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Occurrence of an isolate of maize stripe virus on sorghum in India*

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Summary

A disease characterised by chlorotic stripes and bands, named sorghum stripe disease (SStD), was observed on sorghum in India with an incidence of less than 0.5% to nearly 10%. The affected plants were dwarfed and had poor or no panicle formation. This disease could be transmitted by the delphacid planthopper *Peregrinus maidis* to sorghum but not to *Brachiaria eruciformis; Cenchrus ciliaris; Chloris barbata; Dichantium annulatum; Dichantium aristatum; Digitaria ciliaris; Dinebra retroflexa; Echinocloa colona; Eleusine coracana; Pennisetum glaucum; Pennisetum violaceum; Setaria pallida Fusca; Triticum aestivum* and *Zea mays*. Sorghum stripe disease was shown to be caused by a tenuivirus serologically related to maize stripe virus (MStV). Virus particles were filamentous, less than 10 nm in width. The purified virus preparation contained only one polypeptide of 34 500 D. Eight species of nucleic acids, four ssRNA of 1.21, 0.87, 0.73, 0.47 × 10^6D and four dsRNA of 2.43, 1.69, 1.40, 0.71 × 10^6D, were extracted from purified virus preparations. When the four dsRNA were denatured, they migrated along with the four ssRNA species indicating that dsRNA contained duplex RNA of same molecular weight as the four ssRNA. In enzyme-linked immunosorbent assay and in electro-blot immunoassay it was evident that MStV-Sorg was serologically more closely related to the MStV isolates from Florida, Reunion and Venezuela than to a RStV isolate from Japan. The virus was named MStV-Sorg to distinguish it from MStV which readily infects maize. This is the first report of occurrence of a tenuivirus in the Indian subcontinent.

Key words: Maize stripe virus-Sorg, sorghum stripe disease, *Peregrinus maidis*, tenuivirus, ELISA, electro-blot immunoassay, *Sorghum bicolor*

Introduction

Among the virus diseases of sorghum (*Sorghum bicolor* (L.) Moench) reported from India (Capoor, Rao & Varma, 1968; Mali & Bhagawat, 1975; Garud, 1982) only maize mosaic virus (MMV) has been characterised (Naidu *et al.*, 1989). A survey for sorhgum viruses at several locations in India, revealed the presence of virus disease exhibiting large chlorotic spots, and bands or stripes on leaves. Incidence was nearly 10% in rainy season crops. Similar leaf symptoms were described as freckled yellow disease by Cherian & Kylasam (1937) and as chlorosis of sorghum transmitted by the planthopper *Peregrinus maidis* (Ashn.) (Capoor *et al.*, 1968). Since chlorosis is common to several virus diseases occurring on sorghum (Toler, 1980), and freckled yellowing is not a typical symptom, we named this disease sorghum stripe disease because pale yellow or light green stripes were the most prominent symptoms. In preliminary

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experiments utilising a direct antigen coating enzyme-linked immunosorbent assay (DAC-ELISA) procedure, infected leaf extracts reacted positively with an antiserum produced against a maize stripe virus (MStpV) isolate occurring in Reunion (Peterschmitt, Chatenet & Baudin, 1987), indicating that the disease was caused by a virus related to MStpV. In initial experiments, it was transmitted to sorghum by *P. maidis*. In this paper, symptoms and transmission of sorghum stripe disease, as well as the purification, serological relationships and physico-chemical properties of the causal virus are presented and discussed.

Materials and Methods

Virus maintenance

Sorghum plants showing typical stripe symptoms were collected from the ICRISAT farm. *P. maidis* was used for transmitting the virus. Colonies of *P. maidis* were maintained on sorghum (Naidu *et al.*, 1989). Last instar nymphs and young adults were used for acquiring the virus and then transferred to 3 - 5 wk old sorghum plants. The acquisition access, incubation, and inoculation access periods were 4, 11 and 4 days, respectively (Gingery, Nault & Bradfute, 1981). All exposed plants were maintained in a glasshouse at 20 - 30 °C.

