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Reactions and Virus Titres of Differential Sorghum Inbred Lines Mechanically Inoculated with an Indian Isolate of Sorghum Red Stripe Potyvirus (SRSV-Ind)

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Of the 18 sorghum differentials tested, SRSV-Ind (Tx 2786 isolate) induced systemic necrotic red stripe reactions (DSR = 4) in four sorghum differentials (Redlan, Hegari, QL-3/ India, SCO-175-14E), mild mosaic (DSR = 2) in equal number of sorghum differentials (RTx 09, Martin, Caprock, RTx 7078) and severe mosaic (DSR = 3) in ten sorghum differentials (NM-31, BTx 623, QL-11, PI-35038, BTx 3048, Rio, Tx 2786, Atlas, SA-394, Cargill-40). Positive correlation between infectivity levels (IL), disease severity reactions (DSR) and virus titres (VT) was found only in two sorghum differentials (Cargill-40, SCO-175-14E) infected mechanically by SRSV-Ind. The virus (SRSV-Ind) was found distinct from MDMV-B (SCMV-MDB) in infecting nine sorghum differentials known to be resistant to MDMV-B, from SrMV in infecting three sorghum differentials (Tx 2786, QL-3, QL-11) known to be resistant to well characterized SCMV strains and from MDMV-A in infecting QL-11 sorghum differential known to be resistant to MDMV-A.

KEY WORDS : Sorghum differentials, disease severity reactions (DSR), SRSV-Ind, SCMV, SrMV, JGMV, MDMV

INTRODUCTION

A potyvirus naturally infecting sorghum *(Sorghum bicolor)* crop grown in the proximity of sugarcane (*Saccharum* species hybrids) in Maharashtra, India has been labeled as sugarcane mosaic virus-Jg (SCMV-Jg) (Garud and Mali, 1985; Mali and Garud, 1977, 1978), SRSV-Ind (Mali and Garud, 1994), SrMV-RBS, a resistant breaking strain of SrMV (Mali, 1995), an Indian isolate of sorghum mosaic potyvirus (SoMV-Ind) (Anonymous, 1994). The name SRSV-Ind is preferable over SrMV-RBS and SoMV-Ind as the virus causes systemic necrotic red stripe symptoms in number of sorghum genotypes (Mali, 1994). SRSV-Ind causes systemic symptoms of mosaic followed by necrotic red stripe and temperature dependent red leaf in sorghum. In this respect, it resembles an European isolate of sorghum red stripe caused by sugarcane mosaic potyvirus (Grancini and Mariani, 1974; Dijkstra and Grancini, 1957; Lovisolo, 1957; Pirone, 1972; Persley, 1996) and an Australian isolate of sorghum red stripe caused by Johnsongrass mosaic potyvirus (JGMV) (Shukla and Teakle, 1989). SRSV-Ind is distinct from JGMV as it is not serologically related to JGMV. SRSV-Ind is also not serologically related to SCMV strains reported from the United States and Australia (Teakle *et al.*, 1989) but its serological relationships with European SCMV strains have not been determined (Mali, 1994). However, SRSV-Ind shares common epitopes with a new strain of SCMV (SCMV-N) reported from Tirupati, India (Hema and Sreenivasulu, 1995; Kondaiah and Nayudu, 1984). Recently, Peterschmitt *et al.*, (1991) purified an isolate (CS-3541) of SRSV-Ind and produced a polyclonal homologues antiserum and concluded that SRSV-Ind is caused by a strain of SCMV. SCMV-N also gave positive serological reactions with the antiserum of SRSV-Ind in DAC-ELISA (Hema and Sreenivasulu, 1995). Moreover, the SRSV-Ind does not share common epitopes with MDMV strains (A, D, E, F) (Ford *et al.*,

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Table 1. : Infectivity levels (IL), disease severity reactions (DSR) and virus titres (VT as expressed in OD values) of grain sorghum accessions mechanically inoculated with SRSV-Ind in glasshouse

