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Biochemical Changes in Sorghum *(Sorghum bicolor L.* Moench) Plants Infected with Maize Mosaic Virus*

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Abstract: Biochemical changes in sorghum cultivars naturally infected with maize mosaic virus were investigated. Virus infection reduced plant biomass ranging from 10-53% among different cultivars. Total chlorophyll content in infected leaves was reduced. Reduction in nitrate reductase activity varied from 23-72% in the infected leaves of different cultivars but nitrate reductase activity in the stems of infected plants was significantly higher than in those of healthy plants. The relationship between plant biomass and leaf chlorophyll concentration was positive and that between plant biomass and stem nitrate reductase activity was negative. The relationship between plant biomass and stem N concentration was negative. Concentration of soluble sugars in leaves and stems of infected plants was increased. N concentration in the infected leaves was lower than in the healthy leaves but the N concentration in infected stems was higher. Electrophoretic analysis of soluble leaf proteins revealed the presence of two polypeptides of 21 and 22 kD in the infected but not in the healthy leaves and these were found to be not of viral origin by electro-blot immunoassay.

Key Words : Sorghum, Maize mosaic virus, PR Proteins, Nitrate reductase

Introduction

High incidence of the maize mosaic virus (MMV) infection in sorghum *(Sorghum bicolor* L. Moench) was reported in peninsular India during 1987 post-rainy season (1). Virus diseases causing severe yield losses in sorghum have been reported earlier from India (2, 3). However, the mechanisms causing yield losses in sorghum due to MMV infection are not known. Comparative studies of biochemical changes in virus infected plants in different cultivars would help in understanding the causes for yield losses. The main aim of the present study was to investigate the biochemical changes in the virus infected leaves of different sorghum cultivars and compare with the healthy plants.

Materials and Methods

Thirty three sorghum cultivars were grown on a vertisol field during the 1987-88 post-rainy season with 40 kg N and 17.5 kg P ha^{-1} as a basal fertilizer dose. The crop was irrigated twice, once for ensuring germination and then at 31 DAE (Days after emegence), The crop was top dressed with 40 kg N^{-1} ha after final thinning at 10 DAE. Each cultivar was replicated twice. Nine of the 33 cultivars (Table 1) which were found infected by the MMV were selected for the present study.

Infection percentage and plant dry matter production : The total number of plants grown in in each plot (4m x 3m) and the number of virus infected plants were counted from both the

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replications and the percentage of virus infected plants was computed. At 65 DAE, four MMV infected plants and four healthy plants from each plot were cut at ground level, leaves and stems were separated, oven-dried at 70°C for 48 h and dry weights were recorded. Dried leaf and stem tissue samples were powdered and analysed for N and P concentrations using Technicon autoanalyser and for K by atomic absorption spectrophotometry as per the standard method (4).

Chlorophyll estimation : Deribbed leaves were cut into flakes and subsamples of leafshreads (70-80 mg) were put in 30 ml screw cap bottles. Ten ml dimethyl sulfoxide was added to each bottle and capped sample bottles were incubated at 65°C in a shaking water bath for 3 h. The color intensity was measured at 645 and 663 nm and the total chlorophyll content was estimated (5).

Nitrate reductase activity : Nitrate reductase activity (NRA) in leaves and stems of healthy and MMV infected plants was measured 65 DAE. Four healthy and four MMV infected plants from each plot were cut at ground level, and the leaves and stems separated. Leaf discs of 8 mm diameter representing all the leaves, and stem pieces of 2-3 mm width (2-4 g) representing top, middle and bottom portion of each stem were cut. The Incubation mixture of stem tissue samples was filtered through washed Whatman 1 filter paper to separate the suspended particles and the activity was estimated as described by Joworski (6).

Total soluble sugars : Dried leaf and stem tissue samples were powdered and 200 mg subsample was extracted thrice in 30 ml of 80% hot ethanol. The total soluble sugars were estimated colorimetrically as per the method of Michel *et al.* (7).

Virus inoculation : The original virus source was naturally infected sorghum plant from the fields. Following identification of the plant hopper vector, healthy sorghum plants were inoculated in the green house as described by Naidu *et al.* (1).

