

Full Length Research Paper

Sugarcane mosaic virus: The causal agent of mosaic disease on sorghum (*Sorghum bicolor* L.) in Tehran province of Iran

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During disease diagnosing studies on sorghum fields in Tehran province, Iran through vegetation period in 2005 - 2006, 75 sorghum expressing virus-associated symptoms including mosaic, leaf-redding and necrosis were collected. The virus was inoculated mechanically to Sweet corn (*Zea mays* cv. Pars403) and grain sorghum (*Sorghum bicolor* cv. Kimia). The virus specifically was reacted in Double Antibody Sandwich-Enzyme Linked Immunosorbent Assay (DAS-ELISA) and Dot Immunobinding Assay (DIBA). Also relative molecular mass of virus coat protein was calculated using a densitometer via sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) to determine electrophoretic mobility compared with protein standards. The virus reacted with anti SCMV polyclonal IgG antiserum and was detected with Goat anti-Rabbit IgG alkaline phosphatase conjugate. The total nucleic acids were subjected to reverse transcription-polymerase chain reaction (RT-PCR) using SCMV degenerate and specific primers. Sugarcane mosaic virus (SCMV) was detected in all collected samples. The capsid protein was evaluated approximately 37 kDa in size. Amplification product (approximately 900 bp) was obtained from the collected and inoculated plants but not from healthy plants. This may confirm the presence of SCMV in the symptom-expressing plants.

Key words: SCMV, *Sorghum bicolor*, DAS-ELISA, DIBA, SDS-PAGE, RT-PCR.

INTRODUCTION

Sorghum (*Sorghum bicolor* L.) is one of the most important and world wide grown forage crop particularly in tropical and subtropical areas including Iran. Viral disease of plants occurs in many parts of the world where susceptible species are grown and cause yield loss in their hosts. Like other plants in the family Poaceae, sorghum is a susceptible host to various viruses that infect this crop and cause significant losses. Sorghum is alternative host for many viruses described on the other graminoid plants and is important source for transmitting and spreading into the species of Poaceae. During disease diagnosing studies on sorghum fields in Tehran

province through vegetation period in 2005-2006, symptoms of mosaic, streak mosaic, leaf redding, and sever necrosis are seen in sorghum plants that represent virus infection. Several distinct sorghum infecting viruses including Johnson grass mosaic virus (JGMV), Maize Dwarf Mosaic Virus (MDMV), Sorghum Mosaic Virus (SrMV) and Sugarcane Mosaic Virus (SCMV) causes mosaic symptoms and red necrotic stripes on the sorghum leaves known as SCMV subgroup viruses, which are members of potyvirus genus of the family potyviridae.

Management and development of virus-free clone of disease depends on identifying and characterising the causal agent for potential differences in epidemiology and in resistance of varieties to the virus. Therefore the purpose of this study was to determine the causal agent of mosaic disease in sorghum (*Sorghum bicolor* L.) grown in Tehran province, Iran.

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MATERIALS AND METHODS

Maintenance and propagation

Sorghum expressing virus-associated symptoms including mosaic, leaf-redding and necrosis were collected during survey in 2005 - 2006 from sorghum growing fields in Tehran province, Iran. The gathered samples were transferred to virology laboratory and were kept in 4°C for later use. For virus maintenance, Sweet corn (*Zea mays* cv. Pars403) and grain sorghum (*Sorghum bicolor* cv. Kimia) were inoculated mechanically using the extracts of gathered samples. For this, the seeds of maintenance plants were sown in steam sterilized soil (autoclaved in 121°C for 1 h twice with 24 h distance) in 12 cm pots under glasshouse condition. 0.1 g leaf tissue of each was frozen in liquid nitrogen, pulverized in a mortar and pestle, and homogenized before grinding with 1 ml of 0.1 M potassium phosphate buffer pH 7.0 containing 2% Polyvinyl Pyrrolidone (PVP) cool sap. The extracts from each source samples was mixed with 600 meshes carborundum powder before rubbing onto at least 4 plants at 2 - 3 leaves stage. The mechanically inoculated plants were held in separate glasshouse chambers at 20 ± 3°C with 12 h of illumination per day.