Sorghum Accessions	IL* (%)	DSR**	DAC-ELISA OD value ($A_{620/nm}$)	Ratings**** IL/DSR/ODV
RTx 09	28.6	2	0.99***	LS/LS/MS
Martin	76.9	2	1.04	MS/LS/HS
Caprock	40.0	2	1.34	LS/LS/HS
RTx 7078	53.3	2	0.88	MS/LS/MS
N. Mex. 31	73.3	3	1.05	MS/MS/HS
BTx 623	100	3	1.25	HS/MS/HS
QL-11	100	3	1.21	HS/MS/HS
PI-35038	100	3	0.80	HS/MS/MS
BTx 3048	100	3	1.18	HS/MS/HS
Rio	80.0	3	1.22	MS/MS/HS
Tx 2786	100	3	1.30	HS/MS/HS
Atlas	78.6	3	1.19	MS/MS/HS
SA-394	53.8	3	1.35	MS/MS/HS
Cargill-40	78.6	3	0.73	MS/MS/MS
Redlan	60.0	4	0.98	MS/HS/MS
Hegari	86.2	4	0.82	MS/HS/MS
QL-3 (India)	57.0	4	0.63	MS/HS/LS
SCO-175-14E	100	4	1.57	HS/HS/HS
CK-60A (LE)*****	86.7	4	1.12	MS/HS/HS
CS-3541 (LE)	60.0	3	1.19	MS/MS/HS
CSH-1 (LE)	50.0	4	1.69	MS/HS/HS
CS-3541 (healthy)	0	0	0.04	----------

* – Percentage infectivity based on 20 plants each of the test accession.

** – DSR (disease severity reactions) : 2 – mild mosaic, 3 – strong mosaic, 4 – red stripe/red leaf and necrosis.

*** – OD values based on means of two dilutions of the virus antigen.

**** – Ratings : LS – less susceptible, MS – moderately susceptible, HS – Highly susceptible.

***** – LE : Local entries of sorghum cultivars/hybrids.

1989). Nevertheless, SRSV-Ind shares common epitopes with the strains of sorghum mosaic potyvirus (SrMV-H and M) (Mali, 1995; Giorda *et al.*, 1986; Shukla *et al.*, 1989). The SRSV-Ind is however, distinct from SrMV which causes mosaic and temperature independent red leaf but not necrotic red stripe symptoms in sorghum (Giorda *et al.*, 1986). The SRSV-Ind is also distinct from MDMV which causes mosaic and temperature dependent red leaf but not necrotic red stripe symptoms in sorghum (Toler, 1985). Recently, strains of these viruses (SCMV, SrMV, MDMV, JGMV) have been differentiated using a set of differential sorghum inbred lines based on their disease severity reactions (DSR) (Toler, 1980; Giorda *et al.*, 1986; Persley *et al.*, 1985; Tosic *et al.*, 1990). Knowledge of the relationships among the SCMV-subgroup potyviruses (SCMV, MDMV, SrMV, JGMV) occurring in different areas of the world is important because of the wide spread economic importance of theses viruses in sorghum and because resistance and

Table 2. : Comparative disease severity reactions (DSR) of sorghum accessions to SRSV-Ind, MDMV-A, SCMV-MDB, SrMV and JGMV-O (compiled by R.W. Toler, Texas A and M, Univ., USA)

Sorghum Accessions	SCMV sub-group potyvirus strains/isolates (DSR)*					
	SRSV-Ind	MDMV-A	SCMV-MDB	SrMV-H	SrMV-Sor	JGMV-O
Tx 2786	3	2	0	0	0	2
Cargill-40	3	2	-	5	5	-
Atlas	3	4	EL	5	5	2
Rio	3	5	4	4	4	2
N. Mex. 31	3	5	0	5	5	2
Redlan	4 (RS)	4	0	5	5	2
SCO-175-14E	4 (RS)	4	3	3-4	4	2
Caprock	2	4	5	5	5	2
RTx 09	2	3	3	3	4	3
SA-394	3	3	5	5	5	2
PI-35038	3	-	LL	5	5	4
BTx 3048	3	-	0	5	5	2
QL-11	3	0	0	0	0	0
QL-3 (India)	4 (RS)	(2)**	0	0	0	0
BTx 623	3	3	3	3	3	2
Hegari	4 (RS)	3	3	3	3	2
RTx 7078	2	3	3	3	3	3
Martin	2	3	0	5	5	2

* - DSR (disease severity reactions); 0 - Immune or symptomless; LL - Local lesions only; EL - Elongated lesions; RS - Red stripes; 2 - Mild mosaic; 3 - Strong mosaic; 4 - Slight red leaf (Necrosis); 5 - Severe red leaf and general necrosis; ** - Parentheses indicates rare occurrence

tolerance in sorghum may be strain specific (Toler, 1980). Eighteen grain sorghum accessions (identified as differential sorghum inbred lines for identification of strains of SCMV, SrMV, MDMV, JGMV) (Table-1) received from Dr. R.E. Toler, Texas A&M University, USA were tested for their reactions and virus titres after manual inoculation with SRSV-Ind in the glasshouse and reactions were compared to those produced by the strains of SrMV, MDMV, SCMV and JGMV in order to establish the relationship of SRSV-Ind with these potyvirses.