Host range

After a 7-day acquisition access period followed by a 10-day incubation period on sorghum, *P. maidis* were transferred to at least 15 plants of each test species and was allowed a 5-day inoculation access period. The following species were tested: *Brachiaria eruciformis; Cenchrus ciliaris; Chloris barbata; Dichantium annulatum; Dichantium aristatum; Digitaria ciliaris; Dinebra retroflexa; Echinocloa colona; Eleusine coracana* cvs IE 2214 and IE 2540; *Pennisetum glaucum* cvs WCC 75, P 7 - 4, NHB 3 and IP 5467; *Pennisetum violaceum* cv. P 2662; *Setaria pallida Fusca; Sorghum bicolor* cvs ICSV 1, CS 3541, CSH 6 and IS 2266; *Triticum aestivum* cv. RR 21 and *Zea mays* cvs Ashwini, Ganga, Rohini, DH 103 and Madhuri (Sweet corn cv.). All cultivars and grasses were inoculated when they were 2 to 3 wk old plants. After inoculation, exposed plants were sprayed with malathion and kept for observation for at least 4 wk in the glasshouse at 20 - 30 °C. Irrespective of the symptoms produced, all exposed plants were assayed by DAC-ELISA using homologous antiserum.

Virus purification

The procedure used was a modification of that reported by Lastra & Carballo (1985). Buffers and glassware used in the purification were heat sterilised. 8 to 12 wk old sorghum plants showing typical leaf symptoms were ground in a Waring blender in steril 0.2 M potassium phosphate buffer, pH 8.0, containing 1.2 ml/litre thioglycerol, and 0.001 M DIECA, at a ratio of 1:4 (1 g tissue in 4 ml buffer). The crude extract was squeezed through muslin cloth and treated with 10% (v/v) chloroform. After shaking for 10 min, the emulsion was centrifuged at 8000 ***g*** for 10 min. Polyethyleneglycol 8000 at 60 g/litre and NaCl to give 0.2 M were added to the aqueous phase and, after dissolving, the mixture was allowed to stand for 2 h at 4 °C. The resulting precipitate was collected by centrifuging for 20 min at 13 000 ***g***. The pellets were resuspended in sterile 0.1 M potassium phosphate buffer, pH 8.0 (PB) at a rate of 1 ml for each 6 g tissue, clarified at 2500 ***g***, and the supernatant subjected to rate zonal density gradient centrifugation. Sucrose gradients were prepared in Beckman SW28 rotor tubes by layering 8 ml each of 100, 200 and 300 g/litre and 10 ml of 400 g/litre sucrose prepared in sterile PB. Gradients were stored overnight at 4 °C before use. Approximately 2 ml of virus preparation was layered on each tube and centrifuged for 2.5 h at 5 °C in a Beckman SW28 rotor at 120 000 ***g***. Three light scattering zones observed at heights of 4.2, 5.5 and 6.3 cm were withdrawn, diluted in sterile PB and pelleted at 150 000 ***g*** for 3.5 h in a Beckman R50Ti

rotor. The pellets were resuspended in sterile PB. The yield of purified virus was estimated assuming E(0.1%, 1 cm, 260) = 2.3 (Gingery *et al.*, 1981).

Electron microscopy

Grids with purified preparations were stained with 20 g/litre uranyl acetate.

Production of antiserum

Approximately 1 mg of purified virus was emulsified with an equal volume of Freund's incomplete adjuvant and injected intramuscularly into a New Zealand White inbred rabbit. At 5-days intervals, four further intramuscular injections of 1 mg virus each were given. Starting one week after the last injection, the rabbit was bled at weekly intervals for 2 months. Each bleed was tested by DAC-ELISA utilising healthy and infected sorghum leaf extracts. The titre of the antiserum was determined by the precipitin ring interphase test utilising purified virus (Reddy & Black, 1966).

