or interactions may lead to the occurrence of a synergistic effect which results in more severe damage to the crop than would be expected if an individual virus was present (Gutierrez *et al.,* 2003). The symptoms include severe stunting of the plant, distortion either a chlorotic mottle or vein clearing of leaves. The tuberous root yield of affected plants is also less.

Very little efforts have been made so far on the distribution, occurrence and characterization of viruses in India. Considering this fact, our preliminary work was devoted to investigate the natural incidence of the SPVD in some Indian cultivars and detection of viruses, if any, associated with SPVD using immunoenzymatic assay and virus coat protein characters.

Materials and methods

Field trial: 26 sweet potato cultivars were grown at the Horticultural Experimental Field in randomised block design in two consecutive years from 2004-2005. The natural incidence of SPVD among the cultivars was recorded based on the symptoms expression pattern. The intensity of the foliar symptoms among the cultivars was recorded in each replicated plot at 60 and 90 days after planting and analysed statistically.

Virus indexing by nitrocellulose membrane enzymed linked immunosorbent assay (NCM-ELISA): Detection of sweet potato viruses were carried out by immunoenzymatic method using nitrocellulose membrane enzymed linked immunosorbent assay (NCM-ELISA) following the protocol standardized by the International Potato Centre (CIP), Virology Laboratory, Lima, Peru. For this, sample preparation was carried out by cutting leaf disc of 1 cm diameter and ground in to 3 mL of Tris base (TBS) buffer of pH 7.5. After 30-45 minutes, 15µl of clear supernatant for each macerated leaf disc was dropped at the centre of the individual nitrocellulose membrane with the micropipette and membranes were kept 15-30 minutes for air drying. Then blocking of the membranes was done by TBS buffer of pH 7.5 and Triton X-100 followed by adding of antibody and then T-TBS buffer

solution and colour development solution of NBT and BCIP as by the protocols.

Coat protein: Partial purification of the virus and analysis of the coat protein was performed as per the methods described by Dougherty and Hilbert (1980) with slight modification by Gorsane *et al.* (1999). The leaves showing chlorotic feathery discolouration and vein clearing symptoms were harvested from the plants maintained in net house and immediately after harvest 5 g of diseased leaves were ground in liquid nitrogen and homogenized at a ratio of 1:3 (w/v) in 20 mM HEPES buffer of pH 7.6, containing 200mM urea and 0.1% sodium sulphite. The homogenate was filtered through two layers of cheese cloth and butanol-1 was added to make final concentration of 8%. The mixture was stirred over night at 4ºC and then clarified by centrifugation at 6000 rpm for 20 mins at 4ºC. After centrifugation, Triton X-100, Polyethylene glycol (6000 mol wt.) and NaCl were added to the supernatant separately for the final concentration of 2%, 4% and 0.1M, respectively and stirred at 4ºC for 90 min. This solution was centrifuged at 13000 rpm for 30 mins at 4ºC and pellet was resuspended in 10 mL of 20 mM HEPES buffer. After stirring over night at 4ºC the solution was spined at 8000 rpm for 10 mins. The supernatant was layered on a 20% sucrose cushion and again centrifuged at 21000 rpm for 15 min. The virus pellet was suspended in 300:1 Laemmli buffer and virus coat protein was detected by performing Sodium Dodecyl Sulphate Polyacrylamide gel electrophoresis (SDS-PAGE). In the SDS-PAGE, a broad range protein molecular weight marker (Promega Cat#V 8491) was used as marker protein.

Results and discussion

Disease incidence: Wide ranges of symptoms were noticed in sweet potato cultivars in both the years where chlorotic spot, feathery mottle with purple ring and mosaic symptoms were dominated on the plants. The results on the average percentage of incidence of SPVD for both the years (Table 1) revealed that intensity of the virus disease gradually increased with age of the plants in most of the cultivars. The overall intensity of the disease was high at 90 DAP (days after planting) in both the years. A differential symptom was noticed in the test cultivars. Moderately high incidence of the SPVD was observed in the local cultivars BCSP-10 and Kamala Sundari in the year 2004. It was also observed that SPVD symptoms increased to 9.44 and 7.78% in BCSP-10 and Kamala Sundari, respectively at 60DAP during the year 2005. The cultivar Pol-21-1 showed very high incidence of SPVD with 12.87 and 25.19% at 60 and 90 DAP, respectively, during 2005. However, it was observed that SPVD is consistently associated with the cultivars in both the seasons. Infection rate of viral diseases of sweet potato was 40% in China, (Shang *et al.,* 1999), 54 % in Uganda and 60% in Indonesia (Carey *et al.,* 1999). Studies indicate that there are five major poty viruses

