

SUGARCANE BACILLIFORM VIRUS: SYMPTOMS, DETECTION AND DISTRIBUTION IN THE WORLD GERmplasm COLLECTION AT CANNANORE, INDIA

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ABSTRACT

Sugarcane bacilliform virus (SCBV) was detected in the world collection of sugarcane germplasm at Cannanore in recent years. The characteristic foliar symptoms were freckles, chlorotic stripes of varying length, narrowing of leaves, stunted growth, presence of internodal cracks and a "bunchy top" appearance. Among the different genotypes, those of Saccharum officinarum appear most prone to the virus. Genotypes of S. officinarum collected prior to 1970 were heavily infected with the virus. Most of the S. barberi and S. sinense genotypes were also infected. However only a limited number of genotypes of S. robustum and interspecific hybrids of this had suspected infection. Enzyme linked immunosorbent assay (ELISA) and immunosorbent electron microscopy (ISEM) were standardised for the detection of virus in the suspected genotypes. The presence of the virus in the sugarcane germplasm in India and other countries is a major obstacle to the safe exchange of germplasm. Immediate attention is warranted to enhance the precision of virus indexing by means of DNA probes and the polymerase chain reaction and also for eliminating the virus from infected genotypes.

Keywords: Sugarcane germplasm, sugarcane bacilliform virus, symptoms, detection, germplasm.

INTRODUCTION

The world collection of sugarcane germplasm maintained at Cannanore, Kerala State, consists of more than 300 genotypes of *Saccharum* spp. and related genera of grasses. Genotypes from national and international expeditions are added regularly after being quarantined for one year at Coimbatore. The location of Cannanore, far from commercial sugarcane plantations, facilitates maintenance of the collection in a pest and disease-free condition.

SCBV and other badnaviruses belong to the family *Caulimoviridae*. They are viruses containing a double stranded DNA genome which is replicated via reverse transcription. SCBV has non-enveloped, bacilliform particles averaging 30 x 120-150 nm in size, containing circular, double stranded DNA (7.5 kb) with three open reading frames (Lockhart et al, 1996).

SCBV was first reported in sugarcane from Cuba (Rodriguez-Lema et al, 1985) and Morocco (Lockhart and Autrey, 1988). It was later reported from Mauritius (Autrey, 1985), Florida and Texas (Comstock and Lockhart, 1990), Australia (Teakle and Egan, 1994), India (Viswanathan, 1994) and South Africa and Malawi (Bailey, 1996).

In India, the virus was initially confirmed on a few noble genotypes by electron microscopic and ELISA studies (Viswanathan, 1994). Subsequently, the virus was detected in more genotypes after the standardisation of the

enzyme linked immunosorbent assay (ELISA) and immunosorbent electron microscopy (ISEM) techniques. This paper reports the extent of SCBV infection in the world collection at Cannanore, its symptoms on different sugarcane genotypes and its effects on growth.

MATERIALS AND METHODS

Descriptions of the symptoms of SCBV at Cannanore were based on field observations made from November 1992 to May 1998. Some genotypes were brought to Coimbatore for the observation of symptoms. Samples for all the serological assays consisted of the third youngest leaf from canes 5 to 8 months old.

Direct antigen coating (DAC) ELISA was performed for the detection of SCBV (Viswanathan et al, 1996). In this indirect ELISA, goat anti-rabbit gamma globulin conjugated with alkaline phosphatase (Sigma) was used and the absorbance was measured at 405 nm in an ELISA reader (Model EL 311, Biotek Instruments, USA) 30 min. after colour development. Antigen was diluted to 1:200, antiserum 1:2000 and enzyme conjugate 1:8000. For ISEM, the antiserum (1:100) was coated on collodion-filmed copper grids and the grids were incubated with antigen extracts after washing. The grids were then washed and negatively stained with 2% uranyl acetate and viewed at a displayed magnification of x21000.

RESULTS

Characteristic foliar symptoms of SCBV were observed in 500 out of 764 *Saccharum officinarum*, 38 out of 43 *S. barberi*, 27 out of 29 *S. sinense*, 48 out of 146 *S. robustum*, 15 out of more than 1600 hybrid *Saccharum* genotypes and a few genotypes of related genera. The presence of the virus was subsequently confirmed on many of these genotypes by ELISA and ISEM studies.

