

OCCURRENCE OF MAIZE DWARF MOSAIC VIRUS IN MAIZE

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Abstract

From naturally infected maize hybrid (YH 202) showing mosaic symptoms, Maize Dwarf Mosaic Virus was isolated. It was mechanically transmissible only to hosts belonging to Gramineae. Partially purified preparation showed large number of flexuous rods measuring about 750-800 nm in length. On the basis of host range and particle morphology the virus was tentatively identified as Maize Dwarf Mosaic Virus (MDMV). This is the first report of the occurrence of MDMV in maize from Pakistan.

Introduction

Maize dwarf mosaic virus (MDMV) has a worldwide distribution. It has been reported from the United States (Johnson & Ellet, 1963), Europe (Szirami, 1968), Asia (Chona & Seth, 1960; Klein *et al.*, 1973) and Australia (Penrose, 1974). MDM is considered to be the most important virus disease of sorghum throughout the world and occurs almost every where in sorghum (*Sorghum bicolor*), maize (*Zea mays*) and Johnson grass (*Sorghum halepense*). The virus is serologically related to Sugarcane mosaic virus (SCMV) (Shepherd, 1965; Tosic & Ford, 1974), but SCMV and MDMV are still considered distinct viruses in the United States (Langham, 1986) and the two names continued to be used in literature (Antignus, 1987; Giorda *et al.*, 1986; Louie & Darrah, 1980) and are distinguished by their ability to infect Johnson grass. Robert & Donald (1967), Ayers *et al.*, (1978) and Sehgal (1966) found that MDMV could infect Johnson grass, but strains/isolates of SCMV do not infect this weed (Shepherd, 1965; Penrose, 1974). While describing MDMV, most workers add strain designation i.e., MDMV-A, MDMV-B, MDMV-C, MDMV-D, MDMV-E, MDMV-F (Louie & Knoke, 1975) and MDMV-O (McDaniel & Gordon, 1985). Among these strains only MDMV-A could infect Johnson grass (Mackenzie, 1967; Tosic & Ford, 1972). MDMV-A is transmitted by corn leaf aphid *Rhopalosiphum maidis* (Shepherd, 1965; Sehgal, 1966; Scott *et al.*, 1969), which plays an important role in the spread of virus.

The virus has wide host range since over 200 species of grasses are susceptible (Rosenkranz, 1981). Symptoms of this virus in the field and green house depend upon genotype, virus strain and temperature. The classic symptoms include mosaic, red leaf, red stripe, necrosis, stunting, delay in flowering, reduction in head length, number of heads and grain yield. Rosenkranz & Scott. (1978) reported that under favourable conditions for infection and disease development, MDMV-A has the potential for reducing grain yield by as much as 45% in highly susceptible hybrids. It is also transmitted through corn seeds in low percentage (Shepherd & Holdeman, 1965). The virus under

study was isolated from a corn hybrid showing mosaic symptoms. As a weed, Johnson grass with similar symptoms was also present in the field. In this paper we report some properties of the virus which seems to be the first report of MDMV occurrence in maize from Pakistan.

Materials and Methods

Virus isolation and host range: The virus was isolated from naturally infected maize hybrid (YH 202) in spring 1989 at the National Agricultural Research Centre, Islamabad, showing mosaic symptoms. The virus was mechanically inoculated on several graminaceous hosts including maize (*Zea mays*), sorghum (*Sorghum vulgare*), millet (*Pennisetum americanum*), grasses i.e., Johnson grass, Sudan grass, Elephant grass (*Pennisetum purpureum*) and Lemon grass (*Cymbopogon citratus*), wheat (*Triticum aestivum*), rice (*Oryza sativa*), oats (*Avena sativa*) and on non-graminaceous hosts of Chenopodiaceae, Leguminosae and Solanaceae. Test plants raised in peat/soil mixture and kept in an insect-proof glass house at about 20-24°C were inoculated at 3 leaf stage. Inoculum was prepared in 0.05 M phosphate buffer pH 7 containing 0.5% 2-Mercaptoethanol. Symptoms were observed for one month. The virus was propagated in sorghum and maize cultivars.

Purification: Purification of MDMV is difficult, because the virus is relatively unstable (Sehgal, 1968; Snazelle *et al.*, 1971). However, it was partially purified with the modified method of Jarjees & Uyemoto (1984). Leaves with well developed symptoms were cut into 1/2 inch pieces, chilled and were homogenized with pestle and mortar in 0.1M sodium citrate buffer pH 7.5, 1:2 (w/v) containing 0.5% 2-Mercaptoethanol and quartz sand. The homogenate was filtered through double layer of muslin cloth. Filtrate was emulsified with half volume (50%) of chloroform, and stirred for 20-30 min., at 4°C. The emulsion was broken at 4000 rpm for 10 min. To the supernatant 5% triton X-100 was added and kept O/N, while stirring. Virus was sedimented at 26000 rpm for 2 h in type 30 rotor. The pellet was suspended in 0.02 M borate buffer pH 8 containing 0.001 M EDTA. After clarification at 8000 rpm for 10 min. second high speed centrifugation was done at 27000 rpm for 2 h. The pellet was resuspended in a small quantity of 0.02 M borate buffer. This preparation was used for particle morphological studies.

