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Characterization of a *Watermelon mosaic virus* Isolate Inducing a Severe Disease in Watermelon in Saudi Arabia

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Abstract: In one of the field visits to cucurbits-growing areas in vicinity of Riyadh city during 2013-2015, severe virus disease-like symptoms were observed on watermelon in Al-Ammariyah area. Mechanical inoculation of the different plant species used in the host range study, from the collected symptomatic watermelon samples, produced mosaic symptoms on *Citrullus lanatus*, *Cucumis sativus*, *Cucurbita pepo*, *C. melo*, *C. melo* subsp. *melo* and *Nicotiana benthamiana*, but chlorotic local lesions on *Chenopodium amaranticolor*. No symptoms were observed on the rest of the other inoculated plant species. The virus was transmitted by *Aphis gossypii* and *A. craccivora* in a non-persistent manner. Transmission electron microscopic examination of watermelon samples using the leaf dip method revealed only microscopic filamentous shaped virus particles measuring 750 nm in length and 12 nm in diameter in average. ELISA revealed positive results only to *Watermelon mosaic virus* (WMV) and negative to *Zucchini yellow mosaic virus* (ZYMV), *Papaya ringspot virus* (PRSV), *Cucumber mosaic virus* (CMV) and *Squash mosaic virus* (SqMV). Specific bands of approximately 825 bp were formed on agarose gel following electrophoresis of the reverse transcriptase-polymerase chain reaction (RT-PCR) products of each of the naturally infected *C. lanatus*, and artificially infected *C. lanatus*, *C. pepo*, *C. sativus*, and *N. benthamiana*. The homology tree that was constructed from multiple sequence alignments of the detected Saudi Arabian isolate of WMV (WMV-SA) with 18 other isolates of WMV from nine different countries indicated close relationships between them. Two isolates from Spain and two other isolates from Iran were more closely related to the WMV-SA whereas the isolate from Poland was the least.

Key words: Watermelon, ELISA, nucleotide sequence, RT-PCR, Saudi Arabia, *Watermelon mosaic virus*.

1. Introduction

Watermelon (*Citrullus lanatus* (Thunb.) Matsum & Nakai) is one of the major crops in the Cucurbitaceae. The total production of watermelon in Saudi Arabia reached 216,708 tons from a total area of about 24,558 ha [1]. According to VIDE database, watermelon is susceptible to infection with 27 viruses. Six of those virus species: *Zucchini yellow mosaic virus* (ZYMV), *Watermelon mosaic virus* (WMV), *Papaya ringspot virus type-W* (PRSV-W), *Cucumber green mottle mosaic virus* (CGMMV), *Cucumber mosaic virus* (CMV) and *Squash mosaic virus* (SqMV) are considered the most common and important ones in addition to some newly emerging viruses [2-7]. All of those six viruses had been reported in Saudi Arabia [5] in addition to the newly detected viruses such as *Cucurbit yellow stunting disorder virus* (CYSDV) and *Watermelon chlorotic stunt virus* (WmCSV) [8].

Beside watermelon and other cucurbits, WMV host range also includes legumes, orchids and many weeds as alternate hosts [6]. This virus can be transmitted mechanically by plant sap and also by 35 aphid species in a non-persistent manner, with *Aphis craccivora* Koch., *A. gossypii* Glover, *Macrosiphum*

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euphorbiae Thomas and *Myzus persicae* Sulz. being the most efficient vectors [9, 10]. Yield losses due to WMV infection on cucurbits seem to be related to how early the infection takes place. One study estimated yield losses to reach 43%, 28% and 9% when plants were infected early, mid and late in the season [11]. Yield losses of marketable fruits could reach almost 100% on early and mid season infections and 70% on late season infection according to the same study.

The severe symptoms observed on watermelon including mosaic, leaf malformation and stunting were not encountered in this area before. Therefore, the objective of this study was to characterize the causal agent of the severely affected watermelon crop.

2. Materials and Methods

2.1 Host Range

Samples of severely affected watermelon plants showing mosaic, mottle and leaf malformation in Al-Ammariyah area were maintained in the deep freezer (-86 °C). *Cucurbita pepo* var. Marrow White, obtained from Bonanza Seeds Int., Inc. was grown in the greenhouse (temp. 25-27 °C) used as a propagative host and used to inoculate the 23 plant species used in the host range experiment (Table 1).

