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Identification and Coat Protein Nucleotide Sequence of Turnip Mosaic Potyvirus from *Eruca sativa* in Saudi Arabia

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Abstract: The goal behind this study was the identification, biological and molecular characterization of a virus infecting garden rocket (*Eruca sativa* Mill) and producing severe symptoms on it in Riyadh region, Saudi Arabia. Mosaic, stunting and deformation were observed on field grown garden rocket plants. Mechanical inoculation of twelve plant species with sap prepared from infected garden rocket plants resulted in infection of four of them. These were *E. sativa* Mill, *Raphanus sativus* L., *Brassica rapa* L. and *Chenopodium ammaranticolor* Cost and Reyn L. Symptoms on the first three plant species were systemic mosaic, stunting and deformation, whereas symptoms on *C. ammaranticolor* were local lesions. *Brevicoryne brassicae* L., transmitted the virus to *E. sativa* in a non-persistent manner. Electronmicroscopic examination of carbon-coated grids prepared by the leaf dip method revealed flexuous virus particles typical of potyviruses. Enzyme linked immunosorbent assay indicated that samples collected from symptomatic *E. sativa* plants in both Riyadh and Qassim regions were infected with turnip mosaic potyvirus (TuMV). Positive results were also obtained using a Reverse Transcription-Polymerase Chain Reaction (RT-PCR) method to detect and identify TuMV from nucleic acid extracts of the symptomatic garden rocket plants collected from that area, using a specific oligonucleotide primer for detection of TuMV-CP. Nucleic Acid Spot Hybridization Assay (NASH) using DIG labeled cDNA probe showed high levels of hybridization signal, whereas no hybridization was observed with uninfected tissues. The nucleotide sequence of the CP gene of the Saudi Arabian isolate of TuMV detected in garden rocket and tentatively denoted (TuMV-SA-Ro) was determined to be composed of 862 nucleotides in length.

Key words: TuMV, *Eruca sativa*, electron microscope, host range, NASH, RT-PCR, sequencing

INTRODUCTION

Turnip Mosaic Virus (TuMV) is a member of the genus potyvirus in the family potyviridae. It is the only potyvirus known to infect brassicas (Walsh and Jenner, 2002). It is geographically of widespread and has been reported in North America, Europe, Africa, Asia, Australia and New Zealand (Chen *et al*., 2003; Lehmann *et al*., 1997; Omunyin *et al*., 1996; Petrzik and Lehmann, 1996; Fujisawa, 1990; Henson and French, 1993; Feldman and

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Gracia, 1972; Tomlinson, 1970). Turnip mosaic virus has a wide natural host range including crucifers, legumes, ornamentals and weeds (Chen *et al*., 2003; Farzadfar *et al*., 2005; Green and Deng, 1985). It was ranked second only to cucumber mosaic virus as the most important virus infecting field-grown vegetables in a survey o f virus diseases in 28 countries and regions (Tomlinson, 1987; Walsh and Jenner, 2002). In Asia, TuMV is considered the most important virus of cultivated cruciferous cash crops and has caused significant yield losses in plant species belonging to this group (Provvidenti, 1982), particularly; it has several different strains (Fujisawa, 1990; Green and Deng, 1985; Jenner and Walsh, 1996; Stavolone *et al*., 1998; Thomson *et al*., 1995). Aphids are very important vectors of this virus which help its spread in the neighboring fields. Eighty nine aphid species were reported to transmit TuMV in a non-persistent manner (Walsh and Jenner, 2002). The fact that adds to the problems already imposed by the infections of this virus. In 2002, striking mosaic symptoms were observed on garden rocket for the first time in Riyadh region of Saudi Arabia.

The present study is one of several investigations regarding the significant of this virus and aims at isolation, biological and molecular characterization of the Saudi Arabian isolate of TuMV which was isolated from garden rocket plants in Al-Aoyaynah area, Riyadh, Saudi Arabia.