Electron Microscopy: Partially purified virus was mounted on 200 mesh carbon coated copper grids and stained with 2% PTA (Phosphotungstic acid) pH 6.8. Grids were examined under JEOL 100 CX-11 electron microscope at 80 KV.

Results

Symptomatology and host range: Mosaic symptoms were induced on susceptible hosts which includes grasses, sorghum and maize cultivars. All tested 20 sorghum cultivars showed systemic infection. Out of 17 inoculated maize cultivars, 15 developed severe mosaic symptoms except two Cargill hybrids viz., Bemisal II and Bemisal IV (Table

Table 1. Reaction of maize and sorghum cultivars to Maize Dwarf Mosaic Virus (MDMV).

SORGHUM		MAIZE	
Cultivar	Symptoms	Cultivar	Symptoms
Atlas	Red necrotic lesions	Akbar	Mosaic, slight stunting
BR-123	Mosaic, Pale blotch on the base of leaf	Azam	Chlorotic lines, mild mosaic
BR-319	Veinal mosaic	Bahar	Mosaic
Bagdar	Severe mosaic symptoms	Baimisal-II (HYBRID)	—
CSH-6	Yellow lesions, Mosaic	Baimisal IV (HYBRID)	—
DS-75	Dwarfing, Mosaic	C-15	Mosaic
Giza-3	Severe mosaic, dwarfing	Ehsan	Mottling, stunting
Hegri	Mosaic, Mottling	Faisal	Mosaic
ICSV 107	Pale line pattern	Pool-10	Gohar Mosaic, mottling
ICSV 219	Dark green streak	Popcorn (White)	Severe mosaic, stunting
IC 1039	Yellow lesions	Popcorn (Yellow)	Chlorotic stripes
JS-1	Mosaic	Sarhad (White)	Dark Green lines
MR-839	Severe mosaic	Sarhad (Yellow)	Chlorotic streaks, dwarfing
PU-7	Severe systemic infection	Sarhad (White)	Dark green lines
Potohar-3	Chlorotic line pattern	Shaheen	Mosaic
Pak-SS-II	Green dots in pale streaks, narrow leaf	Sultan	Mosaic
Rio	Pale line pattern	YH202 (HYBRID)	Green dots in chlorotic lines.
Red Janpur	Mosaic		
Sarokartuho	Mottling, mosaic		
Indian-III	Chlorotic streak		

1). From 9 tested millet cultivars (Gahi, Y-72, 18-BY, IC-8206, WC-C75, Ugandi, IVS-P78, Y-84 and C-47) only cv. Ugandi showed severe mosaic and leaf necrosis. Elephant grass and Lemon grass did not show any symptoms. Four cultivars of wheat (Barani 83, Sindh 81, Lyallpur 73 and Punjab 85), and rice (Basmati 370, Basmati 385, KS 282 and IRRI 6), oat and non-graminaceous hosts viz., *Chenopodium amarnticolor*, *C. quinoa*, *Glycine max* cv. Williams, *Vigna unguiculata* cvs. 411 & SA-Dandy, *V. mungo* cv. No. 133, *Phaseolus vulgaris* cv. Top crop, *Capsicum annum*, *Nicotiana tobacum* cvs. Samsun & White burley, *N. glutinosa* and *Solanum nigrum* were not infected.

Symptoms induced by the virus on maize, sorghum cultivars and on grasses are described below:

Maize (Zea mays): On seedlings the symptoms appeared 4-5 days after inoculation in the form of small chlorotic spots near the base of unopened leaves, which developed later



Fig. 1. Mosaic symptoms induced in *Sorghum bicolor* CV. Pak. SS. II. 7 days after inoculation with MDMV.

into generalized yellow green mosaic. Occasionally older leaves showed longitudinal streaks running parallel to the veins or rectangular dark green areas on a chlorotic background. Infected plants showed varying degrees of dwarfing.

Sorghum (Sorghum bicolor): The symptoms consisted of a brilliant yellow green mosaic with pale and dark green streaks. These were very apparent on top leaves. Infected leaves were somewhat narrower compared to healthy ones (Fig. 1).

Grasses: Johnson grass, Sudan grass and Swank grass were infected. The initial symptoms appeared as isolated narrow and elongated streaks on young leaves, which later extended throughout the leaf. On Sudan grass very severe mosaic symptoms were observed.

Purification and Electron microscopy: The purification method gave satisfactory results and yielded a large number of flexuous virus particles with the approximate length of 750-800 nm (Fig. 2).

