Internat. J. agric. Sci. Vol.3 No.1 January 2007 : 68-72

Mixed infection of sugarcane streak mosaic and sugarcane yellow leaf virus infecting sugarcane crops in Andhra Pradesh, India

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ABSTRACT
During survey of sugarcane crops of Andhra Pradesh in 2004-05, incidence of mosaic symptoms and midrib yellowing symptoms were observed in promising commercial sugarcane cultivars. The incidence of 6 to 30% was observed in farmer's fields for the first time in the month of August during the year 2004. Disease incidence was also observed in the range of 2 to 36% in some of the clones in the germplasm. These infected samples were found antigenically similar to sugarcane streak mosaic virus as well as sugarcane yellow leaf luteo virus. The results were confirmed either by DAC-ELISA, DIBA and TIBA together or by any these serological tests in suspected samples.The sugarcane yellow leaf virus particles measured about 25 to 30 nm in diameter and sugarcane streak mosaic virus particles measure about 480-600 nm. The mosaic isolate reacted with sugarcane streak mosaic virus-AP isolate (SCSMV-AP, antiserum), recently reported from South India in DAC ELISA and DIBA test. The mosaic infected leaves with or without midrib yellowing symptoms were also analysed with antiserum of sugarcane yellow leaf luteo virus. The results were positive in DAC-ELISA and TIBA. Hence it was concluded that mosaic and midrib yellowing observed on many varieties of sugarcane in Rudrur, Andhra Pradesh is caused by sugarcane streak mosaic virus and sugarcane yellow leaf luteo virus.

Key words: Sugarcane streak mosaic virus, Sugarcane yellow leaf virus, Mixed infection, Sugarcane

INTRODUCTION

Sugarcane is an important food cum cash crop and is the third largest crop in terms of value next to rice and wheat in India. It is susceptible to several biotic stresses in nature (Rott *et al*., 2000). Among the viral diseases mosaic disease caused by sugarcane mosaic virus and sugarcane streak mosaic virus is widely prevalent in almost all sugarcane growing states of the country (Rao *et al*., 2002). Incidence of the mosaic disease is very severe in major sugarcane growing states of Uttar Pradesh, Maharashtra, Bihar, Tamilnadu, Gujarat, Haryana and Andhra Pradesh. Even 10 to 15% loss due to this disease is highly significant because of excessive cultivation of the crop (Singh *et al*., 1997; Vishwanathan, 2002).

Sugarcane streak mosaic virus (SCSMV) is recently described disease (Hema *et al.,* 1999) and is known to infect sugarcane, wheat and sorghum. During a recent survey of sugarcane crops in AP, mosaic incidence and midrib yellowing was observed in many varieties of sugarcane viz., Co 86010, 83 A 30, Co 85036, Co 92013, Co 6907, CoC 671, Co 7219, 97 R 183, 97 R 272, 97 R 134, 98 R 145. Hence there is a need to characterize these virus isolates from affected sugarcane plants from AP. We report here antigenic relatedness of mosaic and yellow midrib isolates of sugarcane collected from different regions of AP, India.

Incidence of sugarcane yellow leaf luteo virus (SCYLV) has not been reported in Andhra Pradesh except a few earlier reports from South India (Vishwanthan and Balamuralikrishnan, 2004). Hence preliminary observation was undertaken in some of the infected sugarcane varieties in the Northern Telangana zone of Andhra Pradesh on its occurrence detection and observations are reported here.

MATERIALS AND METHODS

Sugarcane growing areas of Andhra Pradesh were survyed. Mosaic and yellow midrib affected samples from different countries listed in Table 1 were collected and subjected to serological assay by direct antigen coated enzyme linked immonosorbent assay (DAC-ELISA), TBIA. The samples showing varying severity with interveinal chlorotic specks, streaks or stripes especially on young leaves of sugarcane has been prevalent in almost all varieties including some showing midrib yellowing of mature leaves with a distinctive strong yellow midrib on the lower (abaxial) surface but gradually spreads laterally into it, drying starting at the tip and progressing down towards the base of leaf. The samples of sugarcane showing both types of symptoms were collected, stored in plastic bags at 4^{o} C and processed with in 7-8 days for leaf dip preparations and serological experiments. Cultures of the virus isolates causing mosaic disease and yellow midrib of commercial varieties around Andhra Pradesh was maintained on sugarcane in earthen pots.