Serological diagnosis

For preliminary screening of each gathered samples and inoculated plants, Double Antibody Sandwich-Enzyme Linked Immunosorbent Assay (DAS-ELISA) and Dot Immunobinding Assay (DIBA) were conducted using polyclonal antisera of four Poaceous potyviruses including Sugarcane Mosaic Virus (SCMV), Maize Dwarf Mosaic Virus (MDMV), Sorghum Mosaic virus (SrMV) and Johnson Grass Mosaic Virus (JGMV) (antisera were received from DSMZ, Braunschweig, Germany) according to protocols previously described by Clark and Adams (1977) and Dijkstra and Jager (1998), respectively.

The infected virion was purified from infested sorghum and inoculated plants following the procedure of Lane (1986). For purification, 2 g of the leaf tissue was homogenized in 7.5 volumes of extraction buffer (0.1 M ammonium citrate grinding buffer, pH = 6.5 containing 0.25% 2-mercaptoethanol, 0.1% Na diethyldithiocarbamate and 1% polyvinyl pyrrolidone). The slurry was then strained through four layers of cheesecloth. The filtrate was clarified by centrifugation at 5900 × g for 15 min. Triton X100 was added to supernatant to final concentration of 1% followed by stirring for 2 h at 4°C. The mixture was centrifuged for 2 h at 85000×g at 4°C through 5 ml of ammonium citrate buffer pH = 6.5 containing 20% sucrose pad using Beckman Spinco L50 centrifuge (Beckman Instruments, Inc. Palo Alto, Ca). The plate was resuspended in 0.25 M citrate grinding buffer. The concentration and purity of the viral suspension was evaluated by UV spectrophotometry, using an extraction coefficient of 2.7 mg/ml/cm as determined for some cereals potyviruses (Mc Daniel and Gordon, 1989).

SDS PAGE and western blotting

Relative molecular mass of virus coat protein was calculated using a densitometer to determine electrophoretic mobility compared with protein standards. The method of Laemli (1970) was used for Sodium Dodecyl Sulphate-Polyacrylamide Gel Electrophoresis (SDS-PAGE). The sample extracts from field, inoculated plants and healthy plant as well as purified virion and protein weight molecular size marker were mixed with SDS-PAGE loading buffer and were kept in boiling water in water bath for dissociation for 5 min, then loaded into 5% stacking gel. Proteins were resolved in 15% gel until the dye front reached the bottom of the gel (Volts and mA set at 400 and 60 respectively) and then visualized by staining with

coomassie blue (coomassie blue R-250, 30% methanol and 10% acetic acid in tap water). Protein sizes were estimated by comparisons with low range protein molecular weight markers.

For Electro Blot Immune Assay (EBIA, Western blotting), the proteins were electrophoretically transferred onto nitrocellulose membrane as described by Dijkstra and Jager (1998). Proteins were reacted with anti SCMV, MDMV, JGMV, SrMV polyclonal IgG antisera (antisera were prepared from DSMZ, Braunschweig, Germany) and were detected with Goat anti-Rabbit IgG alkaline phosphatase conjugate. Bound conjugate was determined with the substrates BCIP/NBT used according to the manufacturer's instructions.