Heterologous antisera

The heterologous antisera used in this study were generous gifts from others working with stripe viruses. They were prepared against different isolates of MStV and an isolate of rice stripe (RStV): MStV Florida isolate (MStV-Fl) from Dr J. H. Tsai, USA; MStV Reunion isolate (MStV-Re) from Dr P. Baudin, France; MStV Venezuela isolate, capsid protein (MStV-Ve) and non-capsid protein (MStV-NC-Ve), from Dr O. Carballo, Venezuela; RStV from Dr S. Yamashita, Japan.

Enzyme-linked immunosorbent assay

A direct antigen coating method (DAC-ELISA) and protein A coating method (PAC-ELISA) were used as described by Hobbs, Reddy, Rajeshwari & Reddy (1987) with a few modifications.

DAC-ELISA: The antigen coating was carried out overnight at 4 °C or for 2 h at 37 °C and followed by a blocking step utilising 10 g/litre bovine serum albumin for 30 min at 37 °C. Antisera were used at dilutions of 1:500, 1:5000 and 1:10 000.

PAC-ELISA: Protein A (Sigma, USA) was added to the plates at a concentration of 5 ng/ml. The first antiserum was always the homologous one used at dilution of 1:20 000. The antigen incubation was carried out overnight at 4 °C. Second antisera were used over a range of dilutions from 1:2000 to 1:100 000.

In both methods plant samples were ground with an homogeniser (SDT Tissumiser) at a 1:10 dilution in carbonate or phosphate buffer depending on the method. Neither sodium diethyldithiocarbamate nor polyvinyl pyrrolidone were added to any buffer. Rabbit Fc-specific globulins prepared in goat (Sigma, USA) and conjugated to penicillinase as described by Sudarshana & Reddy (1989) were used in both methods. The conjugate was used at a 1:10 000 dilution. Substrate (Penicillin G with bromophenol blue) incubation times were 60 or 90 min. Absorbance values were recorded at 620 nm. Values exceeding 0.2 optical density (O.D.) units over that of comparable healthy sorghum extracts were considered to be positive. All assays were duplicated and conducted three times.

SDS-polyacrylamide gel electrophoresis (SDS-PAGE)

Purified virus preparations were solubilised and analysed by SDS polyacrylamide gel electrophoresis (Laemmli, 1970). Electrophoresis was carried out using a 100 g/litre resolving gel and a 40 g/litre stacking gel, at 30 V for 18 h at room temperature. Phosphorylase B (97 400), bovine serum albumin (66 200), ovalbumin (42 699), carbonic anhydrase (31 000),

soybean trypsin inhibitor (21 500) and lysozyme (14 400) (BioRad, USA) were used as molecular weight markers.

Electro-blot immunoassay

Details of the procedure used for electrophoretic transfer of proteins to a nitrocellulose membrane were similar to those described by Naidu *et al.* (1989). The homologous antiserum was used at 1:1000 and all heterologous antisera, at 1:250 dilutions. Rabbit Fc-specific antibodies prepared in goat (Cappel Laboratories, USA) were conjugated to horse radish peroxidase (Sigma, USA) by the periodate oxidation method (Barbara & Clark, 1982). The conjugate was used at a 1:500 dilution. The substrate, 3, 3′, 5, 5′, tetramethyl benzidine was obtained from Kirkegaard & Perry Laboratories, USA.