Protein profile by gel electrophoresis : Leaf samples of nine sorghum cultivars (healthy and infected) were collected from the field and midribs were removed. Two g of fresh leaf tissue were cut into small pieces and placed in 20 ml acetone in a test tube. The acetone was decanted after 1 h and 20 ml of fresh acetone was added to the sample and kept overnight. Acetone was decanted and the samples were air dried. Dried leaf samples were finely powdered in mortar and pestle. Acetone powder subsample (200 mg) was extracted with 2 ml Tris-HCl buffer 50 rnM, pH 7.5, $MgCl_2$ 1 mM, using a polytron homogenizer at low speed. The homogenate was centrifuged for 15 min at 3000 rpm in a clinical centrifuge. The clear supernatant was decanted and the samples were prepared as per the procedure of Laemmli (8) for SDS-PAGE analysis. In case of samples of infected and healthy plants from the green house, no acetone extraction was carried out and the extraction was done directly with the buffer. The electrophoresis was carried out on 12.5% polyacrylamide slab gels according to Laemmli (8). Equal amount of proteins (30-50 Ug) of healthy and infected leaf samples were loaded along with virus sample on the gel. Gels were stained with 0.25% Coomassie Brilliant Blue R 250 (Bio-Rad). The molecular weight standards were also run along with the samples.

Electro-blot Immunoassay : Electroblotting and immunological detection of proteins from healthy and virus infected sorghum leaves and purified virus were done as described by

Burgermeister and Koeing (9). After electrophoretic separation, the proteins were transferred to the nitrocellulose membranes using a BioRad Transblot cell at 150 V for 4 h at 4°C. Membranes were blocked with 1% BSA in TBS (20 mM Tris-HCl, 150 mM NaCl, pH 7.5) and then incubated for 1 h in 1/5000 dilution of antiserum in TBS for 1 h. The antiserum for MMV proteins was raised in rabbits as described by Naidu *et al.* (1). After washing in TBS with 0.05% Tween-20 the membrane was incubated for 1 h in 1/500 dilution of alkaline phosphatase labelled goat anti-rabbit Fc-specific antibodies. The substrate used was 1-naphthol-phosphate (5 mg in 20 ml 0.2 M Tris-HCl, pH 8.3). The colour was developed by the addition of Fast Blue RR salt (0.5% in water).

Results

Infection percentage and plant biomass : The infected plants were stunted with chlorotic leaves and the exertion of the earhead was incomplete with poor grain formation. The percentage infection in the nine field-grown sorghum cultivars varied from 24-40% (Table 1). The plant biomass (above-ground) of most cultivars was severely affected in virus infected plants and the reduction varied from 10-53% amongst the nine cultivars (Table 1) with a mean 31%.

Table 1. Maize mosaic virus incidence on sorghum cultivars and plant biomass of healthy and virus infected plants of sorghum cultivars.

Cultivar	Virus incidence (%)	Plant biomass (g/plant)	
		Healthy	Infected
IS 2954	24	26.2	17.5
E 36-1	27	32.9	23.1
M 35-1	39	38.4	24.4
BJ 111	38	33.3	15.7
Annigeri	24	35.2	22.4
SPV 86	39	33.5	26.2
PQ 58	39	35.4	21.0
PQ 66	25	38.0	34.2
PQ 74-1	40	30.0	23.4
LSD		NS	
Mean		37.7	23.4
LSD		5.38**	

** = P <0.01, NS = Non significant

Total chlorophyll concentration and content in leaves : Chlorophyll concentration was significantly reduced (about 89%) in the leaves of MMV infected plants as compared to the healthy sorghum leaves (Table 2). Total chlorophyll content also showed a similar reduction in infected sorghum leaves.

Table 2. Total chlorophyll concentration and content in leaves of sorghum cultivars infected with maize mosaic virus.

Cultivar	Chlorophyll concentration (mg/g)		Chlorophyll content (mg/piant)	
	Healthy	infected	Healthy	infected
IS 2954	3 75	0.43	74.7	11.3
E 36-1	3.60	0.47	97.0	10.0
M 35-1	3.47	0.38	89.2	8.2
BJ 111	3.90	0.81	69.2	18.0
Annigeri	3.77	0.37	122 0	8.3
SPV 86	3.26	0.16	101.2	5.1
PQ 58	4.18	0.33	85.0	10.6
PQ 66	3.93	0.29	52.6	5.5
PR 74	3.72	0.32	114.4	13.3
LSD	NS		31.5*	
Mean	3.75	0.39	89.5	10.0
LSD	0.26**		10.6**	

** P<0.01, * = P<0.05, NS = non significant

Nitrate reductase activity : NRA in infected leaves was significantly reduced (23-72%) among all cultivars except IS 2954 compared to that in healthy leaves (Table 3). In cultivars IS 2954 the activity was slightly higher in infected leaves (Table 3). However, the total leaf NRA/plant was significantly reduced (34-90%) in all the cultivars compared to that in healthy plants.

