



Natural Infection of Sugarcane by an Immunity Breaking Strain of Sorghum Mosaic Potyvirus (Sr MV-IBS) in Peninsular India

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Serodiagnosis of symptomatic samples of sugarcane collected from number of sugarcane growing localities in Marathwada (Maharashtra), Andhra Pradesh (AP) and Tamil Nadu (TN) indicated that the sugarcane was predominantly infected by sorghum mosaic potyvirus – an immunity breaking strain (SrMV-IBS) in 39 localities out of 41 (95%) whereas mixed infections of sugarcane with SrMV-H was evident only in 5 localities (12%). Moreover, mixed infections of sugarcane with sugarcane mosaic potyvirus strains including SCMV-A (7%), SCMV-B (22%), SCMV-SC (7%) and with maize dwarf mosaic potyvirus – strain A (MDMV-A: 17%) were also evident in some localities. Infections of sugarcane by MDMV-A was detected only in Marathwada but not in AP and TN whereas infections of sugarcane by SrMV-H, SCMV-A, SCMV-B and SCMV-SC besides SrMV-IBS were found in Marathwada, AP and TN by SCMV-D, SCMV-E, infections of sugarcane varieties grown in Marathwada, AP and TN by SCMV-D, SCMV-E, SCMV-MDB and johnsongrass mosaic potyvirus (JGMV) were found to be totally absent in all the surveyed localities. Of these potyviruses detected in sugarcane, only three, SrMV-IBS (100%), SrMV-H (71%) and SCMV-B (14%) were mechanically transmitted from field infected sugarcane to glasshouse grown CK-60A, CS-3541 and Maldandi (M-35-1) sorghum genotypes.

KEY WORDS : Potyvirus surveys, serodiagnosis, mechanical transmission, SCMV, SrMV, MDMV, JGMV, SrMV-IBS.

INTRODUCTION

Recent investigations on the taxonomy of aphid borne potyviruses infecting species of poaceae (Shukla *et al.*, 1989) have shown that virus isolates previously included as strains of sugarcane mosaic virus (SCMV) comprise four distinct potyviruses namely SCMV itself (USA strains A, B, D, E and MDMV-B; Australian strains SC, BC, Sabi, Teakle *et al.*, 1989), johnsongrass mosaic potyvirus, JGMV (Australian SCMV-Jg, USA MDMV-O and Kansas-1) (Shukla and Teakle, 1989; McKern *et al.*, 1991), maize dwarf mosaic potyvirus, MDMV (USA strains A, D, E and F, Ford *et al.*, 1989) and sorghum mosaic potyvirus, SrMV (USA SCMV-H, I and M strains). Besides three strains of SrMV (H, I and M), a new strain of SrMV identified as an immunity breaking strain of SrMV (SrMV-IBS) (Mali, 1995b, 1996, 1999) has recently been described from India

based on serological, biological properties and cylindrical inclusion morphology. SrMV-IBS infects sorghum differentials (QL-3, QL-11) resistant/immune to other SrMV-strains (Mali and Thakur, 1999). These four potyviruses (SCMV, SrMV, MDMV, JGMV) induce similar symptoms in some hosts, have host ranges usually restricted to poaceae and have some aphid vectors in common. However, they are serologically unrelated or only or only distantly related (Shukla *et al.*, 1989). These four potyviruses can also be distinguished by their reactions on some sorghum inbred lines (Giorda *et al.*, 1986, Mali and Thakur, 1999, Persley *et al.*, 1985, Teakle and Grylls, 1973, Tosic *et al.*, 1990, Rao *et al.*, 1998).

Earlier reports indicated that the SrMV-IBS and other potyviruses in SCMV-subgroup (SCMV, MDMV, SrMV, SrMV) were naturally transmitted by corn leaf aphid (*Rhopalosiphum maidis*) vector from sugarcane to sorghum under natural epiphytotic conditions in Marathwada (Mali and Garud, 1994). Therefore, these

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investigations on serodiagnosis of SrMV-IBS were done in order to assess the infections of sugarcane by SrMV-IBS in peninsular India. Mixed infections with the strains of SCMV, SrMV and MDMV of sugarcane were also assessed.