Nucleic acid analysis

Purified virus suspended in PB was treated with equal volumes of 0.06 M NaCl, 0.01 M Tris HCl, 0.003 M EDTA, pH 8.6 saturated phenol containing 100 g/litre m-cresol and 1 g/litre 8-hydroxyquinoline. The aqueous phase was collected and treated with a mixture of chloroform and isoamyl alcohol (24:1, v/v). The aqueous phase was removed and the nucleic acid precipitated with ethanol at −20 °C. Approximately 10 μg of nucleic acid was used (extinction coefficient assumed to be 25) for treatment with RNase. The nucleic acid was suspended either in a low salt buffer (0.01 M Tris, 0.0001 M EDTA pH 7.5) and treated at room temperature for 30 min or in a high salt buffer (0.3 M NaCl, 0.03 M sodium citrate, pH 7.0) and treated at 4 °C for 70 min. Nucleic acid samples were denatured with formaldehyde (Maniatis, Fritsch & Sambrook, 1982) except that the buffer used was 0.02 M HEPES, 0.001 M EDTA pH 7.8 (HEPES/EDTA). Nucleic acids were eluted on a column of CF-11 cellulose (Dodds, 1986). The ssRNA and dsRNA fractions were analysed on agarose gels after denaturation with dimethyl sulfoxide (DMSO) (Maniatis *et al.*, 1982). All samples were electrophoresed in 1% agarose gel in TBE (0.089 M Tris 0.089 M boric acid, 0.002 M EDTA pH 7.6) non-denatured or in HEPES/EDTA when denatured. Electrophoresis was generally carried out at 50 V for about 6 h. Gels were stained with ethidium bromide and destained in distilled water (Sharp, Sugden & Sambrook, 1973). Molecular weights of the nucleic acids were estimated by comparison with brome mosaic virus (BMV) RNA under denaturing conditions and with rice dwarf virus (RDV) RNA.

Results

Symptoms

One week after inoculation, chlorotic spots arranged in lines parallel to the midrib appeared on the youngest leaves. On the younger part of their lamina and on the leaves unfolded after infection, bigger spots and finally chlorotic or pale yellow bands were observed (Fig. 1). In the case of severe infection the entire leaf became chlorotic. Poor panicle exertion (Fig. 1) and severe stunting (Fig. 2) were observed on plants which were early infected.

Host range

All *S. bicolor* cultivars were infected. The number of infected plants out of 15 tested was 13 for ICSV 1, 9 for CS 3541, 14 for CSH 6 and 12 for IS 2266. None of the 15 plants of the other species showed stripe symptoms, and the virus could not be detected in them by DAC-ELISA.

Virus purification

Only leaves showing severe symptoms were used for purification since these contained the maximum virus concentration, as determined by ELISA. In initial experiments, the following

Fig. 1. Symptoms of maize stripe virus, sorghum isolate (MStV-Sorg). Note chlorotic stripes on the leaves and poor panicle exertion.

six buffers employed in extracting tenuiviruses, were compared. (A) 0.1 M dibasic sodium phosphate containing 0.1 M sodium diethyl dithiocarbamate (DIECA), pH adjusted to 7.2 with ascorbic acid (Toriyama, 1982); (B) 0.1 M potassium phosphate pH 7.0 containing 5 ml/litre 2-mercaptoethanol and 0.01 M disodium ethylene diamine tetraacetic acid (Falk & Tsai, 1984); (C) 0.1 M borate pH 8.0, containing 0.01 M sodium sulphite (Hibino *et al.*, 1985); (D) 0.2 M potassium phosphate pH 7.6, containing 1.2 ml/litre thioglycerol and 0.001 M DIECA (Lastra & Carballo, 1985); (E) same as D but pH 8.0; (F) 0.1 M glycine pH 7.4, containing 5 ml/litre mercaptoethanol and 0.01 M EDTA. The highest viral antigen concentration, as measured by ELISA, was obtained with 0.2 M potassium phosphate buffer pH 7.6 or 8.0 (D or E). When tested with phosphate buffer (B), 10% chloroform clarification was preferred over that of 10% carbon tetrachloride because it removed more green material with no difference in the yield of partially purified virus antigens as measured by ELISA. Bentonite treatment at 1.5 g/litre suggested by Hibino *et al.* (1985), resulted in a substantial loss in viral antigen concentration, as measured by ELISA. Several combinations of polyethylene glycol (PEG) and NaCl were tested. PEG at 60 g/litre with NaCl at 0.2 M gave satisfactory preparations. Potassium phosphate buffer (0.1 M) pH 8.0 (PPB) was compared

Fig. 2. Symptoms of MStV-Sorg on early infected sorghum. Note severe stunting.