MATERIALS AND METHODS

Source of Virus Isolates and Host Range

Diseased leaves of garden rocket plants growing naturally and exhibiting systemic mosaic, stunting, chlorosis and deformations were the source of this virus isolate. Twenty four leaf samples showing mosaic symptoms suspected of virus infections were collected from, Riyadh (Al-Aoyaynah area), Saudi Arabia, during Spring of 2008. Seeds of the following plant species: *Eruca sativa* Mill., *Raphanus sativus* L., *Brassica rapa* L., *B. oleracea capitata*, *Spinacea oleracea*, *Lycopersicum esculentum* Mill., *Datura stramonium* L., *Chenopodium ammaranticolor* Cost and Reyn, *Solanum nigrum* L., *Gomphrena globosa* L., *Nicotiana tabaccum* L. and *N. glutinosa* L. were germinated in small pots in a greenhouse. The seedlings were transplanted in large pots filled with soil mixture of sand and peatmoss (1:1). Inoculum was prepared by grinding leaves of *E. sativa* showing mosaic symptoms, in 0.01M potassium phosphate buffer, pH 7.05 in a ratio of 1:4 and then filtered through cheese cloth. Seedlings of the above plants were inoculated with the prepared inoculum after being dusted with carborundum (300 meshes). The seedlings were then rinsed with distilled water and kept in the greenhouse. For biological purification, single local lesion technique (Kahn and Monroe, 1963) using *C. ammaranticolor* as a local lesion host was performed whereas garden rocket was used as a propagation host for the virus (Chen *et al*., 2003).

Electronmicroscopy

A leaf dip method (Hill, 1984) was used for detection of virus particles in plant tissues showing virus-like symptoms. A drop of 0.01M potassium phosphate buffer, pH 7.0 was placed on each of three carbon coated grids (Pelco Inc., CA and USA). Pieces of infected garden rocket leaves were sliced using a razor blade and immersed several times (3-9 times) in the buffer drops on the grids to release the virus particles were. The excess buffer was then removed from the grids using pieces of filter papers. The grids were then negatively stained using phosphotungstic acid. A drop of this stain was placed on each grid for 45-60 sec before it was removed using pieces of filter papers.

Aphid Transmission

The aphid species *Brevicoryne brassicae* L. was collected from noninfected garden rocket plants in Oyaynah area and left starving for an hour in Petri dishes. The aphids in some of these plates were then allowed to feed briefly on pieces of healthy garden rocket leaves subsequent to the starving period, while others were allowed to feed briefly on pieces of garden rocket leaves infected with TuMV and showing mosaic symptoms. After the insects in both groups were fed for about 2 min, they were quickly transferred singly by a brush to healthy garden rocket seedlings (5 insects plant^{-1}). The aphids in both groups continued to feed on these seedlings for 10 min after their transfer. The seedlings were then sprayed with an insecticide to kill the insects. Seedlings were kept in the greenhouse and observations were recorded (Shattuck, 1992).

Enzyme Linked Immunosorbent Assay (ELISA)

Virus detection in the collected rocket leaf samples was carried out using DAS-ELISA as demonstrated by Clark and Adams (1977). ELISA kit's for Radish Mosaic Virus (RMV), Alfalfa Mosaic Virus (AMV), Turnip Mosaic Virus (TuMV) and Cucumber Mosaic Virus (CMV) were purchased from Agdia (Agdia Inc., 30380 Country Road, Elkhart, Indiana 46514 USA). Each of four microtiter plates was coated with antibodies for each of the abovementioned viruses after being diluted with the coating buffer. Subsequent to incubation and washing, aliquots of 100 µL of each of the samples which were extracted in the extraction buffer, were added in two wells of each plate. One hundred microliter of the proper dilutions of the relevant antibody-alkaline phosphatase conjugate were dispensed in the wells of each plate following washing the plates from samples sap. *P*-nitrophenyl phosphate solution was then added in the wells of each plate after washing from the conjugate solution. The plates were incubated for 1 h before the reaction was stopped using 3 M NaOH and the plates were read at 405 nm in the minireader II.