Table1. Incidence of sweet potato virus disease (SPVD) during 2004-2005

Cultivars	Percent infection at two different stage of plant growth					
	60 DAP		Pooled data 2004 -2005	90 DAP		Pooled data 2004-2005
	2004	2005		2004	2005	
Tripty	2.22 (8.56)*	4.44 (12.16)	3.34 (10.53)	5.46 (13.51)	7.50 (15.89)	6.48 (14.74)
IBM-95-229	1.67 (7.42)	2.90 (9.80)	2.28 (8.68)	0.74 (4.93)	3.24 (10.37)	1.99 (8.10)
WBSP-4	2.22 (8.56)	2.96 (9.90)	2.59 (9.26)	4.91 (12.80)	3.35 (10.54)	4.13 (11.72)
IBM-95-220	2.59 (9.26)	3.89 (11.37)	3.24 (10.37)	2.47 (9.04)	8.15 (16.58)	5.31 (13.32)
Pol-20-6-2	5.01 (12.93)	6.66 (14.95)	5.83 (13.97)	4.31 (11.98)	7.59(15.99)	5.95 (14.11)
Pol-4-4-2	2.59 (9.26)	3.89 (11.37)	3.24 (10.37)	8.36 (16.80)	9.63 (18.07)	8.99 (17.44)
BCSP-14	3.14 (10.21)	4.46 (12.19)	3.8 (11.24)	3.33 (10.51)	4.81 (12.66)	4.07 (11.63)
Pol-4-9	2.22 (8.56)	4.26 (11.91)	3.24 (10.37)	2.22 (8.56)	7.13 (15.48)	4.67 (12.48)
BCSP-5	0.77 (5.03)	1.30 (6.54)	1.05 (5.88)	1.46 (6.94)	4.63 (12.42)	3.04 (10.04)
IBM-95-206	3.33 (10.51)	12.43 (4.64)	3.98 (11.50)	3.97 (11.49)	5.93 (14.09)	4.95 (12.85)
Pol-13-4	1.83 (7.77)	3.33 (10.51)	2.58 (9.24)	2.41 (8.93)	7.41 (15.79)	4.91 12.80)
Pol-21-1	2.41(8.93)	3.71 (11.10)	3.06 (10.07)	12.87 (21.02)	25.19 (30.12)	19.03 (25.86)
IGSP-7	1.11 (6.04)	2.41 (8.93)	1.76 (7.62)	0.56 (4.29)	1.67 (7.42)	1.12 (6.07)
NDSP-9	1.66 (7.40)	2.59 (9.26)	2.12 (8.37)	1.11 (6.04)	1.67 (7.42)	1.32 (6.59)
CO-3	2.41 (8.93)	4.26 (11.91)	3.33 (10.51)	3.39 (10.61)	1.11 (6.04)	3.77 (11.19)
IGSP-6	2.59 (9.26)	4.26 (11.91)	3.42 (10.65)	0.56 (4.29)	4.16 (11.76)	0.83 (5.22)
NDSP-10	4.26 (11.19)	6.48 (14.74)	5.37 (13.39)	3.49 (10.76)	6.48 (14.74)	4.98 (12.89)
S-1221	1.66 (7.44)	2.96 (9.90)	2.31 (8.74)	3.71 (11.10)	5.74 (13.86)	4.72 (12.54)
RNSP-1	2.89 (9.78)	5.37 (13.39)	4.13 (11.72)	1.11 (6.04)	5.93 (14.09)	3.52 (10.81)
IGSP-8	2.78 (9.59)	4.16 (11.76)	3.47 (10.73)	2.41 (8.95)	5.01 (13.93)	3.71 (11.10)
IGSP-9	1.67 (7.42)	3.15 (10.22)	2.41 (8.39)	0.74 (4.93)	2.96 (9.90)	1.85 (7.81)
BCSP-7	3.33 (10.51)	3.89 (11.37)	3.61 (10.95)	0.74 (4.93)	3.33 (10.51)	2.03 (8.19)
RNSP-2	0.92 (5.50)	1.85 (7.81)	1.38 (6.71)	3.15 (10.22)	4.91 (12.90)	4.03 (11.58)
RNSP-3	4.25 (11.89)	6.30 (14.55)	5.27 (13.27)	4.26 (11.91)	6.48 (14.74)	5.37 (13.39)
BCSP-10	6.11 (14.31)	9.44 (17.89)	7.77 (15.27)	2.01 (8.15)	3.34 (10.53)	2.67 (9.40)
Kamala Sundari	6.11 (14.31)	7.78 (16.19)	6.94 (15.27)	6.29 (14.52)	11.11 (19.47)	8.70 (17.15)
SEd±	0.03	0.09	1.76	0.11	0.03	0.33
CD(*P*=0.05)	0.05	0.13	2.49	0.14	0.05	0.46