NRA in the stem tissue of cultivars was significantly increased (about 55%) in virus infected plants as compared to that in healthy plants. A maximum increase of 209% was observed in cultivar PQ 58 (Table 3) and there was no change in cultivar PQ 66. However, total stem NRA/plant was not significantly affected due to virus infection (Table 3).

Soluble sugars concentration and content: The concentration and content of soluble sugars in infected sorghum leaves did not show any appreciable change compared to healthy leaves (data not given). But soluble sugars concentration in sorghum stem was significantly increased (11.25%) due to virus infection (Table 4) as compared to healthy plants (8%) in all the cultivars except IS 2954, which showed a significant reduction.

N, P and K concentration and content : N concentration in infected sorghum leaves was lower by 35% in all the cultivars (Table 4), whereas in stem tissues N concentration in infected stems was higher by 20% than in healthy plants. However, total N content in leaves and stems put together was significantly lower in infected plants than in healthy plants (Table 5).

The P and K concentration in leaves and stems was lower in virus infected plants than in healthy plants. Total P and K contents were significantly lower in infected plants than in healthy plants.

Table 3. Nitrate reductase activity in leaves and stem tissues of healthy and virus infected sorghum cultivars.

	Cultivar										
	IS 2954	E 36-1	M 35-1	BJ 111	Annigeri	SPV 86	PQ58	PQ66	PR74-1	Mean	LSD
	Leaf NRA (μ mol NO_2/g fresh wt./h)										
Healthy	6.4	6.1	6.1	7.2	6.8	5.6	6.5	7.0	5.1	6.2	
Infected	7.0	2.3	1.7	3.1	2.0	3.5	1.8	5.4	2.7	3.3	1.07**
LSD	NS										
Percent change	9.4	– 62	-67	-57	-71	-37	-72	-23	-47	-47	
	Total leaf NRA (μ mol NO_2/plant/h)										
Healthy	73	126	63	45	107	173	146	92	150	108	
Infected	44	25	11	30	18	50	14	52	36	31	23.37**
LSD											
Percent change	-39	-80	-80	-34	-84	-71	-90	-44	-76	-71	
	Stem NRA (μ mol NO_2/g fresh wt./h)										
Healthy	0.26	0.29	0.46	0.36	0.18	0.21	0 11	0.45	0.22	0.29	
Infected	0.44	0.42	0.51	0.47	0.50	0.50	0.35	0.45	0.41	0.45	0.14**
LSD	NS										
Percent change	69	45	11	31	117	138	209	0	86	55	
	Total stem NRA (μ mol NO_2/plant/h)										
Healthy	15	32	47	33	24	31	12	41	17	28	
Infected	19	27	31	18	33	38	18	39	19	97	NS
LSD											
Percent	27	-14	-33	-45	39	21	50	-6	13	-4	

** = $P < 0.01$, * = $P < 0.05$, NS = Non significant

Relationship between plant biomass and leaf stem or NRA and chlorophyll concentration : There was a positive correlation between plant biomass produced and leaf chlorophyll concentration (r=0.73**, n=17), leaf N concentration (r=0.48*, n=17), and leaf NRA (r=0.40, n=17); A negative relationship was observed between plant biomass and stem N concentration (r=-0.57**, n =17), and stem NRA (r=-0.45, n=17). A step-wise regression between percentage reduction in plant biomass (Y) due to MMV incidence and chlorophyll content (X_1) and percentage change in stem NRA (X_2) indicated that 60% variation in plant biomass was due to MMV incidence if quantitatively correlated with the changes in these two parameters ($y= 187.1-1.695\ X_1-0.72\ X_2$, $R^2=0.60$, n=17).

Table 4. Nitrogen concentration and content in leaf, N concentration in stem tissues and total soluble sugars concentration in stems of healthy and MMV-infected plants of sorghum cultivars.