Total RNA Extraction and RT-PCR

Total RNA was extracted from the field collected sample and two of the inoculated garden rocket plants that were showing symptoms using SV-Total RNA Isolation System (Promega). The used oligonucleotide primers designed according to Sanchez *et al*. (2003) were as follows, the upstream primer Tu 8705- 8726: 5`- caa gca atc ttt gag gat tat g- 3` and the downstream primer Tu 9690-9669: 5`-tat ttc cca taa gcg aga ata c-3` were used. Reverse transcription-polymerase chain reaction was performed using the QIAGEN One Step RT-PCR Kit (Promega). The reaction was set up according to the manufacturer's recommendations. 10 µL of 5x QIAGEN One Step RT-PCR Buffer, 2 µL of 10 mM dNTP Mix, 10 µL of 5x Q-Solution, 2 µL 0f 10 pmol of each complementary and homologous Primers, 2 µL of QIAGEN One Step RT-PCR Enzyme Mix, 5-10 Units/reaction of RNase inhibitor, A total of 5 µL (200 ng) of RNA was added to the one-step mix and the mix was completed to 50 µL with RNase-free water. The master mix was mixed gently, by pipetting up and down a few times. RT-PCR reaction mixture was amplified using the following cycling parameters: hold at 50°C 30 min (RT step), hold at 95°C 15 min (hot start to PCR), then subjected to 35 cycles of amplification: 30 sec at 94°C for denaturation, 30 sec at 54°C for annealing and 60 sec at 72°C for extension, followed by a final hold at 72°C for 10 min. Aliquots of 5 µL from each of RT-PCR amplified DNA products were mixed with gel loading buffer (0.25% bromophenol blue, 0.25% xylene cyanol and 30% glycerol). Separation was done on a 1% agarose gel in 1x TBE buffer pH 8.3 (1x = 89 mM Tris, 89 mM borate and 2 mM EDTA). The DNA was stained with ethidium bromide added to the gel at a concentration of 0.5 µg mL^{-1}. The DNA was visualized on a UV transilluminator and photographed using DNA documentation gel

analysis. PCR DNA marker (Promega) was used to determine the size of RT-PCR amplified cDNA products (Sambrook *et al*., 1989).

Molecular Hybridization for Detection of TuMV

The amplified ~985 bp fragment from the virus was eluted and purified from agarose and labeled with DIG labeling system according to the manufacturer directions (Boehringer Mannheim GmbH, Mannheim, Germany). Normally for DNA fragments the random primed labeling method is preferred to enzymatically synthesize DIG labeled DNA probes (Feinberg and Vogelstein, 1983; Holtike and Kessler, 1990). For optimal results, the template DNA should be purified as follows: An equal volume of phenol/chloroform (1:1 V/V) was added, vortexed and centrifuged in a microcentrifuge for 2 min at 12,000 rpm at 4°C. The aqueous phase was transferred to fresh Eppendorf tubes of 1.5 mL and 2.5 volumes of ice cold absolute ethanol were added, mixed by inversion and incubated at -70°C for 30 min. The samples were centrifuged at 4°C for 5 min, the supernatants were discarded and the pellets were dried under vacuum. The pellets were dissolved in 50 μL distilled water. Template DNA (PCR products) was diluted to 0.5-3 μg to a total volume of 15 μL and denatured by heating for 10 min in a boiling water bath and quick chilling on ice, 2 μL of hexanucleotide mixture, 2 μL DTP labeling mixture, 1 μL Klenow enzyme, mixed and centrifuged briefly and incubated for at least 60 min at 37°C, 2 μL of 0.2 M of EDTA was added to stop the reaction. The labeled DNA was precipitated by adding 2.5 μL of 4 M LiCl and 75 μL of prechiled (-20°C) ethanol. The mixture was mixed very well, then left for 30 min at -70°C or for 2 h at -20°C, then centrifuged for 15 min. The pellets were washed with 50 μL cold ethanol 70% then dried briefly under vacuum and dissolved in 50 μL TE buffer.