* Figures in the parenthesis are angular transformed values

that could affect sweet potato production: sweet potato feathery mottle virus (SPFMV), sweet potato mild mottle virus (SPMMV), sweet potato latent virus (SPLV), sweet potato vein mosaic virus (SPVMV) and sweet potato yellow dwarf virus (SPYVD) (Moyer and Salazar, 1989).

Table 2. Detection of sweet potato viruses by NCM-ELISA in some Indian sweet potato cultivars

Cultivars	Sweet potato viruses					
	SPFMV	SPMMV	SwPLV	SPCFV	C-6	SPMSV
Tripty	+		+++	+++	++	
IBM – 95 – 229	+				+	
WBSP – 4	+				++	
IBM – 95 – 220	+				+	+
Pol – 20 – 6 – 2						
Pol – 4 – 4 – 5		+			+	
BCSP – 14			+		+++	
Pol – 4 – 9			+	++	+	
BCSP – 5		+++		+	+	+
IBM – 95 – 206				++	+++	+++
Pol – 13 – 4					+++	
Pol – 21 – 1	+++				++	
IGSP – 7		+				
NDSP – 9		+	+++		++	
CO – 3		+++	+		++	++
IGSP – 6				+++	+	+
NDSP – 10						
S – 1 – 221						
RNSP – 1					+	+
IGSP – 8						
IGSP – 9		+	+++	++	++	+++
BCSP – 7						
RNSP – 2	++	+			++	
RNSP – 3					+	

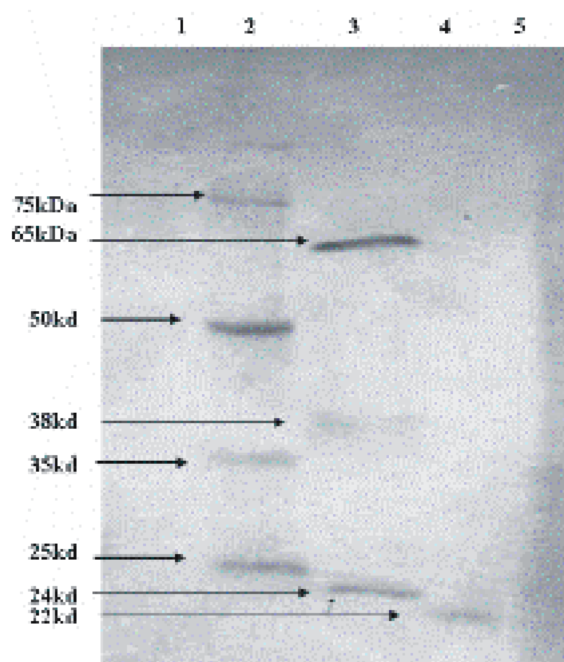

Fig. 1. SDS-PAGE of partially purified virus coat protein (Sample taken from SPVD infected plant)

Nitrocellulose membrane enzymed linked immunosorbent assay (NCM-ELISA): The results on indexing of eight viruses infecting 26 promising sweet potato cultivars are presented in Table 2. The serological tests by NCM-ELISA confirmed the viruses present in the cultivars and many of the samples which showed virus like symptoms did not show the positive reaction to the test. All the cultivars except Pol-20-6-2, NDSP-10, S-1221, IGSP-8, BCSP-7 and BCSP-10 showed positive indication for all the viruses alone or mixed infection. Among the cultivars IBM-95-220, Pol-4-4-5, IGSP-6, RNSP-1 and Kamala Sundari resulted in light purple colour for SPFMV, C-6, SPMSV and SPCaLV. Leaf samples (73.07%) gave positive results to C-6 virus followed by SPCaV (34.61%), SPFMV and SPMSV (26.92%) and SPMMV (23.07%). BCSP-5, RNSP-2, IGSP-9 and CO-3 showed moderate to high infection to different viruses in NCM-ELISA test. NCM-ELISA is found to be the most effective method for virus detection in sweet potato among NCM-ELISA, ELISA and ISEM which have also been tested by Yang *et al.* (1991).