Chlorotic stripe symptoms

Fine chlorotic specks appeared initially and eventually developed into chlorotic stripes, 0.5-2.0 mm wide, that were confined between the veins. In some genotypes, including Black Tanna, HO43, HO52, Iscambine, Listada, Port Mackey Black (all noble canes) and 28NG104 (*S. robustum*), the chlorotic stripes merged to form long stripes running the full length of the leaf lamina, but in many others the stripes remained much as at first appearance until the leaf dried. Usually the stripes progressed downwards after first appearing at the leaf tip. In some genotypes the symptoms were present on all the leaves while in some *S. officinarum* genotypes, including 28NG44, 51NG92 and 51NG96, the symptoms were present only on mature leaves. Genotypes Black Tanna, Castilla, D109, D1135, Guam A, HO43 and Listada also developed an interveinal chlorosis. In total, about 240 *S. officinarum*, 18 *S. robustum*, 11 *S. barberi*, 8 *S. sinense* genotypes and 22 foreign hybrids expressed chlorotic stripe symptoms.

Mild mottling symptoms

Totals of 202 *S. officinarum*, 28 *S. barberi*, 19 *S. sinense* and 28 *S. robustum* genotypes expressed a pale green freckle or mild mottling symptom. In some genotypes, including Fiji 38 and Fiji 43, mature leaves showed a more intense mottling than younger leaves. Leaves of the secondary tillers produced after 8-10 months exhibited a more conspicuous mottling than the leaves of primary shoots. The freckles in the older leaves sometimes turned pale yellow and finally the entire leaf became yellow.

Effect of virus infection on cane growth

The genotypes Black Tanna, Boetato Bilatoe, Castilla, Cerum Red, Guam A, HO52, Listada and Port Mackey Black (*S. officinarum*), Kuswar Aligarh, Kuswar Ottur, Mangwa, Mungo 237, Pararia and Pararia 257 (*S. barberi*), D1135, D1135 str., D4/4 and D109 (hybrids) were stunted from an early stage. In genotypes with

stunted growth there were few tillers and the main shoot had a reduced number of and also shorter internodes. The above genotypes were distinctly stunted compared with adjacent genotypes.

The genotypes D1135, Castilla, Black Tanna, Cerum Red and Listada also had a bunchy top appearance due to failure of the upper leaves to unfurl and a shortening of the upper internodes. In these genotypes and in Port Mackey Black, Gros Genoux, D109 and D4/4, a reduction in the length and breadth of the leaves was conspicuous.

Deep longitudinal cracks on the internodes were noticed in Black Tanna, Boetato Bilatoe, Boengaja Bali, Khajuria, Listada and D1135. In these genotypes, apical growth ceased after about eight months and the stalks gradually died back from the apex. Axillary buds then developed in a basipetal succession. These axillary shoots exhibited marked symptoms of SCBV on the leaf lamina and leaf sheath and the leaves had a twisted and curled appearance. On planting, buds of these severely infected canes germinated poorly (30-50%) and the chlorotic stripe symptoms were present on the first formed and later leaves of the developing shoots.

Detection of SCBV by ELISA and ISEM

The results of a total of 15 ELISA tests showed that 190 of the 314 genotypes of *S. officinarum*, *S. barberi*, *S. sinense*, *S. robustum* and hybrids were positive for the virus (Table 1). Even though leaves with apparent SCBV symptoms were taken for ELISA tests, many of the genotypes were virus-negative.

Table 1: Detection of SCBV by ELISA and ISEM in sugarcane germplasm showing suspected SCBV foliar symptoms.

Genotypes	ELISA		ISEM	
	No. clones tested	No. positive clones	No. clones tested	No. positive clones
<i>S. officinarum</i>	250	154	25	14
<i>S. barberi</i>	3	3	2	2
<i>S. sinense</i>	15	6	3	1
<i>S. robustum</i>	4	2	4	2
<i>Pennisetum sp.</i>	—	—	1	1
Foreign hybrids	30	23	10	4
Indian hybrids	12	2	12	3
Total	314	190	57	27

ISEM using leaf extracts from genotypes suspected of being infected revealed bacilliform virus particles of 108-118 x 20-21 nm in size. Of 57 genotypes of *S. officinarum*, *S. robustum*, *Pennisetum* spp, foreign commercial hybrids and Indian hybrids, 27 showed virus particles with ISEM (Table 1). As with ELISA, some clones with marked foliar symptoms tested negative for the virus by ISEM. With a few samples, lyophilization was necessary before screening by ISEM and ELISA showed virus-positive reactions, indicating a low concentration of virus. The virus was also trapped by ISEM in *P. purpureum* genotypes IK76-68 and IK76-84.

DISCUSSION

This study has revealed the presence of SCBV in many genotypes of *S. officinarum*, *S. robustum*, *S. barberi*,

S. sinense and in some of the hybrid genotypes in the world sugarcane germplasm collection maintained at Cannanore. Symptoms varied widely among the different genotypes.

It was not possible to diagnose infection solely on the basis of symptom expression since some symptomless genotypes also showed virus particles by ISEM. Similar findings with some commercial hybrids and a few *Saccharum* spp genotypes have been reported previously (Autrey et al, 1992; Lockhart et al, 1996). In this study, the detection of the virus was not possible without lyophilization due to a low virus concentration.