The electron microscope examination of the leaf dip preparation on infected leaves showing the above mentioned symptoms were found to contain flexious filamentous particles measuring about 400-600 X 13 nm and the particles of member of the luteo viride group are hexagonal in out line with diameter varying from 30 to 45 nm. The identification by leaf dip preparations resembled mixed infections of both viruses. Keeping this in view the identification and distribution of the casual virus was attempted in the present investigation.

Leaf dip preparation :

Small pieces of infected leaf tissues were cut and

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Table 1 : Incidence of streak mosaic and midrib yellowing disease and their serological diagnosis through DAC-ELISA, DIBA and TBIA tests.

Sl No.	Variety /Clone	Incidence%	DAC-ELISA Results		DIBA	TBIA
			SCSMV	SCYLV	SCSMV	SCYLV
1	Co 737	-	-	-	-	-
2	Co 7219	6	+	-	+	-
3	Co 997	-	-	-	-	-
4	Co 92020	-	-	-	-	-
5	Co 92021	-	-	-	-	-
6	Co 92024	-	-	-	-	-
7	Co 92003	-	-	-	-	-
8	Co 85036	15	+	+	+	+
9	Co 85012	2	+	+	+	+
10	Co 86032	-	-	-	-	-
11	Co 92013	17	+	+	+	+
12	Co 93024	8	+	+	+	+
13	CoC 671	6	+	+	+	+
14	Co 6907	36	+	+	+	+
15	83 A 30	3	+	+	+	+
16	Co 86010	6	+	+	+	+
17	90 R 5	3	+	+	+	+
18	83 R 23	-	-	-	-	+
19	85 R 186	4	+	+	+	+
20	95 R 3	5	+	+	+	+
21	94 R 72	10	+	+	+	+
22	93 A 11	19	+	+	+	+
23	97 R 183	18	+	+	+	+
24	97 R 199	16	+	+	+	+
25	97 R 383	8	+	+	+	+
26	97 R 174	20	+	+	+	+
27	97 R 15	30	+	+	+	+
28	97 R 134	26	+	+	+	+
29	97 R 272	28	+	+	+	+
30	97 R 123	6	+	+	+	+
31	97 R 7	16	+	+	+	+
32	97 R 401	2	+	+	+	+
33	98 R 57	-	+	-	+	-
34	98 R 268	6	+	+	+	+
35	97 R 395	15	+	+	+	+
36	97 R 199	8	+	+	+	+
37	97 R 6	10	+	+	+	+
38	98 R 272	12	+	+	+	+
39	98 R 268	6	+	+	+	+
40	98 R 67	12	+	+	+	+
41	98 R 145	32	+	+	+	+
42	98 R 278	8	+	+	+	+
43	94 R 73	10	+	+	+	+
44	Co Vc 9963	22	+	+	+	+
45	98 R 229	-	-	+	-	+

crushed in a pestle and mortar in 0.1 M phosphate buffer. A drop of extracted sap was placed on a farm war carbon coated copper grid. After few minutes the excess was removed and blotted off by a what man No.1 filter paper and the grid was washed with few drops of distilled water and further dried using filter paper. The staining was done using a drop of 2% uranyl acetate, drained and before examination using TEM Model Hitachi H 7500 at Ruska labs, ANGRAU, Rajendranagar.

The antigenic relationship between the virus isolate purified from an isolate causing Sugarcane streak mosaic disease of sugarcane and sugarcane yellow leaf virus were determined by employing DAC-ELISA and TBIA.

Serology :

Serological methods like DAC-ELISA and TBIA were performed to determine the serological relationship of virus under study. Antigens for these tests were used only as virus infected leaf sap extracts at dilution of 1:10 (i) Direct antigen coating enzyme linked immunosorbent assay (DAC-ELISA) described by Clark and Bar-Joseph (1984) was performed on polysterene plate (Corning, USA) to determine serological relationships.

Procedure :

Programme sheet was prepared according to the member of antigen samples. The samples were prepared in carbonate buffer and 0.2 ml was added to each well of the plate (Tarson, India) and incubated for 2 hours at 37^{o}C. The plate was washed 3 times with PBS-TO (by keeping 3 minutes interval between each wash). Antigens for these tests were used only as virus infected sap extracts. Samples were prepared in coating buffer (1:10 dilution W/V, 0.05 M sodium carbonate ph 9.6) containing substrate polyvinyl pyrolidine (2% W/V, MW 40,000) and incubated overnight at 4 ^{o}C. Antiserum dilutions were prepared with PBS-To (1:500, 1:1000, 1:2000, 1:5000, 1:10000) and added to the wells (0-2 ml/well). Antiserum to SCSMV obtained from Andhra Pradesh and SCYLV obtained from S. Saumatally, Mauritius was used at 1:1000 dilution. Antirabbit immunoglobulin alkaline phosphate conjugate (Sigma, USA) was used at 1:1000 dilution. The reaction was read at 405 nm after 1 hour of adding substrate (P-nitrophenyl phosphate, Sigma, USA) by ELISA reader.