with 0.1 M potassium borate buffer pH 8.0 (PBB) (Hammond & Lawson, 1988) for the resuspension of the PEG pellets and for preparing density gradients. Based on spectrophotometric measurement of purified virus pelleted from the sucrose gradient zones, PPB consistently gave preparations with higher concentrations of virus. Only convoluted filamentous particles of less than 10 nm width (Fig. 3) were detected by electron microscopy, in all three light scattering zones following sucrose gradient analysis. The bottom zone (B) contained less virus than the top (T) and the middle (M) zones. Virus from all the three zones was combined for yield determination, which was 10 - 20 mg from 100 g tissue, assuming an extinction coefficient identical to that of MStpV (Gingery *et al.*, 1981). The A_{260}/A_{280} ratios were 1.54 $\pm$ 0.1, 1.44 $\pm$ 0.08 and 1.55 $\pm$ 0.1 for the T, M and B zones, respectively.

Serology

The homologous antiserum had a titre of 1/1280 in the precipitin ring interphase test. In the DAC-ELISA, the antiserum diluted to 10^{-4} detected the virus in crude sorghum extracts diluted up to 10^{-5}. In comparative DAC-ELISA tests, crude infected leaf extracts cross-reacted with antiserum to MStV-Fl, MStV-Re, MSt-Ve, RStV (Table 1) and with antiserum to

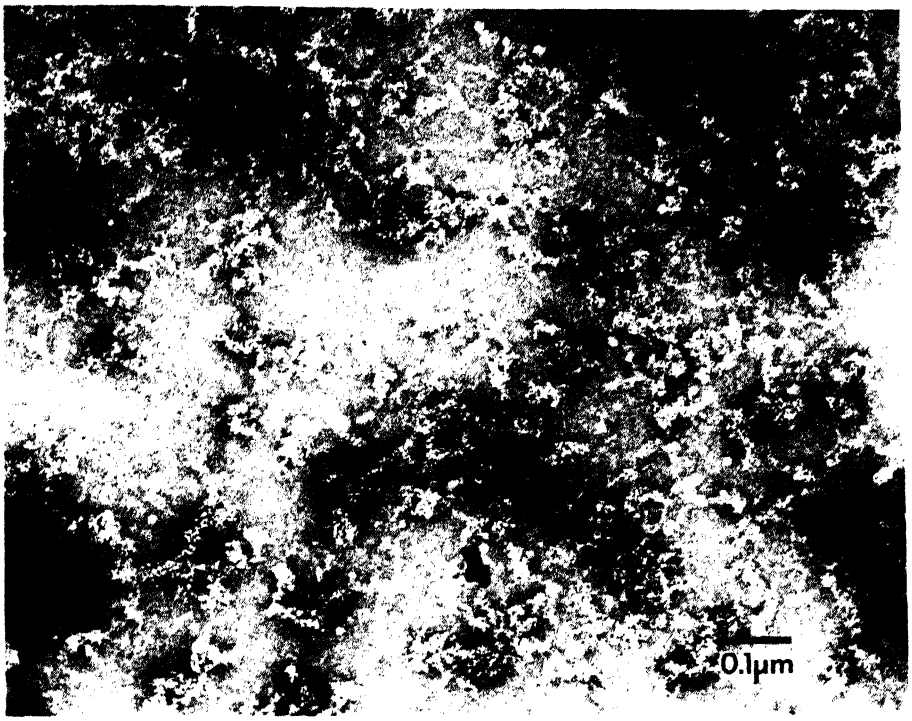

Fig. 3. MStV-Sorg particles from a purified preparation stained with 20 g/litre aqueous uranyl acetate. Bar represents 100 nm.