Coat protein: Major protein bands of 65 and 38 kDa (Fig.1) were obtained which are assumed to be viral coat protein and a 24 kDa of minor protein band was observed. Total degeneration of coat protein of the isolated virus particle was observed upon exposure to high temperature (boiling temperature) whereas storage at low temperature (4ºC) gave apparently single 22kDa protein band. The results indicated that the isolated virus particle from infected plant would have the mixed infection with SPFMV and sweet potato ring spot nepo virus (SPRV) which has high sensitivity to freezing and thawing. Similar observations of three major coat proteins of 32, 30 and 29 kDa have been reported in *Ipomoea* sp infected with SPFMV (Usugi *et al.,* 1994).

A detailed description of the SPRV has been given by Brunt *et al.* (1996) and it suggested that SPRV had four different major protein subunits and the second largest version protein was identified as 65 kDa, which was not glycosylated or phosphorylated. However, our present investigations on coat protein of sweet potato feathrery mottle and purple ring producing agent have the affinity to other similar strains of SPFMV reported so far. It is also predicted that the occurrence of SPRV might have the component virus in producing SPVD syndrome on sweet potato plants infected in this area and similar observation was also reported by Usugi (1994).

The present study has clearly indicated the relative abundance and magnitude of virus diseases infection among the sweet potato varieties in West Bengal, which would be useful in further investigations on sweet potato viruses.

Acknowledgement

We are thankful to Department of Virology, International Potato Centre (CIP), Lima, Peru for kindly providing NCM-ELISA kit.

References

Brunt, A., K. Crabtree, M. Dallwitz, A. Gibbs and L. Watson, 1996. *Viruses of Plants: Descriptions and Lists from the VIDE Database.* C.A.B. International, U.K. 1484 pp.

Carey, E.E., R.W. Gibson, S. Fuentes, M. Machmud, R.O.M. Mwanga, G. Turyamureeba, L. Zhang, D. Ma, F. Abo. El-Abbas, R. Ei-Bedewy and L.F. Salazar, 1999. The causes and control of virus diseases of sweet potato in developing countries: Is sweet potato virus diseases the main problem? *CIP Technical Progress Report (1997-1998),* p. 421-428.

Dougherty, W.G. and E. Hilbert, 1980. Translation of potyvirus RNA in a rabbit reticulocyte lysate: cell free translation strategy and a genetic map of the potyviral genome. *Virology*, 104: 183-194.

Geddes, A.M. 1990. The relative importance of crop pests in sub-Saharan Africa. Bulletin No. 36, Natural Resources Institute (NRI), Chatham, GB.

Gibson, R.W., I. Mpembe, T. Alicai, E.E. Carey, R.O.M. Mwanga, S.E. Seal and H.J. Vetten, 1998. Symptoms, etiology and serological analysis of sweet potato virus disease in Uganda. *Plant Pathology,* 47: 95-102

Gorsane, F., H. Fakhfakh, T. Collete, M. Makni and M. Marrakchi, 1999. Some biological and molecular properties of pepper veinal mottle virus isolates occurring in Tunisia. *Plant Molecular Biology Reporter,* 17: 149-158.

Gutiérrez, D.L., S. Fuentes and L.F. Salazar, 2003. Sweet potato virus disease (SPVD): distribution, incidence, and effect on sweet potato yield in Peru. *Plant Disease,* 87: 297-302.

Karyeija, R.F., J.F. Kreuze, R.W. Gibson and J.P.T. Valkonen, 2000. Synergistic interactions of a potyvirus and a phloem-limited crinivirus in sweet potato plants. *Virology,* 269: 26-36.

Shang, You. Fen, Y. C. Liang, Zhao Jiuhua, L.I. Chang Song, Lu Xignbo, Ji Wang Sheng, Qi Xin Xiang, Y.F. Shang, C.L. Yang, J.H. Zhou, Li C.S., X.B. Lu, S.J. Wang and X.Q. Xin, 1999. Progress in the researches and application of virus free sweet potato in Shadong province. *Research progress in plant protection and plant nutrition,* p.104-108.

Moyer, J.W. and L.F. Salazar, 1989. Virus and virus-like diseases of sweet potato. *Plant Disease*, 74: 451-455.

Usugi, T., M. Nakano, M. Onuki, T. Maoka and T. Hayashi, 1994. A new strain of Sweet Potato Feathery Mottle virus that causes russet crack on fleshy roots of some Japanese cultivars of sweet potato. *Annals of the Phytopathological Society of Japan,* 60: 545-554.

Yang, Y.J. and J. Song, 1991. Yield increase effect of virus free sweet potato. *Jiangsu Agriculture Science*, 1: 45-48.