Leaves of the maintained plants were ground in a mortar and pestle using 0.01 M potassium phosphate buffer pH 7.0 (1 g tissue: 5 mL). The homogenate was then filtered through cheese cloth, the tested plants leaves were dusted with carborundum (400 mesh) before being mechanically inoculated with the sap [12]. The inoculated plants were rinsed with distilled water, maintained in the greenhouse at 25-27 °C and examined for symptoms expression during a period of four to five weeks. The host range experiment was repeated two times. DAS-ELISA and reverse transcriptase-polymerase chain reaction (RT-PCR) were used for infection confirmation.

2.2 Aphid Transmission

Virus-free melon aphids (*A. gossypii*) were reared on healthy watermelon plants maintained at 25-27 °C. The aphids were starved for 60 min then given acquisition access periods of 5 min on the symptomatic watermelon leaves, followed by inoculation access period for overnight on eight healthy watermelon plants kept at room temperature (23-25 °C) according to Hill, 1984 [12] and Hull, 2002 [13]. Confidor aphicide was then sprayed on the watermelon plants at the rate of 25 mL/100 L to kill the aphids. The plants were then kept in an isolated area in the same greenhouse and examined for symptoms appearance for a month. Eight healthy watermelon plants were treated with the same procedure with virus-free aphids feeding on healthy watermelon plants as control. The same procedures were applied using virus-free cowpea aphid (*A. craccivora*) that was reared on healthy *Vicia faba* L. plants and maintained at the same conditions as mentioned earlier.

2.3 Electron Microscopy

The watermelon samples collected from the field were prepared on formvar-coated electron microscope grids. The leaf dip method was used and grids were negatively stained with 0.2% potassium phosphotungstate solution, pH 6.5 [12, 14]. The grids were then examined using transmission electron microscope (TEM) JEOL-JEM 1011 (JEOL Ltd., Japan) at 60 kV at the Department of Soil Sciences, College of Food and Agriculture Sciences, King Saud University.

2.4 Serology

ELISA kits for detection of the five most common viruses affecting watermelon (ZYMV, PRSV, WMV, CMV and SqMV) were purchased from Agdia Inc., 30380 Country Road 6, Elkhart, Indiana 46514, USA. Samples from symptomatic *C. lanatus* that were collected from the field were tested by ELISA. Samples from *C. lanatus*, *C. pepo*, *Cucumis sativus* and *Nicotiana benthamiana* that expressed symptoms in the host range experiment were also tested four

weeks after inoculation; four replicates were used for each sample. The procedure to perform ELISA was as provided by the manufacturer.

2.5 Reverse Transcriptase-Polymerase Chain Reaction (*RT-PCR*)

The SV Total RNA Isolation System kit from Promega Corp. (USA) was used to extract RNA from field *C. lanatus* plants showing virus-like symptoms. RNA was also extracted from plant species artificially inoculated with sap extracted from the field watermelon plants that were found showing symptoms in the field including *C. lanatus*, *C. pepo*, *C. sativus*, *N. benthamiana*, and from healthy *C. pepo* (as negative control). Forward primer (WMV-F: 5'-GAATCAGTGTCTCTGCAATCAGG-3') and reverse primer (WMV-R: 5'-ATTCACGTCCCTTGCAGTGTG-3'), designed to amplify an 825 bp fragment of WMV coat protein (CP) gene, was used in RT-PCR assay [15]. The amount of master mix for one PCR reaction was prepared according to a procedure provided by BioScript™ One-Step RT-PCR Kit (Bioline, USA), which consisted of 12.5 uL of 1× One-Step RT-PCR buffer, 1 uL of 10 mM dNTP mix, 0.25 uL of each of 10 mM reverse and forward primer, 0.5 uL RNase inhibitor, 1 uL of one-step enzyme mix, 1.25 uL of dimethyl sulfoxide (DMSO) and 7.25 uL of diethyl pyrocarbonate (DEPC)-treated water. The amplification procedure consisted of 30 min reverse transcription step at 42 °C, 10 min of first denaturation at 95 °C, followed by 35 cycles of denaturation for 1 min at 94 °C, annealing for 1 min at 60 °C, and extension for 1 min at 72 °C. The final extension step was performed for 10 min at 72 °C. RT-PCR products were then analyzed by electrophoresis in 1.7% agarose gel and visualized on UV after ethidium bromide staining [15].