Sap extracts from twenty one garden rocket samples were prepared by grinding 50 mg of fresh plant tissue in 100 μL of AMES buffer (0.5 M sodium acetate, 10 mM $MgCl_2$, 20% ethanol, 3% SDS and 1 M NaCl) (Laulhere and Rozier, 1976; Podleckis *et al*., 1993). The homogenates were incubated for 5 min at 37°C before extraction with an equal volume of chloroform. The aqueous phase was collected and reserved in fresh microcentrifuge tubes. Five ul aliquots were spotted onto a nitrocellulose membrane. The membrane was air dried and irradiated with UV cross linker and kept at room temperature until hybridized. Membranes were prehybridized (for blocking) in hybridization tube at 68°C for at least 1 h. The membranes were hybridized with 20 mL per 100 cm^2 of hybridized solution containing 5-25 ng of freshly heat DIG labeled denatured cDNA probe (Boiling water bath). The membranes were incubated for at least 6 h to overnight at 68°C. The membranes were washed 2×5 min at room temperature with at least 50 mL of 2xSSC, 0.1% SDS (w/v) per 100 cm^2 membranes and 2×15 min at 68°C with 0.1 x SSC, 0.1% SDS (w/v). Membranes were equilibrated in Genius buffer 1(100 mM Tris-HCl , 150 mM NaCl , pH 7.5) for 1 min with at least 20 mL of prehybridization solution per 100 cm^2 of membranes. The buffer was discarded, then 100 mL of Genius buffer 2 (2% blocking reagent dissolved in Genus buffer 1 and diluted 1:10) was added and membranes were incubated for 30 min at minimum. Anti digoxigenin alkaline phosphatase was diluted 1: 5000 in Genius buffer 2 and incubated in this buffer for 30 min. The membranes were equilibrated in Genius buffer 3 (100 mM Tris-HCl, 100 mM NaCl, 50 mM $MgCl_2$, pH 9.5) for 2 min, then immunologically detected. The membranes were incubated for 5 min to 16 h in 10 mL of freshly prepared color solution (45 μL Nitro blue tetrazolium and 35 μL X-Phosphate solution (5-bromo-4chloro-3 indolyl phosphate) were added to a 10 mL of Genius buffer 3) in box in the dark (avoiding shaking during color development) when the spot or band intensities were achieved, the reaction was stopped by washing the membranes for 5 min with 50 mL of water. The results were documented by photographing the wet filter.

Table 1: GenBank accession numbers for isolates included in this study

GenBank accession No.	Isolate	Original host	Geographical origin
AF103785	R9-10	Radish	South Korea, Andong
AF103786	CA7	Chinese cabbage	South Korea
AF103788	CJ	Chinese cabbage	South Korea, CheJu-Do
AF103789	RG	Radish	South Korea, GeoChang area
AF103790	RH	Radish	South Korea, Suwon
AF103791	TU	Turnip	South Korea
AF103792	Stock	Stock	South Korea
AF539411	TuMV-SD5	Radish	China
AJ831809	ZJ2	Turnip	China: Hangzhou: Zhejiang
AJ831812	JX	Radish	China: Nanchang: Jiangxi
AJ831817	YN2	Radish	China: Kunming: Yunnan
AF434720	BEL 1	*Rorippa nasturtium-aquaticum*	Belgium
AF434721	CHN 1	*Brassica* sp.	Taiwan
AF434722	DEU 2	Radish	Germany
AF434723	ITA 6	*Matthiola incana*	Italy
AF434724	JPN 1	Radish	Japan
AF434725	PRT 1	*Brassica oleracea*	Madeira (Portugal)
AF434726	PV 377	*Alliaria officinalis*	Italy

Sequencing and Computer Analysis

An amplified DNA fragment of expected size (985 bp) of the coat protein gene included 54 bp of the 3`-end of Nib gene and 65 bp of the 3`-UTR for TuMV isolate obtained from Al-Aoyaynah. The amplified PCR product was purified using the Wizard PCR clean up kit (Promega). The nucleotide sequence of the isolated gene of TuMV was carried out in one direction with the specific complementary primer at King Faisal Specialist Hospital and Research Center, Biological and Medical Research Department, Riyadh, Kingdom of Saudi Arabia using AB3730xI DNA Analyzer, Applied Biosystem-HITACHI. Sequence analyses were performed and the homolog tree analyses were done using DNAMAN trial version 5.2.10 program (Lynnon BioSoft., Quebec, Canada, www.Lynnon.com). The GenBank accession numbers for the different isolates shown in Table 1 were used in the comparison.