	Cultivar										
	IS 2954	E 36-1	M 35-1	BJ 111	Annigeri	SPV 86	PQ 58	PQ 66	PQ 74-1	Mean	LSD
	Leaf N concentration (%)										
Healthy	2.9	2.6	2.5	2.6	2.5	2.3	2.6	3.0	2.8	2.7	
Infected	2.4	1.9	1.8	2.3	1.9	1.9	2.1	1.9	2.2	2.0	0.15**
LSD					NS						
	Leaf N content (mg/plant)										
Healthy	192	219	254	190	277	239	254	241	270	237	
Infected	170	158	139	138	157	171	165	187	232	169	36.58**
LSD					NS						
	Stem N concentration (%)										
Healthy	1.4	1.0	0.8	0.8	0.9	1.0	0.1	0.9	1.0	1.0	
Infected	1.7	1.1	1.0	1.1	1.3	1.1	1.2	1.0	1.2	1.2	
S					NS						
	Total soluble sugar concentration in stem (%)										
Healthy	7.5	10.3	9.5	11.9	5.7	7.1	6.5	7.8	5.5	8.0	
Infected	5.2	12.8	16.8	12.8	13.1	10.7	7.4	16.2	6.1	11.2	2.06**
LSD					4.5*						

** = P <0.01, * = P <0.05, NS = Non significant

Table 5. Total N, P and K contents in healthy and MMV-infected plants of sorghurm cultivars.

	Cultivar										
	IS 2954	E 36-1	M 35-1	BJ 111	Annigerl	SPV 86	PQ 58	PQ 66	PQ 74-1	Mean	LSD
	N content (mg/plant)										
Healthy	460	460	500	400	480	470	470	500	460	470	
Infected	345	315	300	250	319	350	320	440	390	340	79.5**
LSD					NS						
	P content (mg/plant)										
Healthy	95	85	91	90	108	75	100	101	90	93	
Infected	59	59	65	45	65	81	7ϕ	84	79	68	17.7**
LSD					NS						
	K content (mg/plant)										
Healthy	420	500	550	490	730	650	620	480	450	550	
Infected	290	290	375	450	240	435	350	440	325	360	98.8**

** = P <0.01, NS = Non significant

SDS profile of protein : The protein profile of the virus infected and healthy leaves of sorghum cultivars is shown in Fig. 1. In infected leaves of all the cultivars, two additional polypeptide bands of 21 kD and 22 kD (with about 6% variation) were observed which were totally absent in the healty leaves. In addition, reduced concentration of several polypeptides normally present in healthy leaves was also compared using fresh leaves of healthy and those inoculated with purified MMV virus (infected) grown in the green house. Interestingly, none of the polypeptides accumulated in virus infected leaves correspond to viral polypeptides (Fig. 2),

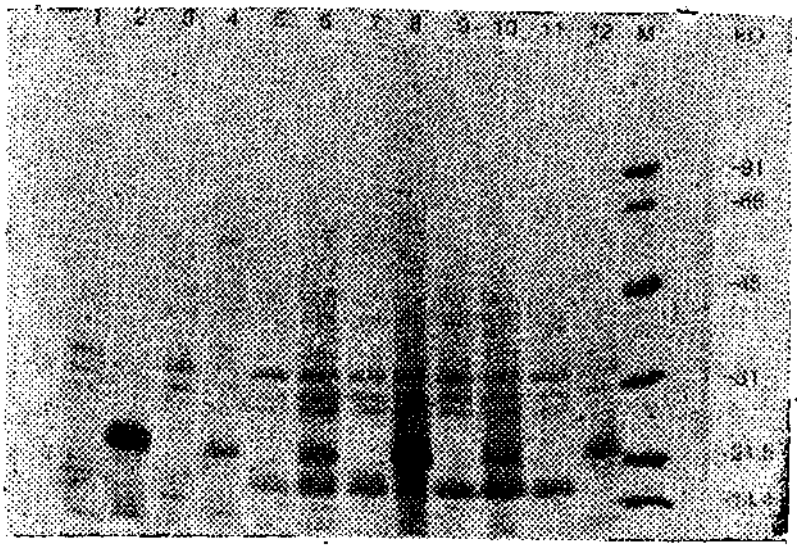

Fig. 1. Electrophoretic pattern of leaf proteins from healthy and MMV-infected sorghum cultivars. Leaves of virus-infected and healthy sorghum from the field were treated as in materials and methods. The lanes with odd numbers contain samples from the healthy leaves and those with even numbers have samples from the infected leaves. Lanes 1 and 2-IS 2954; 3 and 4-M 35-1; 5 and 6-PQ 58; 7 and 8-PQ 74-1; 9 and 10-SPV 86; 11 and 12-E 36-1; M-mo.wt.std. The mol. wt. of the standards are indicated on the right.