Irey et al (1992) reported that SCBV was detected in 94% of genotypes in the USDA germplasm collection. In Mauritius, SCBV infection was found in all but one of 128 noble canes and in 424 of 437 hybrid genotypes in the germplasm collection maintained at Reduit (Autrey et al, 1991). All hybrids more than 50 years old and four of 21 current varieties were found to be infected in Australia (Teakle and Egan, 1994).

The noble genotypes Akoki 22, Badila, Barbados White Port, Green Ribbon, HP43, Iscambine, Jamaica Red, Louisiana Striped, 28NG2, 51NG131, 51NG153, 57NG239 and SS60-1 in the USDA germplasm collection in Florida were reported to be infected by SCBV and some had yellow freckles on the leaves (Autrey et al, 1992). All these genotypes exhibited SCBV symptoms at Cannanore and they tested virus-positive by ELISA. Some of the hybrid genotypes found to be infected with SCBV in Florida, including C279, CP31-588, POJ2725 and Q69, were found to be virus-positive in India in an earlier study (Viswanathan and Alexander, 1995). The genotypes Bois Rogue, Badila, Gros Genoux, Iscambine, Korpi, Mignonne, D 109, D 1135, Tombiapa and 57NG57 reported to be infected with SCBV in Mauritius (Autrey et al, 1992) had clear foliar symptoms at Cannanore.

Genotypes from the world collection of sugarcane germplasm maintained by the USDA at Canal Point, Florida were sent to the Indian germplasm collection in batches from 1956 onwards. Subsequent collections made on expeditions in 1957, 1976, 1977 and 1984 were also made available from the USDA. Certain *S. officinarum* genotypes, especially those originating in New Guinea, exhibited poor vigour at Coimbatore and showed poor root development and a sparse, shallow root system. These symptoms were associated with SCBV and it can be presumed that these genotypes were infected on arrival in India.

Of the 764 *S. officinarum* genotypes at Cannanore, 297 are named (inclusive of Hawaiian originals and genotypes from New Caledonia and Fiji), and of these 236 (80%) had either chlorotic stripes or mild mottle symptoms of SCBV. There is a progressive reduction in the percentage of genotypes with SCBV symptoms in accessions from 1921 onwards. This may be due to the presence of the virus in the 1921 collections, or prior to that in the named genotypes, and subsequent spread. About 90% of the *S. robustum* genotypes collected up to 1951 had foliar symptoms of SCBV, whereas in those collected from 1957 onwards, only 23% were apparently infected (Viswanathan and Premachandran, 1998). The higher incidence in the earlier collections of material might have been due to spread of the virus at the germplasm maintenance centres.

It has been reported that of the 1260 genotypes collected in the period 1875 to 1957, only 338 (27%) remained in the USDA world collection in Florida in 1984 (Berding and Roach, 1987). As SCBV was not identified there until 1988, a large number of genotypes that had been discarded or lost because of poor growth might have been infected with SCBV. At Cannanore, genotypes were maintained irrespective of vigour and difficulty in field establishment. This might be one of the reasons for the higher incidence of SCBV infection in the named varieties and the earlier collections at Cannanore. The named noble canes which had very severe symptoms at Cannanore were those received from the USDA collection in 1956 and 1957, whereas other named noble canes had only mild symptoms.

Before SCBV was identified, the leaf freckles now known to be associated with infection were considered to be non-pathogenic in origin and no restrictions were placed on the movement of such genotypes. This permitted

the virus to spread unchecked, especially in noble canes (Autrey et al, 1992). Three mealy bug species, *Saccharicoccus sacchari*, *Desmicoccus boninsis* and *Planococcus citri*, have been shown experimentally to transmit SCBV (Lockhart et al, 1996). The secondary spread of SCBV to new genotypes might have been due to such insect vectors.

An association of sugarcane mild mosaic virus (SCMMV) with SCBV was reported in the USA (Lockhart et al, 1996). SCMMV was identified in *S. officinarum*, *S. barberi*, *S. sinense* and a number of commercial *Saccharum* hybrids from Mauritius, Malawi, Florida and Australia and was always identified in mixed infection with SCBV, with which it shares a common mealy bug vector. However, detailed work on its geographical distribution is lacking. The presence of SCMMV was suspected in some genotypes of *Saccharum officinarum*, *S. barberi* and *S. sinense* at Cannanore (Alexander and Viswanathan, 1996). Extracts from some *S. officinarum* genotypes showed flexuous particles resembling a closterovirus in ISEM studies (Viswanathan, unpublished).