MATERIALS AND METHODS

The virus isolate of SRSV-Ind from a naturally infected sugarcane (cv. Co. 740) grown in Maharashtra was mechanically transmitted and maintained in an insect free glasshouse on a SrMV immune sorghum inbred line, Tx 2786. Inoculum for mechanical transmission was prepared by macerating leaf tissue 1:5 (w/v) in 0.05M potassium phosphate buffer containing 0.075% thioglycerol (pH 7.0). Carborundum (800 mesh) was used as an abrasive. Twenty plants for each sorghum accession were raised from healthy seeds in suitable earthen pots containing steam sterilized soil, sand and compost mixture (2:1:1 v/v) at 25 to 31°C in the glasshouse. The plants were inoculated at the 3-leaf stage by conventional leaf rub method. Symptomless plants were indexed on Tx 2786 test plants for detecting latent infections if any. Plants were rated for virus infections with disease severity reactions (DSR) on a scale of 1 to 5 where O = immune or symptomless; 1 = local lesions only; 2 = mild mosaic; 3 = strong mosaic; 4 = slight red stripe/red leaf (necrosis) and 5 = severe red stripe/red leaf and general necrosis.

The virus titres of inoculated and non-inoculated

sorghum accessions were determined using penicillinase based direct antigen coating form of enzyme linked immunosorbent assay (PNC-DAC-ELISA) and the polyclonal antiserum of SRSV-Ind, CS-3541 isolate (supplied by D.V.R. Reddy. ICRISAT, Patancheru, A.P., India), MDMV-A, SCMV-A, B, D and E (supplied by A.G. Gillaspie, USA), MDMV-B, SrMV-H and I, JGMV-O (supplied by R.W. Toler, USA) and SCMV-Sc, JGMV-Type isolate (Supplied by D.S. Teakle, Australia). The procedure followed for PNC-DAC-ELISA was essentially the same as outlined by Sudarshana and Reddy (1989) and Hobbs *et al.* (1987). Plant extracts were prepared in 0.05M carbonate buffer (coating buffer) pH 9.6 with two serial dilutions, 10^{-1} and 10^{-2}. Unfractionated (whole) antiserum cross-absorbed with healthy plant extracts from sorghum (cv. CS-3541) (in 1:2 proportion) was diluted to 1:1000 in antibody buffer. Enzyme conjugate (penicillinase labeled anti-rabbit IgG produced in goat) was diluted to 1:2500 in conjugate buffer. After adding bromothymol blue (BTB) substrate mixture (pH 7.2), the reactions were observed for 30 minutes to 1 hour and results were quantified by measuring a loss of BTB at $A_{620(nm)}$ in an ELISA microplate reader (Multiskan Version 2.03, Labsystems).

RESULTS AND DISCUSSIONS

Of the 18 sorghum accessions tested, all were found to be susceptible to SRSV-Ind and none was found to be resistant or tolerant (Table-1). However, the infectivity levels (IL) of the virus was found to vary between sorghum accessions. It was lowest (28.6%) in RTx 09 and this was rated as a less susceptible (LS) accession to SRSV-Ind based on IL. Similarly another accession, Caprock was rated as LS based on IL (40%). About 56% (10/18) sorghum accessions recorded IL ranging from 53 to 87% including Tx 2786 (53%), SA-394 (54%), Redlan (60%), QL-3/India (57%), NM-31 (73%), Martin (77%), Atlas (79%), Cargill-40 (79%), Rio (80%) and Hegari (87%) and these were rated as moderately susceptible (MS) to SRSV-Ind based on IL. Over 33% (6/18) sorghum accessions (BTx 623, PI-35038, BTx 3048, QL-11, Tx 2786, SCO-175-14E) had 100% IL and these were rated as highly susceptible (HS) accessions to SRSV-Ind.

Similarly the DSR (disease severity reactions) caused by the virus were also found to vary between sorghum accessions (Table-1). The virus was found to cause mild mosaic (DSR = 2) in 22% (4/18) accessions (RTx 09, Martin, Caprock, RTx 7078) and these were rated as LS accessions to SRSV-Ind. The virus was found to cause strong mosaic (DSR = 3) in 56% (10/18) sorghum accessions (NM-31, BTx 623, QL-11, PI-35038, BTx 3048, Rio, Tx 2786, Atlas, SA-394, Cargill-40) and these were rated as MS accessions to SRSV-Ind. The virus caused red stripe and red leaf followed by necrosis (DSR = 4 or 5) in 22% (4/18) sorghum accessions (Redlan, Hegari, QL-3/India, SCO-175-14E) and these were rated as HS accessions to SRSV-Ind.