RESULTS

Host Range

Symptoms were observed on four out of the twelve inoculated plant species. These were *Eruca sativa*, *Raphanus sativus*, *Brassica rapa* and *Chenopodium ammaranticolor* (Fig. 1). The first three plant species showed systemic mosaic, however symptoms on *E. sativa* and *B. rapa* were more severe and the plants were more stunted and deformed than *Raphanus sativus. C. ammaranticolor* showed local lesions which were initially chlorotic but later on turned into necrotic lesions. The other eight plant species which included: *B. oleracea capitata, Spinacea oleracea, Lycopersicum esculentum*, *Datura stramoniu*, *Solanum nigrum*, *Gomphrena globosa*, *N. glutinosa* and *Nicotiana tabaccum* did not show symptoms in spite of their repeated inoculation.

The aphid, *B. brassicae* which fed briefly on the pieces of garden rocket leaves showing systemic mosaic subsequent to their starvation, transmitted TuMV to eight out of twelve garden rocket seedlings. No symptoms were observed on the tested rocket plants to which non-viruliferous aphids which were fed on healthy rocket plants were transferred. These results indicated transmission of the virus to *E. sativa* by the aphid *B. brassicae* in a nonpersistent manner.

Fig. 1: Symptoms of TuMV on artificially inoculated host plants: (A) Mosaic and chlorosis on infected garden rocket leaves, (B) Chlorotic local lesions on *C. amaranticolor*, (C) Symptoms on infected turnip plant showing mosaic, chlorosis and epinasty and (D) Electron micrograph of TuMV showing filamentous; flexuous shaped virus particles stained with phosphotungstic acid (Bar = 0.2 µm)

Electron Microscopy and ELISA

The leaf dip method indicated presence of elongated, flexuous virus particles typical of potyviruses, on the grids that were examined in the transmission electron microscope (Fig. 1). Positive results were obtained for *E. sativa* samples collected from Riyadh (Al-Aoyaynah, area) with the ELISA kit of TuMV. All *E. sativa* samples were negative to the tests with the ELISA kits of RMV, AMV and CMV.

RT-PCR and Molecular Hybridization

The electrophoresis analysis of the RT-PCR product obtained from the total RNA extraction of virus infected the field collected sample and two of the inoculated garden rocket plants that were reverse transcripted prior to amplification with PCR using specific primer for TuMV-CP designed to amplify 985 bp of the coat protein gene showed a single amplified fragment of the expected size (985 bp). The obtained data illustrate the agarose gel electrophoresis of RT-PCR amplified TuMV-CP cDNA from these samples of garden rocket that infected with TuMV from Riyadh (Al-Aoyaynah area) (lane 1- 3 (Fig. 2). No amplified fragments of cDNA were obtained from uninfected garden rocket leaves (lane 4).

Nucleic acid spot hybridization with TuMV cDNA probe was also used to detect the virus in infected plant materials which gave a blue reaction, while no signal was observed with uninfected plant materials. Five microliter of extracts from infected and healthy plants were spotted onto a nitrocellulose membrane. After hybridization, samples containing viral RNA were easily detected. Figure 3 showed dot blot hybridization of Dig-labeled cDNA probe with nucleic acid extracts from plant tissues infected with TuMV. A moderate blue signal reaction resulted from infected garden rocket leaves infected with TuMV (spot in lane