Reverse Transcription-Polymerase Chain Reaction (RT-PCR)

For nucleic acid extraction and Reverse Transcription-Polymerase Chain Reaction (RT-PCR), the total nucleic acids were extracted from the frozen infected leaves from collected and inoculated samples as well as healthy plants using RNAWIZ buffer and RNeasy plant mini kit according to the manufacturer's instruction. First-strand cDNA and PCR amplification was carried out with a SCMV-specific primer (SCMV F3; 5'-TTTYCACCAAGCTGGAA-3' and SCMV R3; 5'-AGCTGTGTGTCTCTCTG TATTCTC-3') according to the method described previously by Yang and Mirkov (1997). 10 µl of PCR-amplified product was analyzed by electrophoresis on 1% agarose gel (Sambrook et al., 1989)

RESULTS AND DISCUSSION

Out of 75 collected samples of sorghum plants expressing virus-associated symptoms reinoculated into *Zea mays* cv. Pars 403 and *Sorghum bicolor* cv. Kimia seedling showed the same type of systemic mosaic after a month. From the result of DAS-ELISA and Dot immunoblotting Assay (DIBA) tests, positive reactions were obtained only for SCMV (data not shown). Tosis (1990) demonstrated that serological detection method such as ELISA can exactly distinguish SCMV from other mixed infecting SCMV subgroup viruses. Also Balamuralikrishnan et al. (2004) revealed that anti body-based techniques can be employed for screening SCMV. So the presence of the other tested virus's antisera including Johnson Grass Mosaic Virus (JGMV), Maize Dwarf Mosaic Virus (MDMV), Sorghum Mosaic Virus (SrMV), were screened out. The protein bands from infected samples, purified virions and healthy plants were visible on coomassie blue-stained SDS-PAGE gels; it showed the estimated size, approximately 37 kDa in both infected samples and purified virions, and were absent from corresponding healthy preparations (Figure 1). This findings are in general agreement with Shukla et al. (1994) who showed a single coat protein band on SDS-PAGE with varied molecular weight from 33-40 kDa depending on the strains for SCMV. Also this result is in agreement with previous data obtained on the size of capsid protein of SCMV by Jensen et al. (1986). This band reacted specifically in immunoblots with anti SCMV polyclonal antibody; no antigen was detected in immunoblots from corresponding healthy samples preparation (Figure 2). Using F3/R3 primers in RT-PCR, the expected

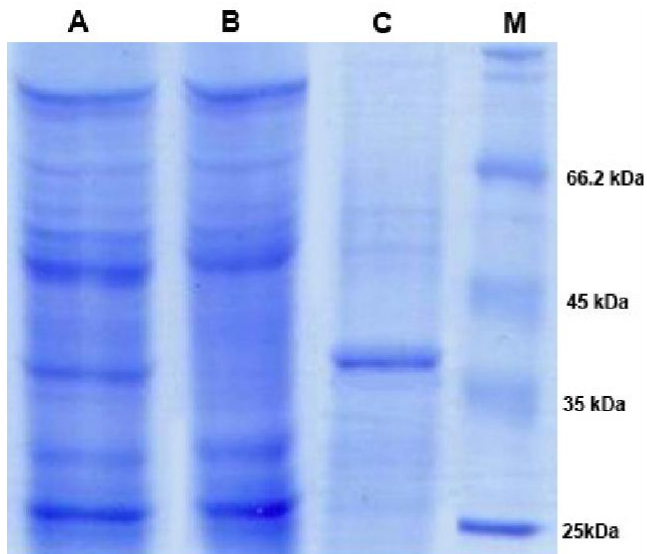


Figure 1. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Lane A, preparation from infected sorghum leaf; Lane B, preparation from healthy sorghum leaf; Lane C, purified virion coat protein; and Lane M; protein molecular weight marker.

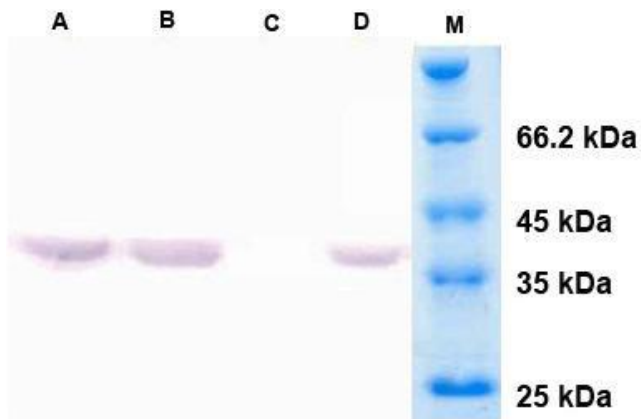


Figure 2. Western immunoblots analyses. Lane A, preparation from collected sorghum leaf; Lane B, preparation from infected sorghum cv Kimia leaf; Lane C, preparation from healthy sorghum leaf; Lane D, purified virion coat protein; and Lane M, protein molecular weight marker.