2.6 Nucleotide Sequence and Phylogenetic Analyses

Fifty microliters of RT-PCR product obtained from symptomatic watermelon samples collected from the fields in Riyadh region were purified subsequent to electrophoresis and sequenced at King Faisal Specialist Hospital and Research Centre, Riyadh, Saudi Arabia using Applied Biosystem AB3730xI DNA analyzer (Life Technologies Co., USA). Partial nucleotide sequence of WMV CP gene used in this study was analyzed using Blastn program and compared with other published WMV nucleotide sequences which are available in GenBank. The phylogenetic analyses and construction of homology tree was performed using DNAMAN trial version 5.2.10 program (Lynnon Biosoft, Canada).

3. Results and Discussion

3.1 Host Range of the Virus

Mosaic symptoms were observed on four cucurbit species, *C. lanatus*, *C. sativus*, *C. pepo*, *C. melo*, *C. melo* subsp. *melo*, as well as on *N. benthamiana*. Chlorotic local lesions were observed on *Chenopodium amaranticolor* (Fig. 1). No symptoms were observed on three other cucurbits, *Lagenaria siceraria*, *Luffa acutangula* and *Momordica charantia*. These results, agree with what was obtained by other investigators [16-19] who were dealing with WMV isolates and found that some of these isolates failed to infect these three cucurbit species. Lack of infection of these cucurbits with WMV isolates in those studies, and with the isolate detected in this study, is probably due to resistance of some of these cultivars to the tested isolates, such as that reported in *L. siceraria* [18, 19].

Results of the inoculated *N. benthamiana* in this study are in agreement with those reported earlier [20, 21]. It was reported that *N. benthamiana* and *L. acutangula* can be used to distinguish WMV and PRSV [22]. *N. benthamiana* was susceptible to WMV but was not susceptible to the tested PRSV isolates. However, *L. acutangula* was not susceptible to isolates of WMV, but it was susceptible to isolates of PRSV.

Fig. 1 Symptom expressions on plants infected with Saudi Arabian isolate of *Watermelon mosaic virus* (WMV-SA).
(A) mosaic on *Citrullus lanatus*; (B) mosaic on *Cucumis sativus*; (C) mosaic on *Cucurbita pepo*; (D) mosaic on *Nicotiana benthamiana*.

Production of chlorotic local lesions on leaves of inoculated *C. amaranticolor* agrees with earlier investigations with WMV [16, 20]. It was also reported that some WMV isolates produce local lesions on *C. quinoa*, some produce systemic symptoms, whereas others do not infect it at all [20, 23]. This may probably help to explain the lack of infection of this plant species with the virus isolate dealt with in this study.

The isolate detected in this study also failed to infect *Pisum sativum* cv. Progress #9 and *Vigna unguiculata* subsp. *unguiculata* cv. California #5, although other cultivars of both species were reported to be infected with WMV [6]. It was reported that *P. sativum* cvs. Alaska was infected with WMV, whereas *P. sativum* cvs. Little Marvel was not [22]. Also, WMV isolates that were collected from South-Eastern France showed similar phenomena that they were able to infect *P. sativum* var. Colmo but failed to infect *P. sativum* var. Douce Provena [20]. These facts may help to explain the failure of the isolate tested in this study to infect both *P. sativum* cv. Progress #9 and *V. unguiculata* subsp. *unguiculata* cv. California #5. The different interactions reported in these studies suggest that WMV seems to have several isolates that could be distinguished biologically.

The agreement of the results obtained in the host range of the detected isolate with earlier results obtained for WMV isolates, suggests that this isolate which was found infecting watermelon in Riyadh region is probably an isolate of that virus.