SCBV is serologically related to badnaviruses occurring in banana, citrus, pineapple, canna and Kalanchoe (Lockhart et al 1996, Viswanathan et al, 1996) and it has yet to be confirmed whether these badnaviruses can infect sugarcane.

Because of the absence of conspicuous symptoms in many genotypes when infected with SCBV, especially hybrids, the safe movement of germplasm cannot be achieved unless precise detection methods are employed in quarantine. Also, once genotypes are infected, the virus is retained in germplasm collections. Work done elsewhere has indicated that attempts to eliminate the virus from some infected genotypes were unsuccessful (Lockhart et al, 1996). Additionally, the lack of visible symptoms in many clones is associated with low concentrations of the virus, preventing detection by ELISA or ISEM. It is necessary to develop more sensitive detection methods, for example, using specific primers for PCR diagnosis, to confirm infection and so that techniques for eliminating SCBV from infected genotypes can be developed.

CONCLUSIONS

Our studies show the presence of SCBV in many *Saccharum* spp. clones and in few hybrid clones with clear symptoms. Clear variation in virus symptoms was observed among different clones. The symptoms observed on many clones are unique and not resemble to those described from other countries. Growth retardation was noticed in certain virus positive clones. Clones which are added recently to the germplasm collections had comparatively less SCBV infection. More sensitive detection techniques are warranted for more precise detection of SCBV and other possible viruses.

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EL VIRUS BACILIFORME DE LA CAÑA DE AZÚCAR: SÍNTOMAS, DETECCIÓN Y DISTRIBUCIÓN EN LA COLECCIÓN MUNDIAL DEL BANCO DE GERMOPLASMA DE CANNORE, INDIA

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RESUMEN

En los últimos años el virus baciliforme (SCBV) ha sido detectado en la colección mundial del banco de germoplasma de caña de azúcar establecido en Cannanore. Los síntomas foliares característicos han consistido en punteaduras amarillas, rayado clorótico de diferente longitud, hojas más angostas, crecimiento raquíptico, presencia de rajaduras en los entrenudos y una apariencia de "bunchy top". Entre los diferentes fenotipos estudiados, los de *Saccharum officinarum* aparecen como los más afectados; los genotipos de *S. officinarum* colectados antes de 1970 se encontraron fuertemente afectados por el virus. La mayoría de genotipos de *S. barberi* y *S. sinense* también se encontraron afectados. Sin embargo solamente un número limitado de genotipos de *S. robustum* e híbridos interespecíficos de éste se sospecha están afectados. Las técnicas de ELISA e inmunoabsorción-microscopio electrónico (ISEM) se estandarizaron para la detección del virus en los genotipos que se sospecha de estar afectados. La presencia del virus en el germoplasma de caña de azúcar en India y en otros países es el principal obstáculo para el intercambio seguro de material. Se requiere de una inmediata atención que garantice el uso de técnicas de diagnóstico más precisas como las sondas de ADN y la reacción en cadena de la polímera y también de aquellas que permitan la eliminación del virus en los genotipos infectados.

Palabras claves: virus baciliforme de la caña de azúcar, síntomas, detección, germoplasma

**LE VIRUS BACILLIFORME DE LA CANNE A SUCRE: SYMPTÔMES,
DETECTION ET DISTRIBUTION DANS LA COLLECTION
MONDIALE DU GERMPLOSM EN CANNANORE, L'INDE**

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RÉSUMÉ

Dans les années récentes, le virus bacilliforme de la canne à sucre (SCBV) a été détecté dans la collection mondiale de la germplasm de la canne à sucre à Cannanore. Les caractéristiques symptomatiques foliaires étaient taches de resseur, les bandes chlorotiques de différentes longueurs, les feuilles faibles, le grosseur rabourogé, la présence de la fente et une apparence de 'buncy top'. Entre les différents génotypes, les *saccharum officinarum* apparaissent comme les plus enclins aux virus. Les génotypes de *s. officinarum* rassemblés avant 1970 étaient infectés gravement par le virus. La majorité des génotypes de *S. barberi* et *S. sinense* étaient aussi infectés. Cependant, seulement, un nombre limité de génotype de *S. robustum* et les hybrides interspécifiques de région se soupçonnaient d'avoir infectés. L'ELISA et l'ISEM ont été standardisés pour dépister le virus dans les génotypes qui se soupçonnaient d'avoir infectés. La présence du virus dans le germplasm de canne à sucre en Inde, et dans autre pays est l'obstacle majeur à l'échange de germplasm. Il y a besoin d'une attention immédiate qui garantit l'usage de la technique du diagnostic plus précise comme les sondages de ADN et la réaction en chaîne de la polymérisation et aussi pour éliminer le virus et des génotypes infectés.

Mots clés: virus bacilliforme de canne à sucre, symptômes, détection, germplasm.