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Antigenic and biological diversity among sugarcane mosaic isolates from different geographical regions in India

G P Rao[1*], Maneesha Singh[3], R K Gaur[3] and R K Jain[2]

[1]Sugarcane Research Station, Kunraghat, Gorakhpur 273 008, India
[2]Division of Plant Pathology, Indian Agricultural Research Institute, New Delhi 110 012, India
[3]Institute of Sugar Crop Research and Allied Industries, A-155, Phase-IV, Ashok Vihar, New Delhi110 052, India

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A recently characterized sugarcane mosaic virus isolate (SCM-UP) from eastern Uttar Pradesh, India was found antigenically similar to sugarcane mosaic virus isolates of West Uttar Pradesh, Bihar, Haryana, Gujarat, Maharashtra and Tamil Nadu. The results were confirmed either by DAC-ELISA, EBIA, DIBA and ISEM together or by any of these serological tests. The SCM-UP isolate reacted with AP isolate of sugarcane streak mosaic virus (SCSMV-AP, antiserum), a member of Tritimovirus and a proposed genus in the family Potyviridae, which is recently reported from South India in ISEM test. Hence, it was concluded that sugarcane mosaic disease in India, observed on many varieties of sugarcane, is caused by pathotype of sugarcane mosaic virus which is antigenically similar to other virus isolates causing sugarcane mosaic disease all over India. These virus isolates showed more or less similar biological reactions on sorghum, sugarcane and Johnsongrass.

Keywords: sugarcane mosaic virus, isolates, serology diversity, biological diversity

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Introduction

Sugarcane is an important food-cum-cash crop in India and ranked the third largest crop, next to rice and wheat in terms of value. The crop is susceptible to several biotic stresses in nature[1]. Among them, mosaic disease caused by sugarcane mosaic virus (SCMV) has been found widely prevalent in almost all the sugarcane-growing areas of the country. Incidence of the disease is generally severe in major sugarcane growing states of Uttar Pradesh (UP), Maharashtra, Bihar, Tamil Nadu, Gujarat, Haryana and Andhra Pradesh. Even 10 to 15% loss due to this disease is highly significant because of excessive cultivation of the crop[2].

SCMV, currently ascribed to the 'Potyvirus' genus of the family 'Potyviridae'[3], has been known to infect sugarcane, maize, sorghum and other Poaceous plant species[4,5]. The virus isolates causing mosaic disease from UP were recently characterized on biological and serological levels[5]. A high titre polyclonal antiserum raised against one of the isolates from UP (SCMV-UP) was found capable of detecting SCMV isolates from North, East and West regions of UP. Interestingly, a new Tritimovirus strain, designated as sugarcane streak mosaic virus (SCSMV-AP) and antigenically related to SCMV-UP, was also isolated from mosaic affected sugarcane plants from Andhra Pradesh, Karnataka and Tamil Nadu[6]. Hence, the authors report here biological and antigenic relationship among various virus isolates from mosaic affected sugarcane plants of different geographical regions of India.

Materials and Methods

The sugarcane growing areas of UP, Haryana, Maharashtra, Bihar, Tamil Nadu, Gujarat and Andhra Pradesh were surveyed. Mosaic affected samples (82) from different cultivars listed in Table 1 were collected and subjected to serological assay by direct antigen coated enzyme linked immunosorbent assay (DAC-ELISA).

The virus isolates causing mosaic disease of commercial sugarcane cultivar around UP was propagated on *Sorghum bicolor* cv. CSH-9 by sap

*Author for correspondence:
Tel: 91-551-2273284; Fax: 91-551-2330969
E-mail: gprao_gor@rediffmail.com

Table 1—Cross reactivity of various SCMV isolates with antiserum of SCMV-UP in DAC-ELISA