No symptoms were observed on *Carica papaya*, *Gomphrena globosa*, *Datura stramonium*, *Solanum nigrum*, *Nicotiana tabacum*, *N. glutinosa*, *N. occidentalis*, *Lycopersicon esculentum*, *Capsicum annuum* and *S. melongena* (Table 1).

The host range results also indicated that the isolate used in this study has a limited host range, mostly cucurbits, although it also infected two out of several other inoculated plant species (Table 1). However, the ability of this isolate to produce systemic symptoms on *C. lanatus*, *C. pepo*, *C. sativus*, *C. melo*, *N. benthamiana*, chlorotic local lesions on *C. amaranticolor*, and its failure to produce any symptom on *L. acutangula* can be considered as a biological evidence not only to confirm that the tested virus is probably a WMV isolate but further that this isolate is similar to those reported earlier [9, 17, 22].

Table 1 Symptoms expressed on several plant species 35-40 d post-inoculation with the Saudi Arabian isolate of *Watermelon mosaic virus* (WMV-SA).

Plant species	Symptoms
Citrullus lanatus (Thunb.) Matsum & Nakai cv. Sugar Baby	Mt, Mo, LM, St
Cucumis sativus L. var. Beit Alpha	Mt, Mo
Cucurbita pepo L. var. Marrow White	Mt, Mo, LM, St
Cucumis melo L. var. Ananas	Mt, Mo, LM
Cucucmis melo subsp. *melo* Naudin var. Hybrid Ananas	Mo
Nicotiana benthamiana L.	Mt, Mo
Chenopodium amaranticolor Coste & Reyn	CLL
Lagenaria siceraria (Molina) Standl. cv. Supreme	-
Luffa acutangula L.	-
Momordica charantia Descourt. cv. PS-33	-
Carica papaya L.	-
Pisum sativum L. cv. Progress #9	-
Vigna unguiculata subsp. *unguiculata* L. cv. California #5	-
Chenopodium quinoa Willd.	-
Gomphrena globosa L.	-
Datura stramonium L.	-
Solanum nigrum L.	-
Nicotiana tabacum L.	-
Nicotiana glutinosa L.	-
Nicotiana occidentalis L.	-
Lycopersicon esculentum Mill. var. Sultana	-
Capsicum annuum L. var. Cayenne Long Slim	-
Solanum melongena L. var. Long Purple	-

Mo = mosaic; Mt = mottle; LM = leaf malformation; St = stunting; CLL = chlorotic local lesions; - = no symptoms.

3.2 Aphid Transmission

The virus isolate detected in this study was transmitted by each of *A. gossypii* and *A. craccivora* in a non-persistent manner. Symptoms observed on infected watermelon plants, in this test, were similar to those expressed on watermelon plants collected from the field as well as on the mechanically inoculated watermelon plants. Transmission by aphids in a non-persistent manner is a typical character of genus *Potyvirus* [24]. *A. craccivora* and *A. gossypii* are considered among the most efficient vectors of Potyviruses including WMV [10]. Occurrence of these aphid species in Saudi Arabia [25], which were reported to transmit cucurbit viruses to healthy cucurbits, may help to explain the wide spread of virus-like symptoms on cucurbits in the central region of Saudi Arabia [5] and probably in other regions of this country.

3.3 Physical Proprieties of WMV

Filamentous flexuous rod-shaped virus particles, with average size of 750 nm in length and 12 nm in diameter, were observed in the TEM (Fig. 2). The shape and size of the virus isolate particles detected in this study are typical of Potyviruses [9, 26].

3.4 Serological Proprieties of WMV

ELISA was positive to WMV only and negative to ZYMV, PRSV, CMV and SqMV in all symptomatic samples collected from the field. These results proved that the virus that was found infecting watermelon plants in Al-Ammariyah area in Riyadh region, Saudi Arabia was WMV, and there was no mix infection of this virus with any of the other suspected viruses that were tested. WMV, ZYMV and PRSV were included in the test because they are among the viruses that commonly infect watermelon, and since they belong