Table 1. *Determination of serological relationships of a sorghum isolate of maize stripe virus by a direct antigen coating form of enzyme-linked immunosorbent assay utilising penicillinase*

	Dilution of sorghum leaf extracts					
	Healthy			*Infected*		
Antiserum dilution	10^{-1}	10^{-3}	10^{-5}	10^{-1}	10^{-3}	10^{-5}
Homologous						
1/500	0.26*	0.29	0.10	1.57	1.62	1.36
1/5000	0.02	0.02	0.00	1.40	1.60	0.96
MStV-Fl						
1/500	0.00	0.01	0.00	0.77	1.37	0.37
1/5000	0.00	0.00	0.00	0.08	0.30	0.01
MStV-Re						
1/500	0.35	0.22	0.01	1.43	1.61	1.02
1/5000	0.05	0.04	0.01	1.39	1.59	0.77
MStV-Ve						
1/500	0.07	0.11	0.00	0.68	1.11	0.15
1/5000	0.00	0.00	0.00	0.18	0.35	0.02
RStV						
1/500	0.33	0.88	0.17	0.65	1.40	0.26
1/5000	0.04	0.09	0.00	0.46	0.91	0.11

MStV-Fl = maize isolate of MStV from Florida.
MStV-Re = maize isolate of MStV from Reunion.
MStV-Ve = maize isolate of MStV from Venezuela.
RStV = rice stripe virus from Japan.

* O.D. values at 620 nm, after deducting from buffer controls. Each value is an average of three replications.

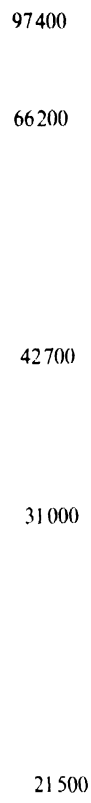

Fig 4 SDS polyacrylamide slab gel showing the single polypeptide of MStV-Sorg with molecular weight markers

MStV-NC-Ve. At a dilution of 1:5000 the reactions were faint with antiserum to MStV-Fl and MStV-Ve. In PAC-ELISA 1 μg/ml of the virus could be detected with homologous, MStV-Re and RStV antisera diluted up to 1.100 000 Antisera to MStV-Ve and to MStV-Fl were not tested by PAC-ELISA.

SDS-PAGE

A single polypeptide of MW 34 500 (average of five estimations) was resolved by SDS-PAGE (Fig. 4) from purified preparations derived from all the three zones following sucrose gradient analysis.

Electro-blot immunoassay

Homologous and MStV-Re antisera gave more intense reaction than that with the antisera to MStV-Ve and MStV-Fl. A very faint reaction was noted with antiserum to RStV (Fig. 5).

Nucleic acid analysis

Examination of the nucleic acid derived from each of the three sucrose gradient zones (T, M and B) revealed the presence of 6 – 8 bands under non-denaturing conditions (Fig. 6). The

B C D

Fig 5 Immunoblot of the MStV-Sorg coat protein after probing with homologous antiserum (A), antisera to MStV-Ve (B), MStV-Fl (C) and RStV (D)

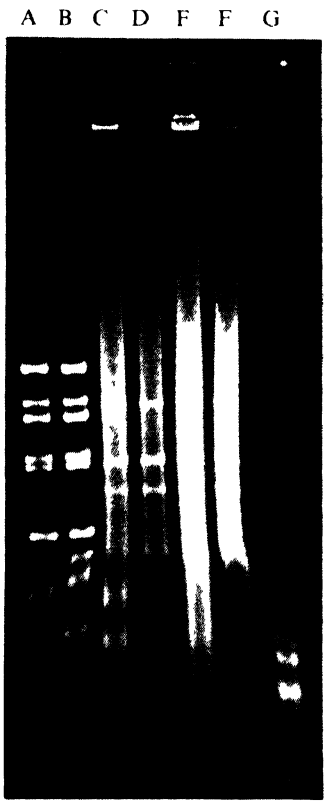

Fig 6 A 10 g/litre agarose gel showing electrophoretic separation of RNase digested (high salt at 4 °C) and non-digested nucleic acid Lanes A, C, F, G non digested nucleic acid from RDV, MStV-Sorg middle (M) and top (T) components and BMV respectively Lanes B, D, F RNase digested nucleic acid from RDV, MStV-Sorg, M and T, respectively