amplification product, approximately 900 bp, was obtained from the collected and inoculated plants but not from healthy plants (Figure 3). Similar results in RT-PCR with F3/R3 SCMV specific primers were obtained by Balamuralikrishnan et al. (2004). The anti SCMV IgG antiserum reacted well with all the collected virus-associated, inoculated samples and matched the RT-PCR results with R3/F3 specific SCMV primers. This confirms the presence of SCMV in the symptom-expressed plants. SCMV, a member of Potyvirus genus of the family

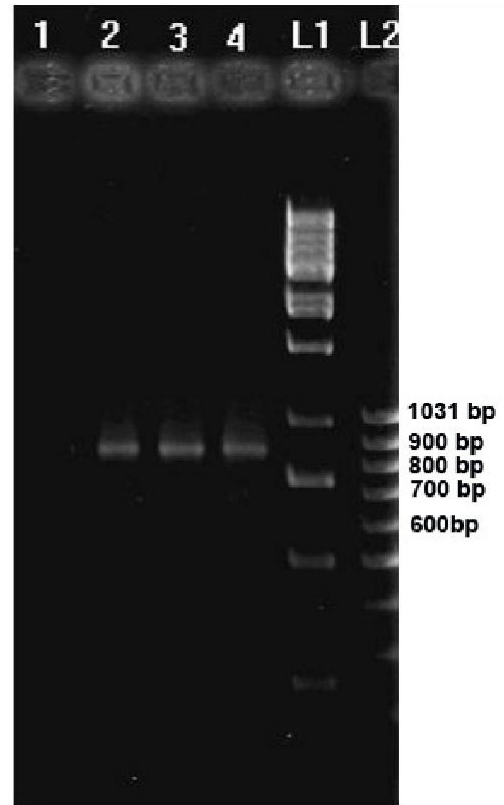


Figure 3. Agarose gel electrophoresis of reverse transcription-polymerase chain reaction products. Lane 1, healthy sorghum leaf; Lane 2, infected sorghum leaf; Lane 3, inoculated sorghum cv. kimia leaf; Lane 4, inoculated corn cv. pars 403 leaf; Lane L1, 1000 bp ladder; Lane L2, 100 bp ladder.

Potyviridae, is recently classified as one of the SCMV subgroup consisting four distinct viruses based on serological properties, coat protein region and genome sequence (Shukla et al., 1989; Frenkle et al., 1991; Shukla et al., 1992) and it is one of the most common and economically important viruses in sugarcane (*Saccharum officinarum* L.) cultivars causing severe effect on sugarcane production world wide. Also the virus is important in corn (*Zea mays* L.), Johnsongrass (*Sorghum halopens* L.) and sorghum (*Sorghum bicolor* L.) and can naturally infect these crops in fields as well as wild grass species as overwintering and virus infection source. It is well known that SCMV is a single stranded positive RNA long rod-shaped virus transmitted in a non-persistent manner by at least 12 species of aphids. However, there are three principal modes of spread of SCMV (aphid vectors, infected seeds and mechanical inoculation); it seems that only aphid vectors and infected seeds are important in the fields and mechanical transmission for the most part is important only in greenhouse and laboratory research. Though SCMV had previously been reported in Iran (Masumi et al., 2007), in the present study SCMV is

reported for the first time from sorghum (*Sorghum bicolor* L.) in Tehran province of Iran.

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