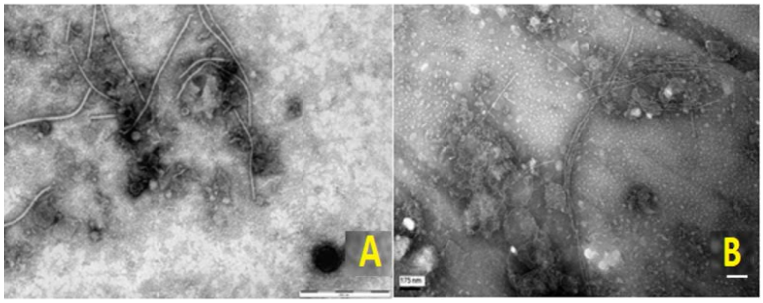

Fig. 2 Electron micrographs of virus particles prepared through leaf dip method and negatively stained with 0.2% potassium phosphotungstate solution (pH 6.5).
(A) virus particles from infected *C. lanatus*; (B) virus particles from infected *C. pepo*; scale bar legend: A = 500 nm, B = 175 nm.

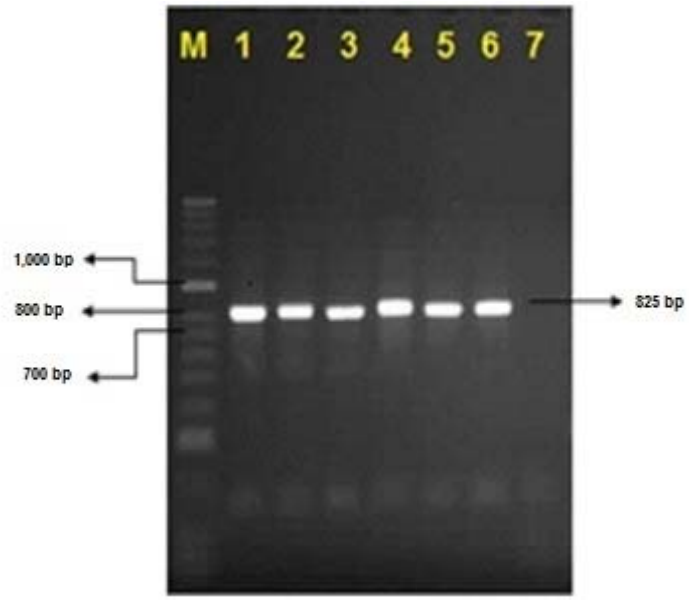

Fig. 3 Electrophoresis gel of reverse transcriptase-polymerase chain reaction (RT-PCR) amplification product using specific primers designed to amplify an 825 bp fragment of WMV coat protein region.
Samples from symptomatic *C. lanatus* collected from the field (lane 1); samples from inoculated symptomatic *C. lanatus* (lane 2); samples from symptomatic *C. pepo* (lane 3 and 4); sample from symptomatic *C. sativus* (lane 5); sample from symptomatic *N. benthamiana* (lane 6); negative control from healthy *C. pepo* plant (lane 7); 1000 bp marker (Bioline, USA) (lane M).

to the same genus *Potyvirus*, they are similar in biological properties and in particle shape [6]. CMV and SqMV were included because they are also among the viruses that frequently infect watermelon, easily transmitted by mechanical inoculation, and cause similar symptoms. DAS-ELISA also gave positive results with all plants that showed symptoms in the host range test confirming their infection with the isolate of WMV. However, none of the tested plants that did not show symptoms was positive to ELISA, confirming lack of latent infection in these plants. Hence, ELISA gave a conclusive evidence of the identity of the detected *Potyvirus* isolate to be WMV and confirmed the results obtained in the host range, insect transmission and the electron microscope studies.

3.5 Molecular Proprieties of WMV

Specific bands of approximately 825 bp fragment were formed on 1.5% agarose gel for the RT-PCR products of the symptomatic watermelon samples collected from the field using a specific primer to amplify a fragment of WMV CP region, whereas no bands were formed for control from healthy squash

plants (Fig. 3). These results confirmed infection of the watermelon plants collected from the field as well as each of the systemically-infected plants in the host range experiment including *C. lanatus*, *C. pepo*, *C. sativus* and *N. benthamiana* with WMV. The isolate used in the current study was then designated the Saudi Arabian isolate of WMV (WMV-SA). Molecular methods had been used in the identification and characterization of WMV and other Potyviruses and cucurbits-infecting viruses in plants and their aphid vectors in earlier studies [27-33].