MATERIALS AND METHODS

- 1. Survey and sampling :** To assess the infections of sugarcane by SrMV-IBS and other strains of SRMV, SCMV and MDMV, a survey was conducted in 41 localities of sugarcane growing areas of Marathwada (Maharashtra), Andhra Pradesh (AP) and Tamil Nadu (TN) in peninsular India (Table-1 and 2). Fresh samples of symptomatic and symptom free leaves of sugarcane were collected from these localities and transported to ICRISAT lab for further processing. Samples were collected from sugarcane varieties grown by the cultivators in these localities as well as those grown on Sugarcane Research Stations at Perumallapalli, AP and Basmatnagar, Marathwada.
- 2. Serodiagnostic assay :** This was accomplished by using penicillinase (PNC) based DAC-ELISA and polyclonal anisera of SrMV-IBS (homologous polyclonal antiserum produced at ICRISAT, Patancheru, Andhra Pradesh, India), SrMV-H, SCMV-A, SCMV-B, SCMV-D, SCMV-E, MDMV-A, MDMV-0 (JGMV-0) (supplied by R. W. Toler, Texas University and A. G Gillaspie, USDA, Maryland, USA), SCMV-BC, SCMV-SC and JGMV-type strain (D. S. Teakle, Univ. Queensland, Australia). The procedure followed for PNC-DAC-ELISA was essentially the same as outlined by Sudarshana and Reddy (1989) and Hobbs *et al.* (1987). Leaf extracts Unfractionated (whole) antiserum cross absorbed with healthy extracts (in 1:20 proportion) was diluted to 1:1000 in antibody buffer, PBS-Tween-PVP (0.02M potassium phosphate buffer, pH 7.3, 0.15M NaCl, 0.003M KC1, 0.05% Tween-20, 2% polyvinyl pyrrolidone). Enzyme conjugate (penicillinase liked anti-rabbit IgG in goat) was diluted to 1:2500 in conjugate buffer, PBS-Tween-20-PVP-ovalbumin (Gillaspie *et al.*, 1995). After adding bromothymol blue (BTB) substrate mixture, the reactions were observed for 30 to 60 minutes and the results were quantified by measuring a loss of BTB A_{620nm} in an ELISA microplate reader (Multiskan Plus Version 2.03, Labsystems). The positive (virus) and healthy (healthy leaf extracts) controls were also

Table - 1 : Serodiagnosis of SrMV-RBS naturally infecting sugarcane in Andhra Pradesh, Tamil Nadu and Marathwada (Maharashtra)

S. No.	Field Samples/locality	SrMV-RBs/ DAC-ELISA	
		Reac- tions	Mean OD values (A_{620})
1.	Mallavarum	+	1.09
2.	Perumallapalli, COT-8201	+	0.96
3.	Perumallapalli, Co 7808	+	1.15
4.	Perumallapalli, Co 7805	+	0.70
5.	Perumallapalli, Co 81037	+	0.70
6.	Perumallapalli, Co 671	+	0.99
7.	Perumallapalli, Co 7219	+	1.25
8.	Perumallapalli, Co 8041	+	1.24
9.	Perumallapalli, Co 87040	+	1.00
10.	Perumallapalli, Co 3021	+	0.44
11.	Perumallapalli, Co COC. 88061	+	0.92
12.	Perumallapalli, Co 62175	+	1.20
13.	Damas-Maduga (Nellore)	+	0.94
14.	Cuddapah	+	1.22
15.	Kottur (Cuddapah)	-	(0.22)
16.	Rajupalem (Nellore)	+	1.62
17.	Nellore	+	1.30
18.	Gundrullamita	+	1.25
19.	Madhavamala	+	0.40
20.	Naidupet	+	0.86
21.	Tiruvellur, Co 9208	+	1.36
22.	Tiruvellur, Co 6711	+	1.10
23.	tirumuchi (near Madras)	+	0.88
24.	Hyderabad	+	1.22
25.	Basmatnagar, Co 7527(M-1)	-	(0.17)
26.	Basmatnagar, Co 7527 (M-1)	+	1.44
27.	Basmatnagar, Co 7125 (M-1)	+	1.08
28.	Basmatnagar, Co 7125 (M-2)	+	1.31
29.	basmatnagar, Co 7125 (M-3)	+	1.34
30.	Basmatnagra, Co 740	+	1.44
31.	basmatnagar, Co 7219	+	1.38
32.	Basmatnagar, CoC 671	+	1.30
33.	Basmatnagar, COM 8014	+	1.31
34.	Parbhani, Co 740 (M-1)	+	1.31
35.	Parbhani, Co 740 (M-2)	+	1.12
36.	Nandgaon, M-89/ Ratoon	+	1.96
37.	Tandaon, M-45	+	1.49
38.	Rahati, M-56	+	1.85
39.	Rahati, M-57	+	1.08
40.	Asola, M-69	+	1.94
41.	Asola, M-70	+	1.96
42.	SrMV-RBS (+ve control)	+	1.67
43.	Healthy control	+	0.04

