DETECTION OF SUGARCANE BACILLIFORM VIRUS IN SUGARCANE GERMPLASM

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Received December 12, 1996

Summary. – Sugarcane bacilliform virus (SCBV), a badnavirus was found in sugarcane genotypes of *Saccharum officinarum* L., *S. barberi* Jesw., *S. sinense* Roxb., *S. robustum* Brand and Jesw., and *Saccharum* hybrids. In most of the suspected genotypes the virus was found associated with clear foliar symptoms. However, certain symptom-free clones carried the virus too. The virus was detected by immuno-electron microscopy (IEM) and enzyme-linked immunosorbent assay (ELISA) in sucpected clones. The virions measured about 108-118 x 20-21 nm in size. The virus was serologically closely related to another badnavirus, banana streak virus (BSV). Virus titer was low in most of the genotypes. However, a close correlation between symptoms expression and virus titer existed in some genotypes.

Key words: sugarcane bacilliform virus; detection; immuno-electron microscopy; ELISA

Introduction

SCBV was first identified in the sugarcane hybrid Mex. 57-473 in Morocco in 1986 (Lockhart and Autrey, 1988). The virus was subsequently found to occur world-wide in most of the clones of *S. officinarum* commonly referred to as noble canes, and in fairly large number of commercial hybrids. The presence of SCBV infection in sugarcane collection maintained at Regional Centre of Sugarcane Breeding Institute, Cannanore, Kerala, was suspected during 1992-1993 and was later confirmed by electron microscopic studies (Viswanathan, 1994). The Sugarcane Breeding Institute maintains one of the world's biggest sugarcane collections having 3345 genotypes in Cannanore, Kerala, and 961 genotypes in Coimbatore and Wellington, Tamil Nadu. The viral infection was thought to be confined to just a few clones in 1992 but recent studies showed presence of the virus in many sugarcane genotypes in the germplasm collection. This paper reports the viral symptoms on the host, and the extent of virus incidence in the sugarcane germplasm determined by IEM and ELISA.

Abbreviations: BSV = banana streak virus; ELISA = enzyme-linked immunosorbent assay; IEM = immuno-electron microscopy; PBS = phosphate buffered saline; PVP = polyvinylpyrrolidone; SCBV = sugarcane bacilliform virus

Materials and Methods

Sugarcane hosts were represented by *S. officinarum, S. barberi, S. sinense, S. robustum, S. spontaneum,* related genera and sugarcane hybrids. The clones were observed for foliar and other symptoms from planting (February to March) till harvest (March, next year) during 1992-1995.

Antisera. Rabbit anti-SCBV- and anti-BSV-serum were supplied by Dr. B.E.L. Lockhart, University of Minesota, St. Paul, USA. For ELISA tests the antisera were diluted 1:500, 1:1000, and 1:2000 in PBS pH 7.2 with 2% polyvinylpyrrolidone (PVP, M_r 40,000), 0.5% Tween 20 and 1% ovalbumin. For IEM studies the antisera were diluted 1:100 with saline.

Virus extracts. Leaf samples (minus midrib) from suspected host plants were homogenized in 2.5 volumes (w/v) of PBS pH 7.2, containing PVP and Tween 20. The homogenates were centrifuged for 10 mins at 10,000 x g and the supernatants were used for IEM and ELISA.

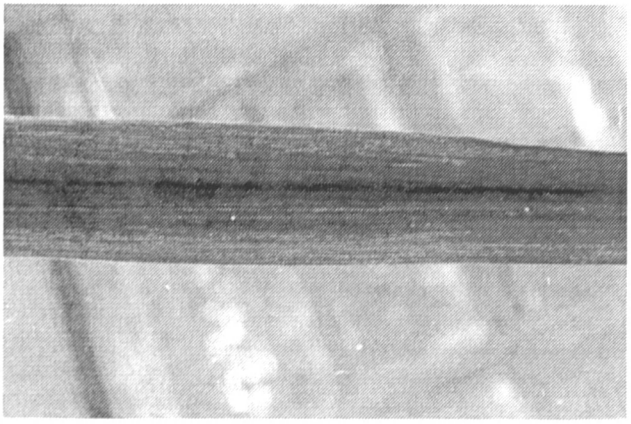

Fig. 1
Foliar symptoms of SCBV infection on sugarcane genotype D 1135

Electron microscopy and IEM. Collodion filmed copper grids were coated with 1:100 dilutions of SCBV or BSV antisera by floating on antisera drops for 25 mins followed by washing with 30 drops of distilled water and draining briefly by touching the grid edge with filter paper. Trapping of virions was carried out by immediate transfer of antisera-coated grids onto drops of crude virus extracts in humid Petri dishes which were incubated at 37°C for 1 hr. Grids were then washed and drained as mentioned above and negatively stained with 2% aqueous uranyl acetate. The grids were viewed at a displayed magnification of 21,000 x. Three holes per grid were observed to ascertain the relative concentration of the virus (Garg and Khurana, 1992).