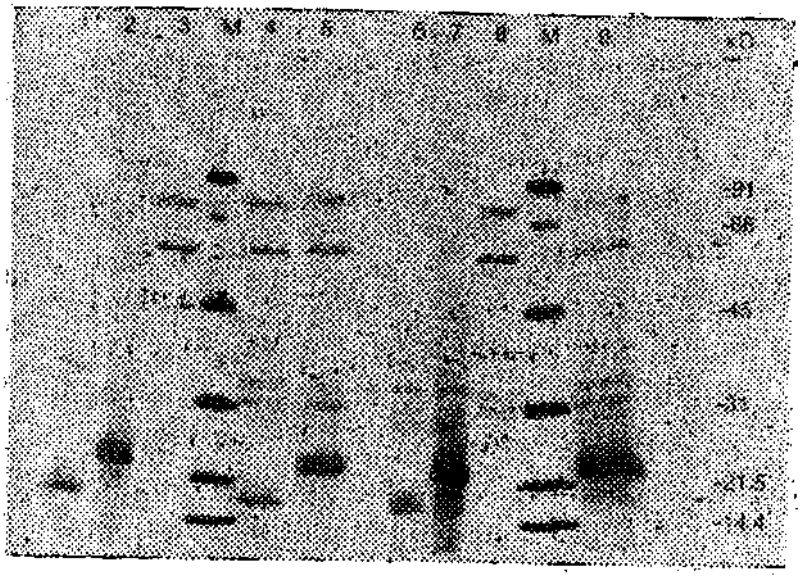

Fig. 2. Comparison of electrophoretic pattern of soluble leaf proteins of healthy, MMV-infected sorghum and purified MMV particles. Leaves of virus-inoculated (infected) and healthy sorghurm from the green house were treated as in Materials and Methods. Lane 1-CSH 6 (healthy) and lane 2-CSH 6 (infected); lanes 3 and 8-Purified virus; Lanes M-Mol. wt. standards; lane 4-CSH 6 (healthy) mixed with purified virus; lane 5-CSH 6 (infected) mixed with purified virus; lane 6 PQ 74-1 (healthy); lane 7-PQ 74-1 (infected); lane 9 PQ 74-1 (infected) with purified virus. The mol. wt. of the standards are indicated on the right.

To confirm, that these additional polypeptides were not related to viral polypeptides, infected and healthy leaf proteins were probed with the virus antiserum in electro-blot immuno assay. Results showed (Fig. 3) that none of the accumulated polypeptides in virus infected leaves (lane 2, 5 and 7) reacted with virus antiserum. The viral polypeptides alone reacted positively with the antiserum (lane 2 and 4), which served as the positive control.