Table - 2 : Serodiagnosis of SCMV subgroup potyviruses naturally infecting sugarcane in Andhra Pradesh, Tamil Nadu and Marathwada (Maharashtra)

S. No.	Field Samples/Locality	DAC-ELISA/OD value (A620)					
		SrMV-IBS	MDMV-A	SrMV-H	SCMV-A	SCMV-B	SCMV-SC
1.	Mallavaurm	1.09*	-	-	0.28	-	-
2.	Perumallapalli /COT 8021	0.96	-	-	0.23	-	-
3.	Perumallapalli/Co 7808	1.15	-	-	-	0.62	-
4.	Perumallapalli/ Co 671	0.99	-	-	-	0.21	-
5.	Perumallapalli/ Co 7219	1.28	-	-	0.20	0.76	-
6.	perumallapalli/ Co 8014	1.24	-	-	-	0.55	-
7.	Perumallapalli/ CoC 88061	0.92	-	-	-	0.54	-
8.	Damas-Modugu	0.49	-	-	-	0.20	-
9.	Cuddapah	1.22	-	-	-	0.19	-
10.	Kottur (Cuddapah)	-	-	1.26	-	-	-
11.	Basmatnagar/ Co 740	1.44	0.63	0.54	-	-	-
12.	Basmatnagar/Co 7219	1.39	0.49	-	-	-	0.57
13.	Basmatnagar/ Co 7125	1.31	0.23	0.20	-	-	0.64
14.	Basmatnagar/ Co 7125	1.30	0.47	-	-	-	0.72
15.	basmatnagar/ COM 8014	1.31	0.43	-	-	-	-
16.	Parbhani/ Co 740/ M-1	1.31	0.28	0.36	-	0.40	-
17.	Parbhani/ Co 740/ M-2	1.12	0.21	0.46	-	-	-
18.	SrMV-RBS (+ve control)	1.62	-	0.82	-	-	-
19.	Healthy control	0.09	0.05	0.07	0.03	0.05	0.06

*- Figures represent mean of OD values based on two dilutions of the viral antigen.

included.

RESULTS AND DISCUSSION

1. Serodiagnosis of potyviruses naturally infecting sugarcane :

The results on DAC-ELISA (Table-1) indicated that of the 41 samples processed, 39 (95%) were ELISA positive for SrMV-IBS thereby indicating its wide prevalence in sugarcane varieties grown in Marathwada, AP and TN. It was however, found to be absent in Co 7527 sugarcane variety grown at the Regional Sugarcane Research Station (RSRS), Basmatnagar (Marathwada) and in a sugarcane variety

grown by a cultivator at Kottur/Cuddapah (AP). In a sugarcane varietal trial conducted at RSRS, Basmatnagar, the SrMV-IBS infected all the test varieties (Co 7125, Co 740, Co 7219, COC 671, COM 8014) excepting Co 7527. These test varieties were found to be highly susceptible (HS) to SrMV-IBS as they supported virus multiplications at higher levels. This was evident from the high virus titres (as inferred from OD values : 1.08 - 1.44 at A_{620nm}) recorded in these varieties. In a sugarcane varietal trial conducted at the Regional Agricultural Research Station (RSRS), Perumallapalli in AP, the SrMV-IBS was also found to infect all the test sugarcane varieties recording OD values ranging from 0.44 – 1.28. The lowest OD value

was recorded in Co 3021 (0.44) which was rated as less susceptible (LS) variety to SrMV-IBS whereas highest OD value recorded in Co 7219 (1.28) and was rated as the HS to SrMV-IBS whereas highest OD value recorded in Co 7219 (1.28) and was rated as the HS to SrMV-IBS as it supported virus multiplication at the highest level. The OD values recorded in Co 7219 grown at RARS, Perumallapalli (1.28) was comparable to the one recorded in Co 7219 (1.38) grown at RSRS at Basmatnagar. Low OD values were recorded in sugarcane varieties grown by cultivators at Madhavamala (0.40) and at Damas-Moduga (Nellore) (0.49) and were rated as LS to SrMV-IBS as they supported virus multiplication at the lowest level. Intermediate OD values were recorded in sugarcane varieties grown by cultivators at Naidupet (0.88) and Tirumuchi (0.88) and were rated as moderately (MS) susceptible to SrMV-IBS as these varieties supported virus multiplication at level. High OD values were recorded in sugarcane varieties grown by cultivators at Mallavarum (1.09), Tiruvellur in Co 6711 (1.10), and Co 9208 (1.36), Cuddapah (1.22), Hyderabad (1.22), Gundrullamita (1.25), Nellore (1.30) and Rajupalem (Nellore) (1.62). These sugarcane varieties were rated as HS to SrMV-IBS as these supported virus multiplication at high level. In Marathwada, in all surveyed localities, the OD values recorded in sugarcane varieties grown by cultivators were towards higher side (1.08 – 1.96) including those grown at Parbhani in Co 740 (1.12 – 1.31), Tadgaon (1.49), Rahati-M-56 (1.85), Asola-M-69 (1.94), Asola-M70 (1.96) and Nandgaon-M-81/Ratoon (1.96). These sugarcane varieties were rated as HS to SrMV-IBS as these supported virus multiplication at high level.