Tissue Blot Immunoassay :

TBIA is performed by using the protocol of Schenck (Schenck ***et al.,*** 1997). Sugarcane yellow leaf virus infection was determined by assaying for the presence of virus in the youngest fully emerged leaf by a tissue blot immunoassay using antiserum to SCYLV obtained from S.Saumatally, Mauritius was used at 1:1000 dilution. Antirabbit immunoglobulin alkaline phosphate conjugate (Sigma, USA) was used at 1:1000 dilution. Fresh cross sectioned basal portion of leaf midrib applied directly onto nitrocellulose membrane. Tissue blots were obtained by pressing with a firm but gentle force, the newly cut surface onto 0.45 mm pore size nitrocellulose membrane. One impression per leaf midrib was made. The membrane was processed for the immunological localization of virus. Tissue blots were first immersed in PBS containing 2% bovine serum (Sigma) in 0.05 M Tris-Hcl buffer, Ph 7.5, for 1 hour, then the nitrocellulose membrane was then incubated 1 hour with 1:1000 antirserum in the same buffer and 1 hour with antigen specific primary antibodies diluted in PBS at room temperature for 2 hours or 4^{0}C over night. The membrane was thoroughly washed in Tris-Hcl buffer containing 0.8% Nacl and 0.5% Tween – 20. Following three successive washings in PBS tween solution (PBS containing 0.05% Tween 20), 10 to 15 min each time, the membrane was incubated with alkaline phosphatase labeled secondary antibodies for 2 hours at room temperature. Finally the alkaline phosphatase substrate (nitro blue tetrazolium at 165 mg/ml and 5 -bromo – 4 - chloro- 3 – indolyl phosphate at 85 mg/ml in 0.1M Tris-Hcl, Ph 9.5, containing 0.8% Nacl and 5 mM $Mgcl_2$ was added and incubated for 15 to 25 min. The alkaline phosphatase reaction was stopped by washing thoroughly in Tris-Hcl, buffer containing 5mM EDTA and in distilled water. A positive result was indicated by the development of a blue colour on the tissue blot. The membrane was serologically developed by BL Lockart University of Minnesota (Minnepolis) according to Schenck *et al.* (1997). Fast blue was used as the enzyme substrate. A stereo microscope was used to examine the leaf prints, because SCYLV is located in the phloem bundles with in the leaf print stained blue as the positive indication for the presence of the virus under study.

RESULTS AND DISCUSSION

Symptoms of SCSMV and SCYLV were recorded on 45 different cultivars. SCSMV initially first symptom was seen as interveinal chlorotic specific streaks or stripes especially on young leaves of sugarcane has become evident in the month of August. (Plate 1a). The symptoms

Plate1a: Interveinal chlorotic specks or stripes on leaves

of SCYLV in Co 6907 and Co C 671 become evident in mature leaves in that begins in the month of September, symptoms were recorded with varying degrees of streaks and stripes and midrib yellowing in different cultivars (Table

1). The incidence of disease varied from 3-36 per cent in different cultivars with maximum incidence in Co 6907 and Co C 671. In some cultivars midrib yellowing followed by top drying towards base of the leaf with varying intensities of yellowing. (Plate 1b).

Plate1b : Midrib yellowing followed by tip drying towards the base.

Some collected samples even without symptoms were also found to consists of the luteovirus and flexuous filamentous particles in leaf dip preparations. Leaf dip preparations of electron microscopy revealed the presence of virus particles in the samples both flexuous filamentous particles of size 400 X 13 nm in size resemblance of SCSMV

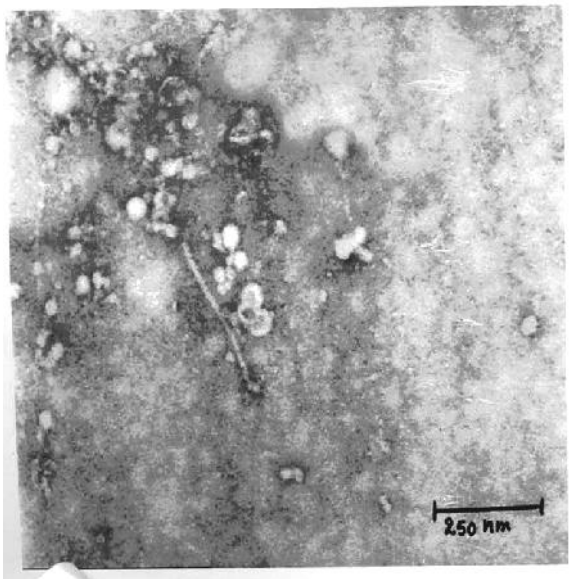

Plate 2a : Electron micrograph showing both flexous filamentous & luteo virus particles under leaf dip preparations size 250nm.