ELISA. Indirect DAC-ELISA was performed according to Mowat and Dawson (1987). Microtiter plates (Corning) were coated with antigen extracts from virus-infected plants diluted 1:5, 1:10, 1:100, 1:200 and 1:500 and incubated overnight at 4°C. After washing with PBS-Tween 20 the plates were loaded with diluted antisera and incubated at 37°C for 3 hrs. After washing, goat anti-rabbit gamma-globulins conjugated with alkaline phosphatase (Sigma) diluted 1:8000 in the buffer were added to the wells and incubated at 37°C for 3 hrs. The plates were then rinsed, substrate solution (1 mg/ml paranitrophenyl phosphate) in 10% diethanolamine pH 9.8) was added and the plates were again incubated for 30 mins at room temperature. A_{405} was measured in an ELISA reader (Model EL 311s, Biotech Instruments, USA). Samples of virus-free genotype (CoC 671) served as control. A test was considered positive if its A_{405} exceeded the 3-fold of control.

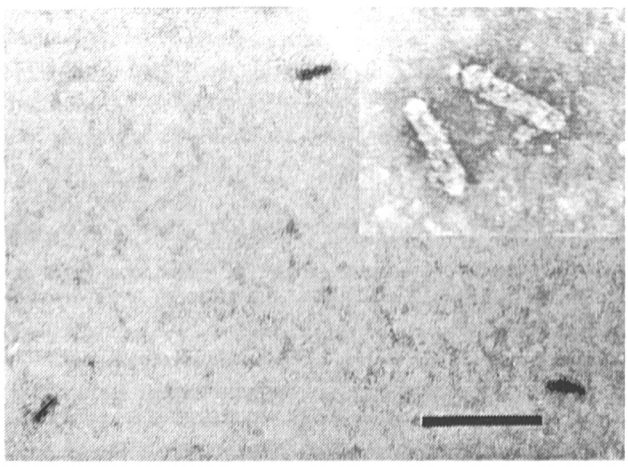

Fig. 2
IEM of SCBV
Virus trapped with antiserum to SCBV. Bar = 400 nm. Insert: enlarged view of virions.

Results

Viral symptoms in the host

Foliar symptoms were chlorotic streaks of varying length confined between veins. Symptoms appeared as chlorotic specks 0.5 – 2.0 mm long and 0.5 – 1.0 mm wide. Later the specks coalesced longitudinally and formed chlorotic streaks (Fig. 1). These specks started appearing on leaf tips and spread downwards. The foliar symptoms were apparent on first formed leaves in genotypes of Black Tanna, Listada (noble cannes) and D 1135 (hybrid). Interveinal chlorosis was also seen in mature leaves of the infected genotypes of Listada and D 1135. Many other associated symptoms were also observed in suspected genotypes. Stunted growth was seen in the severely infected noble cane genotypes of Black Tanna, Castilla, Guam A and Listada, and hybrid clones of D 1135, and D 109. No tillering was noticed in these genotypes and a poor tillering was noticed in many other suspected noble canes. Severely stunted canes showed deep longitudinal cracks on internodal surface. The stunted canes also showed progressive reduction in internodal elongation and the apex leaves failed to open freely and formed a bunchy top. A narrowing of leaf lamina was characteristic for Castilla and Listada. In all suspect clones symptoms expression was clear by 6 to 8 months stage. However, the genotypes showed a variation in expression of disease symptoms. Genotypes which showed clear foliar symptoms were those of Boengaja Bali, Boetata Bilatoe, Iscambine, Iscambine Rayee, HO 36, HO 43, Gros Genoux, Local Pattapatti, Portmackay Black, 57 NG 240, Striped Mauritius and Striped Tanna.