Mixed infection with SrMV-H, MDMV-A and some strains of SCMV were evident in 42% (17/41) sugarcane symptomatic leaf samples from 7-localities (Mallavarum, Perumallapalli, Damas-Moduga, Cuddapah, Kottur, Basmatnagar, Parbhani). In rest of the surveyed (53%) localities, sugarcane was infected by SrMV-IBS alone. MDMV-A infections to 17% (7/41) were evident in samples from two localities (Basmatnagar, Parbhani) in Marathwada. However, sugarcane was not found to be infected by MDMV-A in AP and TN. Nevertheless, sugarcane infections by SrMV-H and SCMV strains were evident in AP, TN and Marathwada. SrMV-H to 12% (5/41) was evident in sugarcane varieties grown at Kottur, Basmatnagar, and Parbhani. Amongst SCMV strains, the infections of sugarcane by SCMV-B were higher 24% (10/41) as compared to SCMV-A (7% : 3/41) and SCMV-SC (7%). The titres of these potyviruses including MDMV-A (OD : 0.21-0.63), SrMV-H (OD : 0.20-1.26), SCMV-

B (OD : 0.20-1.33), SCMV-SC (OD : 0.57-0.72) and SCMV-A (OD : 0.20-0.23) were lower as compared to SrMV-IBS (OD : 0.49-1.44) titres. Thus, SrMV-IBS was found to be a predominant pathogen in sugarcane varieties grown in AP, TN and Marathwada (Maharashtra). The infections of sugarcane varieties grown in these three States by SrMV-I, SCMV-D, SCMV-E, SCMV-MDB, JGMV-T (type strain) and JGMV-O were found to be totally absent. SrMV-M was not included in serological assays.

Natural infection of some sugarcane commercial varieties (CP-44-101, CP-48-103, N. Co. 310, CP-52-68) by MDMV-A has also been reported from the United States (Gillaspie, 1967). MDMV-A caused mild mosaic or symptomless infections in sugarcane. Johnsongrass and corn, natural hosts of MDMV-A also occurred near cane fields (Gillaspie, 1967). In our studies, infections of some sugarcane varieties (Co. 740, Co. 7219, Co. 7125, COM-8014) by MDMV-A was evident in Marathwada but not in TN and AP. In the absence of Johnsongrass, it seems apparent that the MDMV-A has entered sugarcane via virus infected maize grown as an intercrop with sugarcane in Marathwada. Moreover, MDMV-A has also been recovered from maize crops grown in Marathwada (Mali, 1995a).

Natural infection of sugarcane by SCMV strains A, B, D and E has been reported earlier from North India (Bhargava, 1975; Bhargava *et al.*, 1972; Khurana and Singh, 1972; Rishi and Rishi, 1985; Rao *et al.*, 1998). In our studies, sugarcane was found to be naturally infected by SCMV strains A and B but not by D and E. Additionally, sugarcane was also found to be infected by SCMV-SC in peninsular India which has not been reported earlier from India.

In the United States, natural infections of sugarcane by SrMV-H (90-99) have been found at higher level than SCMV-I (1-12%) and SrMV (2-5%) (Grisham, 1994). In our studies, SrMV-H occurred at 12% level but SrMV-I was not detected in sugarcane in Marathwada (Maharashtra), TN and AP and SrMV-M was not included in serological assays. Earlier, natural infections of some sugarcane varieties by SrMV-H has been reported from Gujarat and Tamil Nadu (Joshi and Mishra, 1990) and AP (Konadaiah and Nayudu, 1984). In these cases, the identification of SrMV-H was based on host range and symptomatology and the virus identity was not confirmed by serological tests. Moreover, recent report by Hema and Sreenivasulu (1995) indicates that the sugarcane virus isolate described from AP by Konadaiah and Nayudu (1984) is not serologically related to SrMV-H or any

strains of SCMV but is a distinct sugarcane potyvirus serologically unrelated to the strains of SCMV, SrMV, MDMV and JGMV excepting SrMV-IBS to which it is serologically related. In our studies, the natural infection of sugarcane by SrMV-H was found in a single locality (Kottur) in AP although the SrMV-IBS was predominant in sugarcane in AP like in TN and Marathwada. Moreover, serological relatedness between SrMV-IBS and AP sugarcane has already been established (Mali, 1994).