(Plate 2a) and luteovirus particles of 35 nm in size which are icosahedral in shape and are confined to phloem tissue of the plant (Plate 2b). The size and shape of the particles confirms its identity with SCSMV as earlier reported by Hema *et al.* (1999) and SCYLV (Scaglius and Lockhart,

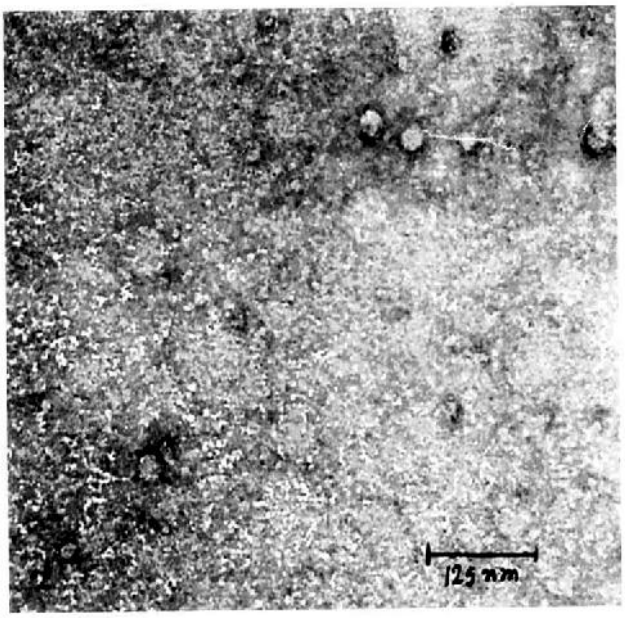

Plate 2b : Maximum luteo virus particles as with symptomless cultivar with 125 nm.

2000).

The results of serological screening through DAC-ELISA and TBIA presented in Table 1 showed that sugarcane streak mosaic virus and sugarcane yellow leaf luteovirus detected in all the symptomatic samples collected of sugarcane with the antiserum of SCSMV and SCYLV. The absorbance for positive reaction's values ranged from 0.332 to 1.785 against 0.106 in healthy control (Table 1). The optical density values calculated in results are the mean values of 12 wells. The ELISA results were further confirmed by DIBA with sugarcane streak mosaic virus isolates and by TBIA with sugarcane yellow leaf virus samples. All the positive ELISA samples showed positive results in DIBA and TIBA test with SCSMV and SCYLV, respectively (Table 1). Absorbance values upto three times higher than healthy were considered as positive in this test. The symptomatology, particle morphology and serological relationship of the virus under study proved that they are SCSMV and SCYLV. Incidence was observed in varying degrees of yellowing and highest per cent incidence was observed in Co 6907, 98 R 145 and 97 R 15 with few varieties showing less than 5% incidence. Twenty clones have an incidence of infection of 10 to 20 per cent. These clones presumably have some resistance to SCYLV infection, since there was equal opportunity for infection with other clones in field trials. The clones with less than 5 per cent incidence apparently had a partial resistance.

However, the existence of sugarcane yellow leaf luteovirus was reported in both symptomatic and asymptomatic leaves. The mixed infections of SCSMV and SCYLV was reported for the first time from AP, India. Looking

the wide spread occurrence of these viruses infecting sugarcane crops, there is need of immediate attention towards checking future spread of virus. We conclude that both sugarcane streak mosaic virus and sugarcane yellow leaf virus are infecting the sugarcane crops in A.P. In most of the infected samples, existence of both the viruses were observed indicating the mixed infection of these viruses in sugarcane crops of AP. However, it should be further confirmed by genome based approach. However, results of occurrence of SCMV, sugarcane streak and sugarcane yellow leaf virus has already been reported from UP, Haryana, Tamilnadu and Maharashtra in India (Rao *et al*., 2002).

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Received : March, 2006; Accepted : September, 2006