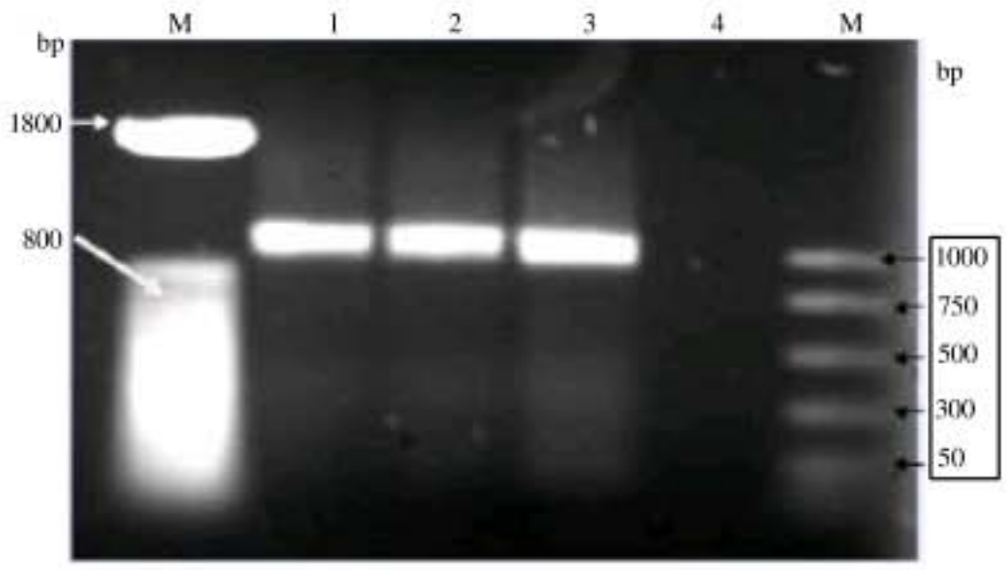

Fig. 2: Gel electrophoresis on RT-PCR amplification of a fragment from TuMV genome using specific primer pair designed to amplify 985 bp fragment of CP gene. Lanes 1, 2 and 3 were loaded from field collected sample and two of the inoculated garden rocket plants with TuMV processed with the SV-Total RNA Isolation System from Riyadh (Al-Aoyaynah area), Lane 4, a healthy tissue control via SV-Total RNA Isolation System kit. Lane M (right) represents 50 bp PCR Marker

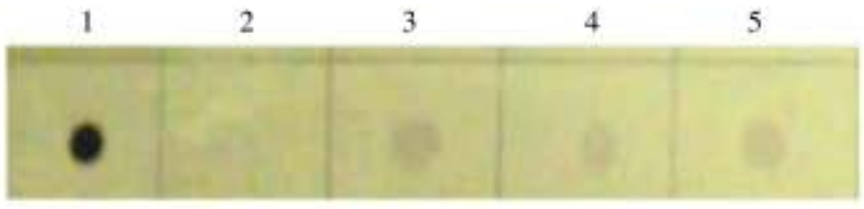

Fig. 3: Dot blot hybridization with TuMV DIG-cDNA probe of total RNA extracted from infected and uninfected garden rocket samples. Spot in Lane (1) shows the positive RT-PCR product as a control, Spots in lanes 3, 4 and 5, show the reaction of the field collected sample and two of the inoculated garden rocket plants with TuMV, respectively. No hybridization reaction was observed with uninfected sample (Spot in lane 2)

3 from field collected sample, spot in lane 4 and 5) were from two of the inoculated garden rocket plants that were showing TuMV symptoms. No hybridization was observed between the cDNA probe and healthy garden rocket leaves lane 2.

Sequencing of TuMV CP Gene

The CP gene sequence of the Saudi Arabian isolate of TuMV obtained from Al-Aoyaynah was found to be composed of 862 nucleotides in length. The nucleotide composition of the cDNA CP sequence revealed the highest contents for A (296) followed by G (196), C (189) and T (181). A multiple alignment was done along with the previously obtained sequences by GeneBank sequence data. As shown in Table 1 and Fig. 4, the comparative sequence analysis of the nucleotides sequence of the Saudi isolate of TuMV (TuMV-SA-Ro) showed 93% similarity with RG strain (#AF103789) and with JPN 1 isolate (#AF434724), both of which were isolated from radish and belonging to South Korea and Japan, respectively.

The nucleotide sequence of TuMV CP also showed 90% similarity with isolates from different plant species with the following GenBank accession number from South Korea (No. AF103785, No. AF103791, No. AF103786, No. AF103790, No. AF103792, No. AF103788),