2. Serodiagnosis of sap transmitted isolates from sugarcane to sorghum :

Six sugarcane potyvirus isolates from Perumallapalli, Malavarum, Kottur (AP), Tirumuchi (TN), Rahati, Parchani (Marathwada, MS) were sap transmitted from field infected sugarcane to glasshouse grown sorghum genotypes, CK-60A and CS-3541 and from Tirumuchi and Kottur to Maldandi (M-35-1) sorghum. The sugarcane test potyvirus isolates caused mosaic and red stripe (M/RS) symptoms in CK-60A, mosaic mottling (M/Mt) in CS-3541 and mosaic (M) in Maldandi sorghum.

Results of DAC-ELISA indicated that four potyviruses, SrMV-IBS (100%), SrMV-H (43%), SCMV-MDB (14%) and JGMV-T (Type strain, 14%) were transmitted mechanically from sugarcane to CK-60A sorghum. Although detected in sugarcane, SCMV-A, SCMV-B and MDMV-A were not mechanically transmitted from sugarcane mechanically transmitted from sugarcane to CK-60A sorghum. Amongst the sugarcane sap transmitted potyviruses to CK-60A sorghum, the titres recorded by SrMV-IBS were the highest (OD: 0.87 – 1.65). The rest of the potyviruses including SrMV-H (OD: 0.21 – 0.23), SCMV-MDB (OD: 0.27) and JGMV-T (OD: 0.18) recorded low virus titres in CK-60A sorghum. Based on OD values, only SrMV-IBS was mechanically transmitted from sugarcane to CK-60A sorghum. Moreover, SCMV-MDB and JGMV are not known to infect sugarcane spontaneously (Shukla and Teakle, 1989, Teakle et al., 1989). As such mechanical transmission of these two potyviruses from sugarcane to CK-60A sorghum is ruled out. Besides this, CK-60 (BTx3197) sorghum genotype is known to be resistant to SCMV-MDB and SCMV-A (Snazelle et al., 1971). Resistant status of CK-60 also accounts for the non mechanical transmission of SCMV-A from sugarcane to CK-60A sorghum.

On the other hand, more number of potyviruses including SrMV-IBS (100%), SrMV-H (71%), SCMV-A (29%), SCMV-MDB (29%), SCMV-B (14%) but not

SCMV-SC were mechanically transmitted from sugarcane to CS-3541 sorghum. However, the titres recorded by SrMV-IBS (OD: 1.03- 1.65) were higher compared to those recorded by SrMV-H (OD: 0.24-0.41), SCMV-B (OD: 0.40), SCMV-MDB (LD: 0.24-0.27), SCMV-A (OD: 0.16 – 0.24), MDMV-A (OD: 0.21), and JGMV-T (OD: 0.21) in CS-3541 sorghum. Based on the OD values, only three potyviruses viz. SrMV-IBS (OD: 1.65), SrMV-H (OD: 0.41) and SCMV-B (OD: 0.40) were mechanically transmitted from sugarcane to CS-3541 sorghum.

Three potyviruses including SrMV-IBS (100%), SrMV-H (50%) and SCMV-MDB (50%) but not SCMV-A, SCMV-B, SCMV-SC, MDMV-A and JGMV were transmitted mechanically from sugarcane to Maldandi sorghum. However, the titres recorded by SrMV-IBS (OD: 0.54-0.57) were high compared to those recorded by SrMV-H (OD: 0.20) and SCMV-MDB (OD: 0.16). based on OD values, only SrMV-IBS was mechanically transmitted from sugarcane to Maldandi sorghum. In all these cases, SrMV-IBS was mechanically transmitted with 100% efficiency from sugarcane to sorghum genotypes, CK-60A (OD: 1.65), CS-3541 (OD: 1.65) and Maldandi (OD: 0.57) recording high virus titres. Nevertheless, titres recorded by SrMV-IBS in Maldandi sorghum were low compared to those recorded in CK-60A and CS-3541 sorghum thereby indicating that the Maldandi sorghum comparatively supported less virus multiplication. These results confirm our earlier findings that more than one potyvirus (SrMV, SCMV) was transmitted from sugarcane to sorghum under natural epiphytotic conditions in Marathwada, India (Mali and Garud, 1994).

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