Isolates	DAC-ELISA OD values	DAC-ELISA Cross reactivity
Tamil Nadu		
T_1 (Co 62198)	0.961*	++
T_2 (Co 92020)	1.019	+++
T_3 (Co 88025)	0.836	+
T_4 (Co 86010)	1.374	+++
T_5 (Co 6304)	0.768	+
T_6 (4-8)	0.734	+
T_7 (CoC 92061)	1.309	+++
T_8 (Co 7514)	0.791	+
T_9 (ISH 268)	1.471	+++
Maharashtra		
M_1 (Co 7427)	1.072	+++
M_2 (Co 8338)	0.605	+
M_3 (Co 94003)	1.009	+++
M_4 (Co 740)	0.92	++
M_5 (Co (8014)	0.76	+
M_6 (Co8369)	0.644	+
M_7 (Co 85004)	0.893	+
M_8 (Co 8370)	1.122	+++
M_9 (83R23)	0.705	+
M_{10} (Co 740)	0.683	+
M_{11} (CoM 7125)	1.118	+++
M_{12} (Co 7219)	0.855	+
M_{13} (CoC 671)	1.429	+++
Haryana		
H_1 (CoC 671)	1.908	+++
H_2 (Co 8021)	0.908	++
H_3 (Co 419)	1.538	+++
H_4 (CoH 115)	1.339	+++
H_5 (SeS 594)	0.69	+
H_6 CoH 117)	1.412	+++
H_7 (Co 8014)	1.002	+++
H_8 (Co 97009)	1.564	+++
H_9 (CoH 119)	1.032	+++
H_{10} (CoH 92)	1.228	+++
H_{11} (CoH 118)	1.432	+++
H_{12} (CoS 8436)	0.632	+
H_{13} (Co 997)	2.549	+++
H_{14} (SAIPUNG)	1.373	+++
H_{15} (Co 98015)	1.065	+++
H_{16} (CoH 107)	0.873	+
H_{17} (CoS 94007)	1.126	+++
H_{18} (Co 85004)	0.886	+
H_{19} (Co 975)	1.587	+++
H_{20} (Co 86032)	1.165	+++
H_{21} (Co 94035)	1.165	+++
H_{22} (CoC 92061)	2.229	+++
H_{23} (CoC 62399)	1.181	+++
H_{24} (BD1Israel)	1.868	+++
Uttar Pradesh		
UP_1 (CoLK8102)	0.772	+
UP_2 (CoS 94270)	1.037	+++
UP_3 (CoS 91269)	1.033	+++
UP_4 (CoSe 92423)	1.271	+++
UP_5 (CoSe 93232)	0.946	++
UP_6 (CoS96260)	1.166	+++
UP_7 (CoSe98231)	0.728	+
UP_8 (Cos 8119)	0.665	+
UP_9 (UP 9530)	0.912	++
Gujarat		
G_1 (CoC 671)	1.152	+++
G_2 (Co 859)	0.885	+
Bihar		
B_1 (BO 91)	0.629	+
B_2 (BO 133)	0.708	+
B_3 (BO 70)	0.925	+
Andhra Pradesh		
AP_1 (Co 740)	1.137	+++
AP_2 (CoC 671)	1.083	+++
Healthy Control	0.176	
Buffer Control	0.165	

+++=very strong reaction; ++=strong reaction; +=positive reaction

*The OD values are the mean of 12 wells

inoculation and also through vegetative propagation of sugarcane CoS 87220.

Five sorghum inbred lines (OKY-8, Atlas, Rio, BT×398, SA 8735) and one each of oat, sugarcane, maize and Johnsongrass were used as differential hosts. Seeds of sorghum differential, Johnsongrass and oat cv. Clintland were obtained from R G Henzell, Queensland, Australia and M Tosic, Belgrade, Yugoslavia.

The test plants were mechanically inoculated at 2-3 leaf stage with inocula prepared by homogenizing infected sorghum CSH-9 and or M P cherry leaves 1:10 (w/v) in borate buffer (0.5 *M*, *p*H 8.0). At least, two plants of each differential were inoculated with each isolate and observed for symptom expression up to six weeks after inoculation. Biological reaction of each isolate was recorded thrice and compared with earlier symptom description[4,7]. The observations were confirmed by leaf dip electron microscopy.

The antigenic relationship between a virus isolate (SCMV-UP), purified from an mosaic disease causing isolate of sugarcane from UP[5], and other mosaic (Potyvirus) isolates from different regions of India were determined by employing direct antigen coating enzyme linked immunosorbant assay (DAC-ELISA). DAC-ELISA[8] was performed on polystyrene plate (Corning, USA). Antigens for the 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 *p*H 9.6) containing polyvinyl pyrolidone (2% w/v, m.w. 40,000) and incubated overnight at 4^0C. Antiserum to SCMV-UP was obtained from Advanced Centre for Plant Virology (ACPV), IARI, New Delhi and used at 1:100 dilutions. Antirabbit immunoglobulin alkaline phosphate conjugate (Sigma, USA) was also used at 1:1000 dilutions. The reaction was read, after 1 hr of adding substrate (*p*-nitrophenyl phosphate, Sigma, USA), at 405 nm by ELISA reader.

For ISEM, 10µl drop of tissue extract (at 1:50 dilution) was put on carbon coated grid and incubated for 30 min at 37°C. The grid was washed with distilled water followed by 2 % aqueous uranyl acetate and was drained and dried. The grid was observed under electron microscope for decoration of virus particles with the antiserum used.