3.6 Nucleotide Sequence and Phylogenetics of WMV

The multiple sequence alignment that was made between WMV-SA and 18 different WMV isolates retrieved from GenBank showed that the CP of WMV isolates are closely related and have a range of similarity of 93.9%-97.5%. Two WMV isolates from Spain (AJ579523 and AJ579521) and two WMV isolates from Iran (GQ421156 and GQ421159) had the highest similarity to WMV-SA and shared nucleotide identity of 97.5%, 97.4%, 97.3% and 97.3%, respectively. However, the WMV isolate from Poland (FJ628395) had the lowest similarity to WMV-SA and shared nucleotide identity of 93.9% (Table 2). The homology tree that was constructed from the multiple sequence alignments among WMV-SA and 18 isolates of WMV from nine different countries showed that the WMV isolates compared in this study can be grouped into three clusters based on their CP gene nucleotide sequence similarity (Fig. 4). This result demonstrated that WMV-SA is not only more similar to Spain isolate but it is also more similar to the isolates from Italy, France, Iran, Turkey and Chile, than other isolates (Table 2). The nucleotide sequence of WMV-SA was deposited in GenBank with accession No. KC447295.

The high similarity among WMV isolates from around the world, especially the ones that were compared in this study, suggests that WMV has low genetic diversity among its population. In another study that compared nucleotide sequences of 44 WMV isolates collected in Spain, it was found that the population of WMV in that country was highly homogeneous, built of a single pathotype, and comprising

Table 2 Comparison between coat protein sequence of the WMV-SA with coat protein sequences of 18 WMV isolates from nine different countries.

No.	Accession no.	Country of origin	Original host	Percentage of similarity between WMV-SA with other isolates
1	AJ579523	Spain	*C. melo*	97.5%
2	AJ579521	Spain	*C. melo*	97.4%
3	AJ579516	Spain	*C. melo*	97.2%
4	AJ579496	Spain	*C. melo*	97.2%
5	AJ579505	Spain	*C. melo*	97.0%
6	GQ421156	Iran	*C. pepo*	97.3%
7	GQ421159	Iran	*C. maxima* Duchesne	97.3%
8	JN166706	Iran	*C. lanatus*	96.9%
9	JN166704	Iran	*C. lanatus*	96.8%
10	EU660590	Italy	-	97.2%
11	AY437609	France	-	97.2%
12	JF273459	France	*C. pepo*	96.9%
13	EU660579	Turkey	-	97.0%
14	EU660582	Chile	-	96.9%
15	AB218280	Pakistan	*C. melo* var. *flexuosus*	95.2%
16	AB127934	Pakistan	*Trichosanthes cucumerina* L.	95.2%
17	DQ399708	China	*C. lanatus*	94.4%
18	FJ628395	Poland	*C. pepo* convar. *giromantiina*	93.9%