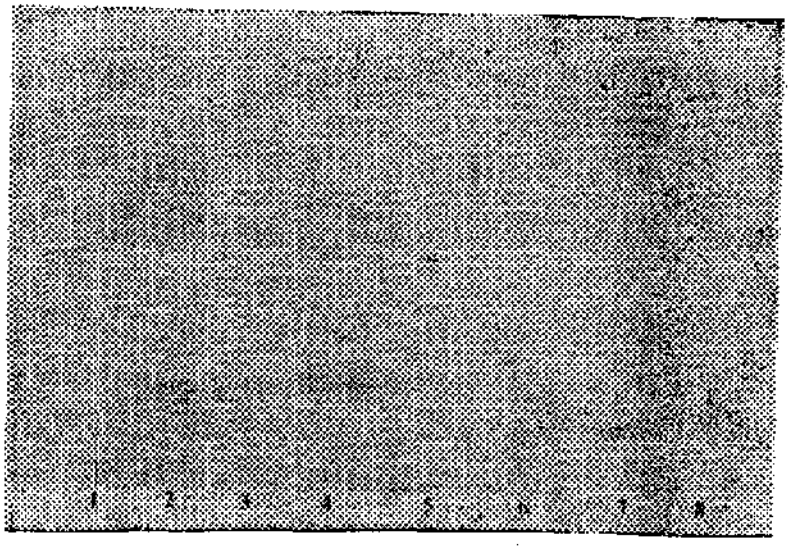

Fig. 3. Electro-blot immunoassay pattern of leaf proteins from healthy and MMV-infected sorghum cultivars from the field. Lane 1-Mol. wt. standards; lane 2-CSH 6 infected from green house; lane 3-CSH 6 healthy from green house; lane 4-purified virus; lane 5-M 35-1 infected; lane 6-M 35-1 healthy; lane 7-PQ 74-1 infected;Iane 8-PQ 74-1 healthy.

Discussion

Both, the incidence of MMV on sorghum cultivars and the reduction in plant biomass showed a large variation (Table 1). Earlier, incidence of chlorosis on sorghum varied from 6-70% in the rainy, and 15-90% in the post-rainy season (2). The varying incidence percentage and reduction in plant biomass suggest that further screening may help in identifying sorghum cultivars resistant or moderately resistant to MMV.

The most striking effect of virus infection on plant is the reduction in the chlorophyll content of leaves (19%) which leads to the chlorotic symptoms and causes a reduction in photo-synthesis and related metabolic processes (10). As a consequence, plant biomass production and grain-filling are drastically affected in the different cultivars. The regression analysis between per cent reduction in plant biomass and leaf chlorophyll content suggested that reduction in chlorophyll content is the main cause of plant biomass reduction due to MMV infection. It is known that the per cent inhibition of photosynthesis in the virus infected plants can vary depending on the position of the leaves and nature of lesion (11).

The reduced leaf NRA due to virus infection accounted for 17% variation in the plant biomass reduction. This result is not in conformity with the observations made earlier (12, 13) where increased NRA was observed in WMV-infected pumpkin and CPMV-infected cowpea. However, our results for increased stem NRA are similar to the observations made earlier (12,13). Total leaf NRA/different plant showed a general reduction but that of stem did not show any definite pattern among the cultivars. The soluble sugars concentration in infected plant leaves was not significantly affected. However, similar to NRA, soluble sugar concentration also increased in stems of infected plants (8-130%) over the sugar concentration in healthy plant stems in all the cultivars (Table 4). This would indicate that there is a higher break down of starch stored in the stem or the photosynthesis machinery was not completely affected. Since chlorophyll content is reduced in infected leaves, the increased sugar concentration could result from higher break down of starch. Among the cultivars, IS 2954 shows a different response with respect to soluble sugar and leaf NRA, the reason for which are not very clear. The results on N, P, and K (Table 5) indicate that physiological and biochemical processes in the sorghum plants are

adversely affected resulting in reduced nutrient supply to the growing parts thus, reducing the plant biomass production, incomplete exertion of earhead and very poor grain-filling.

The SDS-PAGE analysis of proteins in infected leaves revealed two additional polypeptides of MW 21 and 22 kD, which were not found in healthy leaves of any of the cultivars tested. The virus infection results in reduced synthesis of host proteins in same cultivars and the accumulation of large amounts of viral proteins synthesised at the expense of host proteins (Fig. 1). The additional polypeptides observed neither showed any similarity in their electrophoretic mobility to any of the polypeptides of the purified virus (Fig. 2) nor did they react with the antiviral serum in electroblot-immunoassay (Fig. 3).

The extra polypeptides synthesised could be part of the viral proteins like coat proteins or virus induced proteins referred to as pathogenesis related proteins (PR proteins). The PR proteins by definition are normally absent from, or difficult to detect in, normally growing, healthy plants and are expressed only upon infection by virus and are plant specific (15, 16), The vast majority of PR proteins which have been characterised to date are within the range 10-30 kD (10, 11). In TMV infection the coat protein of the virus has been shown to be present in the chloroplasts thus, affecting photosynthesis (14). The absence of these proteins in leaves of healthy sorghum cultivars, their differential electrophoretic mobility and the fact that these polypeptides failed to react with MMV antiserum in immuno-blot assay strongly suggest that these are not the coat proteins of the virus but could be either viral coded or virus-induced plant proteins.

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