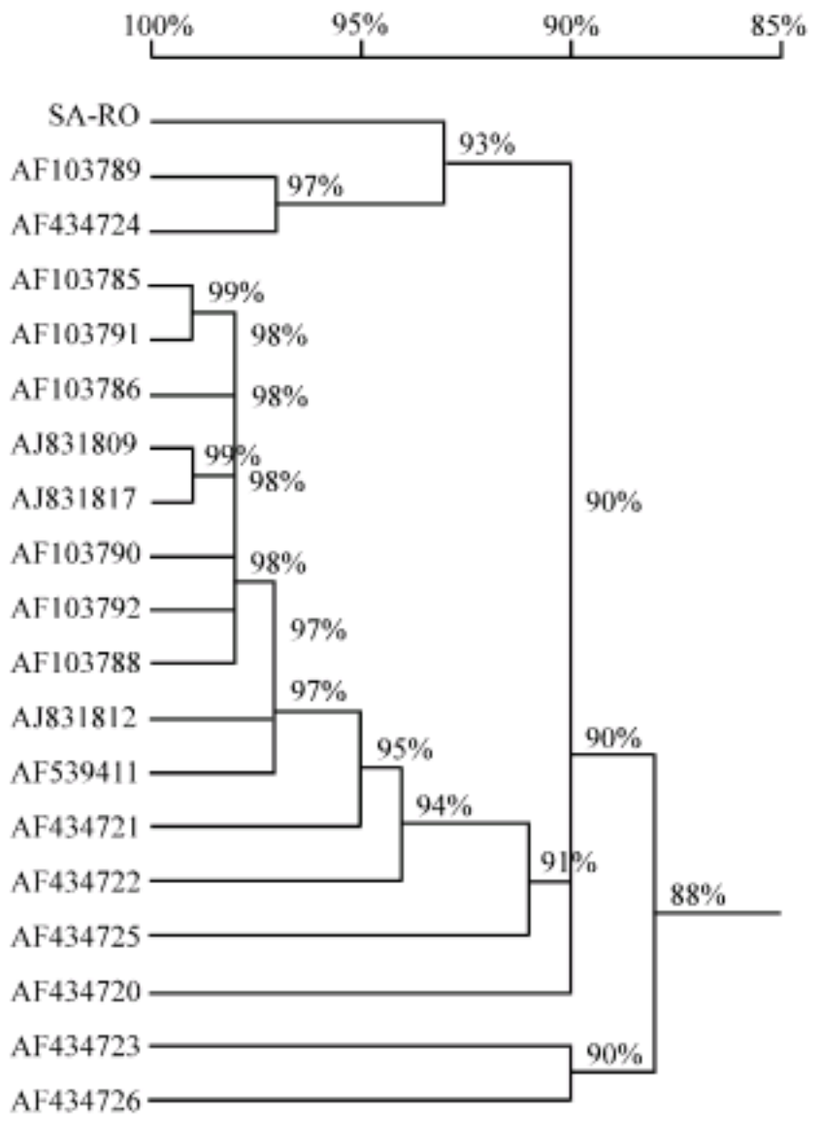

Fig. 4: The Phylogenetic homology tree based on multiple sequence alignments of the Saudi Arabian isolate of TuMV (SA-Ro isolate) compared to previously sequenced isolates

China (No. AJ831817, No. AJ831812, No. AF539411,No. AJ831809), Belgium (No. AF434720), Taiwan (No. AF434721), Germany (No. AF434722) and Portugal (No. AF434725), while the sequence homology was about 88% with Italy isolates (No. AF434723) and (No. AF434726). Previous attempts have been made to analyze TuMV variation at the nucleotide and amino acid levels. These studies have relied on sequence analysis of the 3'-untranslated region (3'-UTR) (Lehmann *et al*., 1997), Coat Protein (CP) gene (Chen *et al*., 2002; Lehmann *et al*., 1997) and on CP plus P1 genes (Ohshima *et al*., 2002). A number of sequences of TuMV are presently available in public databases. Very few cover the whole viral genome and most focus on partial genome stretches, especially the coat protein gene. Although, constantly increasing in number, these sequences probably still do not represent a significant sample of TuMV genetic variability. Furthermore, without an overall view of the genetic structure of the virus species, similar isolates could be repeatedly sequenced, thus biasing the whole picture.

Finally, detection of TuMV and the infection of *E. sativa* with this virus in Saudi Arabia were documented in this study. The spread of this virus is highly likely since different aphid vectors of this virus were reported in the Kingdom of Saudi Arabia (Aldryhim and Khalil, 1996) along with cultivation of several others cruiciferous host of this virus in neighboring and distant locations. The most important of which are *M. persicae*, *Aphis gossyypii* and *B. brassicae*. The later species which was found on garden rocket in the surveyed fields was tested for its transmission of the virus under investigation. It was proved to be very efficient in transmission of this virus that the percent of virus transmission to *E. sativa* was over 65% in spite of the limited number of plants that were used in the test. The minor differences

observed in the nucleotide sequence homology between the Saudi Arabian, Chinese, South Korea, Portugal, Germany, Belgium and Taiwan isolates may suggest that all these isolates are closely related. The results obtained from this study will help managing this disease in this crop and probably in other crops that might be infected as well with this virus.