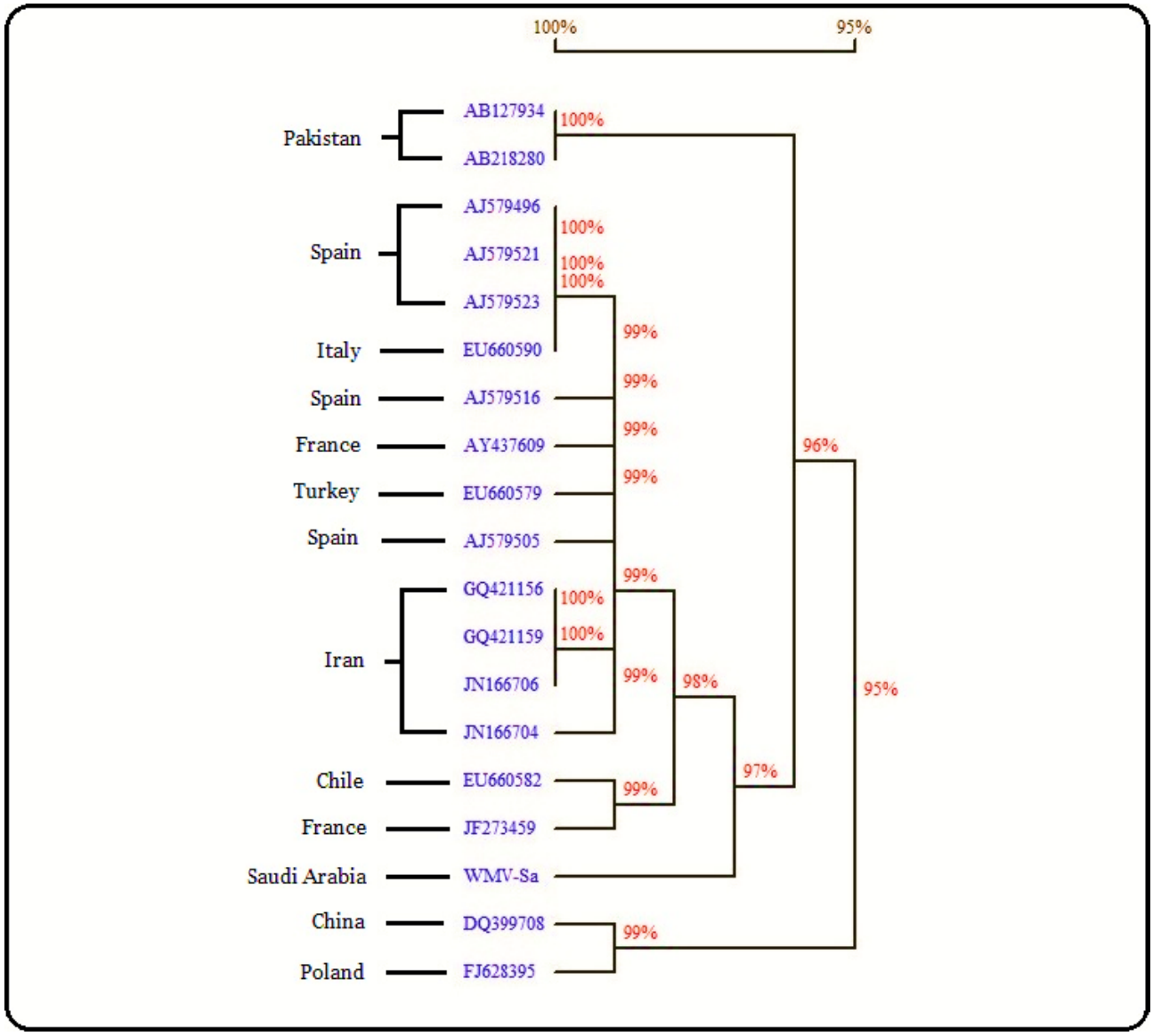

Fig. 4 A homology tree constructed based on multiple sequence alignments between the WMV-SA and 18 different WMV isolates published in the GenBank.

isolates closely related genetically [34]. However, this study only compared similarity among partial CP sequences of WMV isolates, not their whole genome. CP region, particularly the N-terminal part, can be used to assess molecular variability of WMV as that area is well known to be highly variable for Potyviruses [10].

4. Conclusions

Based on host range, insect transmission, electron microscopy, ELISA and RT-PCR results obtained in this investigation, the causal agent of the severe mosaic symptoms observed on watermelon in Al-Ammariyah area near Riyadh, was found to be WMV. Comparison of WMV-SA and isolates from other countries indicated close relationships between them based on nucleotide sequences of their CP genes in which only very low genetic variability of about 3.6% was encountered, and that isolates from Spain and Iran were the most closely related to the Saudi isolate whereas the Poland isolate was the least.

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References

[1] FAOSTAT. 2017. Food and Agriculture Data. Food and Agriculture Organization of the United Nations, Statistics Division. Accessed October 25, 2017. http://www.fao.org/faostat/en/#home.

[2] Abou-Jawdah, Y., Sobh, H., El-Zammar, S., Fayyad, A., and Lecoq, H. 2000. "Incidence and Management of Virus Diseases of Cucurbits in Lebanon." *Crop Protection* 19: 217-24.