Results and Discussion

The results of serological screening of mosaic disease affected sugarcane samples from different regions of India with the antiserum of SCMV-UP isolate in DAC-ELISA test are presented in Table 1. The results show that sugarcane mosaic virus was present in all the symptomatic samples of sugarcane collected from UP (9), Maharashtra (17), Bihar (5), Tamil Nadu (17), Andhra Pradesh (2), Haryana (30) and Gujarat (2). The absorbance for positive reaction values ranged from 0.605 to 2.549 against 0.176 in healthy control (Table 1). The purified sugarcane mosaic isolates from Tamil Nadu, Andhra Pradesh and Gujarat were also reacted well with the antiserum of SCMV-UP isolate and vice versa (Table 2). Most of the samples reacted positively in DAC-ELISA test, also showed positive reaction in ISEM, EBIA and DIBA tests (data not shown).

Irrespective of the isolates, symptoms on five sorghum differential ranged from mild/severe mosaic to chlorotic streaks, necrosis, red stripes and necrotic mid vein (Table 3). Further, all four isolates infected the sugarcane with severe mosaic symptoms with continuous chlorotic streaks. All the isolates also infected Johnsongrass and produced mild/severe mosaic, chlorotic stripes and streaks. However, maize and oat were not infected by any isolates (Table 3). The present results indicate considerable similarity among the sugarcane mosaic isolates from UP, Gujarat, Tamil Nadu and Andhra Pradesh. Most of isolates showed similar host reactions on sorghum differentials.

The virus associated with sugarcane mosaic was decorated with the SCMV-UP antiserum. The electron

Table 2—Cross reactivity of SCMV isolates in DAC-ELISA

Antigen	Cross Reactivity (DAC-ELISA) Antisera			
	SCMV-UP	SCMV-G	SCMV-TN	SCMV-AP
SCMV-UP	+++ (1.155/0.1758)	++ (0.43/0.14)	++ (0.961/0.188)	+++ (1.234/0.387)
SCMV-G	++ (0.50/0.14)	+++ (0.62/0.09)	-	-
SCMV-TN	++ (0.836/0.1758)	- -	++ (0.898/0.083)	++ (2.65/0.23)
SCMV-AP	+++ (1.309/0.1758)	- -	++ (0.776/0.083)	+++ (2.98/0.23)

+++=very strong reaction; ++=positive reaction; - = not tested
SCMV-UP = UP isolates; -G = Gujarat isolates; -TN = Tamil Nadu isolates; -AP = Andhra Pradesh isolates
Values in ( )=OD of infected leaves at 405nm/OD of healthy leaves at 405nm
Source= Rao *et al*[5] (SCMV-UP); Joshi[9] (SCMV-G); Hema *et al*[6] (SCMV-AP); Viswanathan[10] (SCMV-TN)

Table 3—Reaction of SMV isolates on host differentials

Isolates	Reactions on differential hosts								
	Sorghum differentials					Sugarcane	*Zea mays*	Johnson Grass	Oat cv Clintland
	OKY-8	Atlas	Rio	BTx398	SA 8735				
SCMV-UP	MM,NCS	SM,NRS	MM,N	MM,N	MM,RS	SM	NS	MM	NS
SCMV-G	NS	SM,N	N,CS	SM	NS	SM	NS	SM	NS
SCMV-TN	NS	SM,N,CS	N	SM	NS	SM	NS	MM,CS	NS
SCMV-AP	NS	SM	CS,N	NS	NS	SM CCS	NS	MM,CSp	NS

MM=mild mosaic, SM=severe mosaic, CS=chlorotic streaks, N=necrosis, CSp=chlorotic stripes, CCS=continuous chlorotic streaks, RS=Red streak, NS=No symptoms

micrograph at the total magnification of 50,000 indicated a uniform decoration SCMV particles with SCMV anti bodies.

On the basis of above results, it could be concluded that the isolate has wide antigenic similarity to other SCMV isolates from UP, Maharashtra, Bihar, Tamil Nadu, Andhra Pradesh, Haryana and Gujarat states of India as well as SCSMV-AP isolate. However, it requires further confirmation by genome-based approach. Thus, the present study shows amply that the mosaic diseases of sugarcane in India are caused by a common pathotype of SCMV.

The positive relationship of SCMV-UP isolates with SCMV isolates of Gujarat, Tamil Nadu and SCSMV-AP isolate could be attributed to the presence of common epitopes in the core region of coat protein of these viruses[4]. However, the existence of SCSMV-AP has been established on the biological, serological and genomic basis in South India[6].

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