Electron microscopic studies

Electron microscopic examination of crude virus extracts revealed the presence of bacilliform particles in genotypes

Table 1. Detection of SCBV by IEM

Genotype	Group	Trapping[a]
Black Tanna	*S. officinarum*	++
Boetata Bilatoe	*S. officinarum*	–
Fiji 38	*S. officinarum*	++
Guam A	*S. officinarum*	+
Gros Genoux	*S. officinarum*	–
HO 36	*S. officinarum*	+
Ireng Malang	*S. officinarum*	+
Listada	*S. officinarum*	++
Kuswar Ottar	*S. barberi*	+
28 NG 104	*S. robustum*	++
D 1135	Hybrid	+
D 4/4	Hybrid	+
CoS 321	Hybrid	–

[a]No trapping (–); less than 5 virions per 20 cm^2 (+); 5 and more virions per 20 cm^2 (++); magnification of 21,000 x.

of D 1135, Black Tanna and Listada which showed typical foliar symptoms. The virus particles measured about 108-118 x 20-21 nm in size (Fig. 2). The IEM studies showed an enhanced trapping with the antiserum to SCBV. The concentration of the virions varied among different genotypes. The genotypes of Black Tanna, D 1135, Covengerie 972, HO 36 and 28 NG 104 showed higher virus concentration (5 to 6 virions/20 cm^2 of fluorescent screen area at magnification of 21,000 x). Some of the suspect clones were found positive for SCBV (Table 1). In addition, antiserum to BSV showed an equally good trapping of virions.

ELISA

Indirect DAC-ELISA using the antiserum to SCBV was able to detect reliably the virus in crude extracts under the conditions described. The antigen dilutions of 1:5, 1:10 and 1:40 did not give reliable results. Similarly, lower antiserum dilutions (1:500 and 1:1000) gave quick colour development which resulted in false results. The antigen dilution of 1:100 and the antiserum dilution of 1:2000 gave the most reproducible results. Although the antigen dilution of 1:500 gave positive results its A_{405} values were low (Table 2).

Table 2. Detection of SCBV by indirect DAC-ELISA

Genotype	A_{405}[a]
S. officinarum	
Badila	.098
Black Tanna	.110
Iscambine	.061
Iscambine Rayee	.079
Listada	.113
Portmackey Black	.075
Hybrids	
C 279	.093
CP 44-101	.102
D 1135	.177
Q 69	.127
PoJ 2725	.093
CoC 671 (control)	.008

Antigen was diluted 1:200, antiserum 1:2000, and enzyme conjugate 1:8000.
[a]Mean of 6 values.

Discussion

The Sugarcane Breeding Institute maintains one of the largest collections of sugarcane germplasm genotypes. Although the presence of SCBV in sugarcane germplasm was suspected already a few years ago, the present studies have conclusively confirmed the occurence of the virus. The virus produced suspect symptoms on many genotypes of *S. officinarum, S. barberi, S. robustum* and some hybrids. Symptoms comprising interveinal chlorosis, stunting and premature death were observed namely in genotypes of D 1135, Black Tanna and Listada. However, Lockhart *et al.* (1992) reported that SCBV did not induce foliar symptoms in most of the clones tested.

Whereas all clones with foliar symptoms were found positive for the virus in ELISA, a few of them were found negative in IEM. The negative results in IEM might be due to a very low concentration of the virus in those clones (Irey *et al.*, 1992). The enhanced trapping of the virions with the antiserum to BSV in the present studies corroborated the results of studies of Lockhart and Autrey (1988).

A positive relation between the severity of foliar disease symptoms and the concentration of the virus in both the IEM and ELISA was found in some genotypes (viz. D 1135, Black Tanna and Listada) but not in others (viz. Rood Djapara, Lahi 14, Badila and Manjuria).

The incidence of SCBV was higher in the genotypes of *S. officinarum* than in commercial hybrids. Similar observations were reported by Comstock and Lockhart (1990). Nonetheless, all hybrids older than 50 years, and 4 out of 21 current varietes in Australia were found infected with SCBV (Teakle and Egan, 1994).

Acknowledgements. The authors are indebted to Dr. B.E.L. Lockhart, University of Minnesota, St. Paul, USA for supplying the antisera, and to Dr. G.S. Shekhawat, Director of the Central Potato Research Institute, Shimla, for laboratory facilities.

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