DISCUSSION

Slight variations were observed between the results obtained in our study with those obtained in previous investigations. Some of the plant species which did not show symptoms upon their repeated inoculation in this study such as *N. tabaccum* and *N. glutinosa* were reported to show symptoms when inoculated with isolates of the same virus in previous studies (Walsh and Jenner, 2002). The variation of results in these studies could probably be explained by the occurrence of different strains for TuMV (Chamberlain, 1939; Green and Deng, 1985; Henson and French, 1993; Sanchez *et al*., 2007; Stobbs and Shattuck, 1989; Thomson *et al*., 1995).

Although, other and more efficient aphid vectors of TuMV such as *Myzus persicae* Sulzer and *Aphis gossypii* Glover as mentioned by Fujisawa (1985) were reported to occur in the country (Aldryhim and Khalil, 1996), we limited our transmission tests to *B. brassicae* since, it was the only encountered aphid species in the fields we visited during this study. The occurrence of this aphid species on garden rocket and the occurrence of other efficient aphid vectors of this virus on infected crop plants and weeds in the neighboring fields may be responsible for the wide spread of TuMV in this vast area in the central regions of Saudi Arabia. A further study is probably needed to determine the role and the relative importance of these aphid vectors in the transmission of this virus.

Since, PCR is an extremely sensitive and specific technique for the amplification of genomes and became widely used as a diagnostic technique for infection by plant viruses belonging to several different groups such as the geminiviruses, luteoviruses and potyvirus groups (Henson and French, 1993; Langeveld *et al*., 1991; Omunyin *et al*., 1996; Robertson *et al*., 1991; Rojas *et al*., 1993; Sanchez *et al*., 2007; Singh *et al*., 1995; Thomson *et al*., 1995; Wetzel *et al*., 1991). The PCR procedure utilized primers designed to amplify a variable region of the potyvirus genome to distinguish between different viruses within this virus group.

Previous attempts have been made to analyze TuMV variation at the nucleotide and amino acid levels. These studies have relied on sequence analysis of the 3'-untranslated region (3'-UTR) (Lehmann *et al*., 1997), Coat Protein (CP) gene (Chen *et al*., 2002; Lehmann *et al*., 1997) and on CP plus P1 genes (Ohshima *et al*., 2002). A number of sequences of TuMV are presently available in public databases. Very few cover the whole viral genome and most focus on partial genome stretches, especially the coat protein gene. Although, constantly increasing in number, these sequences probably still do not represent a significant sample of TuMV genetic variability. Furthermore, without an overall view of the genetic structure of the virus species, similar isolates could be repeatedly sequenced, thus biasing the whole picture.

Finally, detection of TuMV and the infection of *E. sativa* with this virus in Saudi Arabia were documented in this study. The spread of this virus is highly likely since different aphid vectors of this virus were reported in the Kingdom of Saudi Arabia (Aldryhim and Khalil, 1996) along with cultivation of several others cruiciferous host of this virus in neighboring and distant locations on which this virus has recently being reported (AL-Saleh *et al*., 2009). The most important of which are *M. persicae*, *Aphis gossyypii* and *B. brassicae*. The later

species which was found on garden rocket in the surveyed fields was tested for its transmission of the virus under investigation. It was proved to be very efficient in transmission of this virus that the percent of virus transmission to *E. sativa* was over 65% in spite of the limited number of plants that were used in the test.

The minor differences observed in the nucleotide sequence homology between the Saudi Arabian, Chinese, South Korea, Portugal, Germany, Belgium and Taiwan isolates may suggest that all these isolates are closely related. The results obtained from this study will help managing this disease in this crop and probably in other crops that were reported to be infected with it, lately (AL-Saleh *et al*., 2009) as well.

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