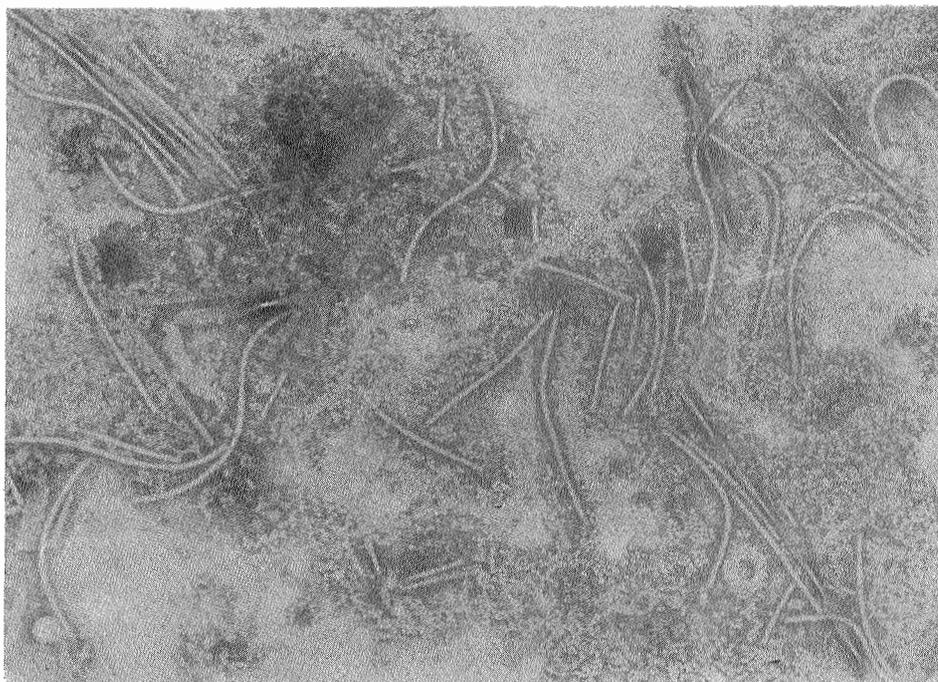


Fig. 2. Electron micrograph of partially purified preparation of MDMV stained with 2% PTA.

Discussion

The viral nature of this disease was confirmed through host range and particle morphology. The virus was readily transmitted by sap inoculation to indicator plants, belonging only to Gramineae. Generally observed symptoms were mosaic type, but streaks, necrosis and chlorosis were also noticed. Symptoms on Johnson grass indicated that virus under study is MDMV, because only MDMV can infect Johnson grass (Robert & Donald, 1967; Ayers *et al.*, 1978; Sehgal, 1966), while strains of SCMV do not have the ability to infect this weed (Shepherd, 1965; Penrose, 1974). Our host range and symptom expression studies also confirmed the findings of Shepherd (1965); Sehgal, (1966) for MDMV that it infects many grass species, Sudan grass, Swank grass, Johnson grass, and several maize and sorghum cultivars. Our isolate did not infect any non-graminaceous hosts which conforms the finding of Shepherd, (1965) and McDaniel & Gordon, (1985).

So far about 7 strains of MDMV i.e., A, B, C, D, E, F (Louie & Knoke, 1975) and MBMV-O (McDaniel & Gordon, 1985) of MDMV are reported in the literature. No details are available except for strain A & B. Strain A and O are somewhat similar the only difference is that strain O also infect Oat (McDaniel & Gordon, 1985). MDMV-A is considered to be Johnson grass infecting strain (Mackenzie, 1967; Snazelle *et al.*, 1971;

Tosic & Ford, 1972; Tosic & Ford, 1974; Antignus, 1987), while strain B does not infect johnson grass (Mackenzie *et al.*, 1966; Giorda *et al.*, 1986; McDaniel & Gordon, 1985; Jarjees & Uyemoto, 1984). On the basis of host range and particle morphology we concluded that the virus reported here is MDMV-A, because it infects Johnson grass and failed to infect oat.

The virus was detected only in few plants and no increase in number of infected plants till the end of growing season was noticed, which suggest that the virus was seed-borne. On the other hand same virus was isolated from Johnson grass present in field as a weed. This indicates that the virus either was transmitted from maize to Johnson grass or otherwise. In both the cases the possibility of a vector cannot be ruled out. Johnson grass is a major weed in all cereals in Pakistan and is reported to act as a reservoir for several years when it is infected with MDMV (Ross & Lembi, 1985). However, only three species of aphids i.e. *obtusirostris Rhopalosiphum padi*, *Myzus obtusirostris* and *Sipha maydis* are reported on maize from Pakistan (Anon., 1987), which does not include *Rhopalosiphum maidis* the reported vector of MDMV. At this stage very little is known about the distribution and incidence of MDMV in maize. This study stresses the need for an extensive survey of maize growing areas of the country to ascertain MDMV distribution and to find out the involvement of a possible vector and seed infection in virus spread to formulate proper control measures.

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(Received for publication 12 May 1990)