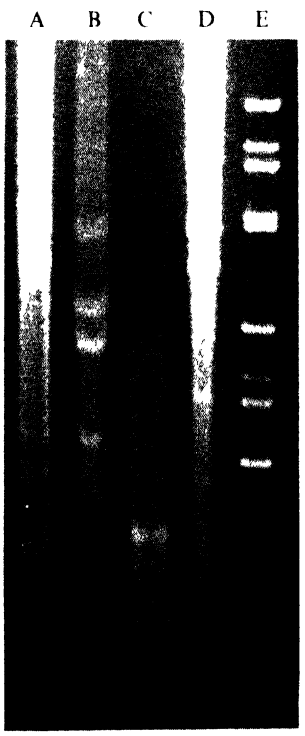

Fig. 7. A 10 g/litre agarose gel showing the electrophoretic separation of formaldehyde denatured nucleic acid from MStV-Sorg (B) and BMV (C) compared to non denatured and RNase digested MStV-Sorg nucleic acid (lanes A and D respectively), lane E, RNase digested nucleic acid from RDV

four high molecular weight bands were sharp compared to the four low molecular weight ones, which were diffuse. The diffuse band corresponding to the highest molecular weight nucleic acid species was evident when the virus was analysed from M but not from T zone. Conversely, the diffuse band corresponding to the lowest molecular weight nucleic acid species was observed when the virus was analysed from T but not from M zone (Fig. 6). All nucleic acid species derived from the B zone were faint because the virus yield from this zone was relatively low.

In order to determine the nature of the nucleic acid, RNAse digestion, denaturation with formamide or DMSO and CF-11 chromatography were carried out. RNAse digested all nucleic acid species at room temperature under low salt conditions, including brome mosaic virus (BMV) and rice dwarf virus (RDV) RNA. This indicated that the MStV genome was composed of RNA. When treated in high salt at 4 °C, the RNAse digested all four bands of lower molecular weight and BMV RNA, while the four high molecular RNA species and RDV RNA were unaffected (Fig. 6). Thus we conclude that purified virus preparations contained four RNAs which behaved as dsRNAs and four which behaved as ssRNAs.

After denaturation with formamide, only four bands were resolved in agarose gel (Fig. 7). Compared to nondenatured nucleic acid, these four bands moved faster than the four dsRNA species and slower than the four ssRNA species. Utilising CF-11 chromatography all the four ssRNA and dsRNA species were separated (Fig. 8). The lowest molecular weight dsRNA species could only be seen when relatively high concentration of sample was loaded (Fig. 6). When both the four dsRNA and ssRNA species were treated with DMSO, they migrated as four species of identical molecular weight (Fig. 8).

Fig. 8. A 10 g/litre agarose gel showing the electrophoretic separation of MStV-Sorg ssRNA and dsRNA obtained by CF-11 chromatography with or without DMSO denaturation. Lanes A, D, E, H: undenatured RNA from MStV-Sorg (ssRNA), RDV, MStV-Sorg (dsRNA) and BMV respectively. Lanes B, C, F, G: DMSO denatured RNA from MStV-Sorg (dsRNA), RDV, MStV-Sorg (ssRNA) and BMV respectively.

The molecular weights of the ssRNA species, determined under denaturing conditions, were 1.21, 0.87, 0.73 and 0.47 $\times$ 10^6D. The molecular weights of the dsRNAs, determined after RNAse digestion in high salt, were 2.43, 1.69, 1.40, 0.71 $\times$ 10^6D.

Discussion

Thread-like filamentous particles, transmitted by *P. maidis*, were shown to be associated with sorghum plants showing pale yellow stripes and chlorosis in India.

The virus was named maize stripe virus sorghum isolate (MStV-Sorg) because it is serologically related to several isolates of MStV. In morphological features MStV-Sorg resembled 3 nm-wide filamentous particles reported for MStV (Gingery *et al.*, 1981). Branched filamentous particles and 8 nm-wide rods noticed with RStV (Toriyama, 1982) were not observed in purified virus preparations stained in 20 g/litre uranyl acetate.