The virus titres in the susceptible test sorghum accessions as determined by PNC-DAC-ELISA and OD values recorded at 620 nm indicated that the accession SCO-175-14E (OD = 1.57) had the highest virus titre and the accession QL-3/India (OD = 0.63) had the lowest virus titre. These accessions were rated as HS and LS, respectively to SRSV-Ind as the former supported the highest and the latter the least virus multiplication. Intermediate virus titres were recorded in 33% (6/18) sorghum accessions including Cargill-40 (OD = 0.73), PI-35038 (OD = 0.80), Hegari (OD = 0.82), RTx 7078 (OD = 0.88), Redlan (OD = 0.98), RTx 09 (OD = 0.99) and these were rated as MS accessions to SRSV-Ind supporting medium virus multiplication. High virus titres were recorded in 61% (11/18), sorghum accession including Martin (OD = 1.04), MN-31 (OD = 1.08), BTx 3048 (OD = 1.18), Atlas (OD = 1.19), QL-11 (OD = 1.21), Rio (OD = 1.22), BTx 623 (OD = 1.25), Tx 2786 (OD = 1.30), Caprock (OD = 1.34), SA-394 (OD = 1.35), SCO-175-14E (OD = 1.57) and these were rated as HS to SRSV-Ind supporting high level of virus multiplication.

Positive correlation between infectivity levels (IL), DSR and virus titres (VT) based on OD-values (ODV) was found in 11% (2/18) sorghum accessions (Cargill-40, SCO-175-14E) whereas negative correlation between IL, DSR and VT was found in case of two accessions (11%) viz., Martin and QL-3/India. Positive correlation only between IL and DSR was found in case of 33% (6/18) sorghum accessions (RTx 09, Caprock, NM-31, Rio, Atlas, SA-394); between IL and VT in case of 39% (7/18) sorghum accessions (RTx 7078, BTx 623, QL-11, BTx 3048, Tx 2786, Redlan, Hegari) and between DSR and VT in case of a single accession, PI-35038 (6%).

Perusal of comparative data on DSR of sorghum accessions to SRSV-Ind and other potyviruses as shown in Table-2 indicates that the SRSV-Ind infected all the seven sorghum accessions (Tx 2786, NM-31, Redlan, BTx 3048, QL-11, QL-3/India, Martin) that are known to be resistant to SCMV-MDB strain (syn. MDMV-B) (Ford and Hill, 1976; Bockholt *et al.*, 1968). Moreover, two accessions (Atlas, PI-35038) reacting only with local lesions to SCMV-MDB were infected systemically

by SRSV-Ind. On the basis of reactions in these nine sorghum accessions, SRSV-Ind and SCMV-MDB were distinct. This has also been confirmed by the lack of serological relatedness between these two potyviruses (Mali, 1994).

SRSV-Ind also showed DSR similar to that of MDMV-A in 22% (4/18) sorghum accessions (Redland, SCO-175-14E, SA-394, BTx 623). However, SRSV-Ind was distinct from MDMV-A in infecting sorghum accession, QL-11 resistant to MDMV-A and for the lack of serological relationship with MDMV-A (Mali, 1994). SRSV-Ind also showed DSR similar to that of JGMV-O in 11% (2/18) sorghum accessions (Caprock, Martin). However, SRSV-Ind was distinct from JGMV-O in infecting sorghum accessions (QL-11, QL-3/India) resistant to JGMV-O and for lack of serological relationship with JGMV (Mali, 1994). SRSV-Ind also showed DSR similar to that of SrMV in 11% (2/18) sorghum accessions (SCO-175-14E, BTx 623) but it was distinct from SrMV in infecting three sorghum accessions (Tx 2786, QL-11, QL-3/India) (Giorda *et al.*, 1986). However, these two potyviruses have been found serologically related to each other (Mali, 1994; 1995). For causing necrotic red stripes in sorghum, SRSV-Ind is also distinct from SrMV (Mali and Garud, 1994).

The sorghum differential, QL-11 has been found to be susceptible to Krish infecting strain of JGMV (serologically related to a type strain of JGMV) in Australia (Shukla and Teakle, 1989) and to an isolate of SCMV-F from Pakistan (Gillaspie and Mock, 1984). SRSV-Ind is not serologically related to JGMV (Type and O strains) and the serological relationships between SRSV-Ind and SCMV-F have not been determined (Mali, 1994). Recently, isolates of SCMV-A have been found to produce systemic necrotic red stripe symptom in mechanically inoculated plants of some sorghum differentials (Atlas, Tx 2786) and isolates of MDMV-A in Rio sorghum differential in India (Rao *et al.*, 1998). However, SRSV-Ind induced only systemic mosaic symptoms in Atlas, Rio and Tx 2786 sorghum differentials and is also not serologically related to SCMV-A and MDMV-A (Mali, 1994).

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