In host range studies, MStV-Sorg failed to infect all the five cultivars of *Z. mays* including the sweet corn cv. Madhuri. However, none of the sweet corn lines, which are known to be highly susceptible to MStV (Gingery *et al.*, 1981; Lastra & Carballo, 1985) were tested in this

investigation. Additionally *P. maidis*, employed in this study is not known to prefer maize (Naidu *et al.*, 1989). These two factors may have contributed to our failure in infecting maize with MStV-Sorg. We were also unable to compare MStV-Sorg with another tenuivirus, maize chlorotic stripe virus (MChStV) reported from Mauritius and Reunion (Autrey & Mowlah, 1984), because MStV-Sorg failed to infect maize.

Purified virus preparations were devoid of host contaminants as judged by electron microscopy and polypeptide analysis in polyacrylamide gel electrophoresis. Since bentonite resulted in substantial loss in virus concentration it was not used in purification. Yields of purified virus were comparable with reported yields for other tenuiviruses (Gingery, 1988). In sucrose gradients virus particles sedimented as three components. As in the case of RStV (Toriyama, 1982) concentration of the B component was lower than those of T and M components. Infectivity of the virus from the three zones was not determined.

Virus particles from all three zones contained one polypeptide and RNA extracted from all the zones consisted of three or four double-stranded and three or four single-stranded species. Altogether we have purified three different samples, and in each case we did not find any variation in the sedimentation of T, M and B components in sucrose gradients. Moreover, we failed to observe any morphological differences among uranyl acetate stained virus particles derived from all the three zones. Thus it was likely that similar virus particles were present in all the zones.

We have utilised two types of indirect ELISA (DAC and PAC forms) and electro-blot immunoassay to determine MStV-Sorg serological relationships. In ELISA and in electro-blot immunoassay, the virus showed close serological relationships with MStV-Re. The antiserum to MStV-Fl and MStV-Ve reacted faintly at a dilution of 1:5000 in ELISA but gave a strong reaction in electro-blot immunoassay contrary to the antiserum to RStV which reacted strongly at a dilution of 1:5000 in ELISA but gave a very faint reaction in electro-blot immunoassay. Therefore this faint reaction with the antiserum to RStV can be explained by a more distant serological relationship between MStV-Sorg and RStV than between MStV-Sorg and the isolates of MStV from Florida and Venezuela. MStV-Sorg and RStV are transmitted by different planthoppers, *P. maidis* and *Laodelphax striatellus* respectively, and thus distant serological relationship can be expected between them.

As in the case of other tenuiviruses, four ss and four dsRNA species were detected in purified MStV-Sorg preparations (Falk & Tsai, 1984; Toriyama & Watanabe, 1989). Slight differences in molecular weights detected between MStV-Sorg and MStV reported from Florida (Falk & Tsai, 1984) could be attributed to different conditions used in the electrophoresis. The size of dsRNA molecules are nearly double the size of ssRNA species. Nevertheless high molecular weight ds and ssRNA detected in MStV-Fl (Falk & Tsai, 1984) were not observed in several purified nucleoptrotein preparations of MStV-Sorg. Only four species as in the case of RStV (Toriyama & Watanabe, 1989) were detected. The observation of a clear high molecular weight ssRNA band in the nucleic acid pattern from M but not T zone and of a clear low molecular weight ssRNA band in the nucleic acid pattern from T but not M zone is consistent with the results of Falk & Tsai (1984). Evidence was presented to show that denatured dsRNA co-migrated with denatured ssRNA species, confirming results with MStV-Fl (Falk & Tsai, 1984) and RStV (Toriyama & Watanabe, 1989).

Evidence presented with MStV-Fl (Falk & Tsai, 1984) and RStV (Toriyama & Watanabe, 1989) showed that ssRNA of both plus and minus polarity are encapsidated and that each ssRNA of RStV (Toriyama & Watanabe, 1989) had a unique sequence.

From our surveys it appears that MStV-Sorg causes the most important virus disease of sorghum in the Indian subcontinent. This report is the first to document the occurrence of a tenuivirus in the Indian sub-continent.

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