[3] Aguair, R. W. S., Rodrigues, A., Portella, A. C. F., Lopes, M. M., Lima, M. F., Resende, R. O., and Nagata, T. 2015. "Serological Identification of Viruses in Watermelon Production Fields in the Tocantins State." *Braz. Arch. Biol. Technol.* 58: 192-7.

[4] Al-Saleh, M. A., Ahmad, M. H., Al-Shahwan, I. M., Brown, J. K., and Idris, A. M. 2014. "First Report of *Watermelon chlorotic stunt virus* Infecting Watermelon in Saudi Arabia." *Plant Diseases* 98 (10): 1451.

[5] Al-Saleh, M. A., and Al-Shahwan, I. M. 1997. "Viruses Infecting Cucurbits in Riyadh, Gassim and Hail Regions of Saudi Arabia." *Arab Gulf Journal of Scientific Research* 15: 223-54.

[6] Brunt, A. A., Crabtree, K., Dallwitz, M. J., Gibbs, A. J., Watson, L., and Zurcher, E. J. (eds.) (1996 onwards). *Plant Viruses Online: Descriptions and Lists from the VIDE Database*. Version: 16th January 1997. Accessed October 25, 2017. http://sdb.im.ac.cn/vide/refs.htm.

[7] Michel, P. 2012. "Vegetable Crops in the Mediterranean Basin with an Overview of Virus Resistance." *Advances in Virus Research* 84: 1-29. https://doi.org/10.1016/B978-0-12-394314-9.00001-4.

[8] Ahmad, M. H. 2017. "Characterization of Two Viruses Associated with a Yellowing Disease of Watermelon and Melon in Western and Southwestern Regions of Saudi Arabia." Master thesis, College of Food and Agriculture Sciences, King Saud University, Riyadh, Saudi Arabia, 65.

[9] Purcifull, D. E., Hiebert, E., and Edwardson, J. R. 1984. "C.M.I./A.A.B. Description of Plant Viruses." In *Watermelon mosaic virus 2,* NO. 293. Commonwealth Mycological Institute, Kew, United Kingdom.

[10] Lecoq, H., and Desbiez, C. 2008. "*Watermelon mosaic virus* and *Zucchini yellow mosaic virus*." In *Encyclopedia of Virology*, 3rd ed., edited by Mahy, B. W. J., and Van Regenmortel, M. H. V. USA: Academic Press Inc., 433-40.

[11] Demski, J. W., and Chalkley, J. H. 1972. "Effect of *Watermelon mosaic virus* on Yield and Marketability of Summer Squash." *Plant Disease Report* 56: 147-50.

[12] Hill, S. A. 1984. *Methods in Plant Virology,* Oxford: Blackwell Scientific Publications, United Kingdom, 167.

[13] Hull, R. 2002. "Chapter 11—Transmission 1: By Invertebrates, Nematodes, and Fungi." In *Matthews' Plant Virology,* New York: Academic Press Inc., USA, 485-531.

[14] Lockhart, B. E., Swenson, A. S., Olszewski, N. E., and Voth, P. 2008. "Characterization of a Strain of *Turnip vein-clearing virus* Causing Red Ringspot of *Penstemon*." *Plant Disease* 92: 725-9.

[15] Moradi, Z. 2011. "Diagnosis and Molecular Variability of *Watermelon mosaic virus* Isolates from North, East, North-East, and North-West Region of Iran." *Asian Journal of Plant Pathology* 5: 115-25.

[16] Adlerz, W. C., Purcifull, D. E., Simone, G. W., and Hiebert, E. 1983. "*Zucchini yellow mosaic virus*: A Pathogen of Squash and Other Cucurbits in Florida." *Proceedings of the Florida State Horticultural Society* 96: 72-4.

[17] Al-Musa, A. 1982. "Some Properties of *Watermelon mosaic virus* in Jordan." *Plant Disease* 66: 330-1.

[18] Ling, K., Levi, A., Adkins, S. T., Kousik, C. S., Miller, G., Hassell, R. L., and Keinath, A. P. 2013. "Development and Field Evaluation of Multiple Virus-Resistant Bottle Gourd (*Lagenaria siceraria*)." *Plant Disease* 97: 1057-62.

[19] Provvidenti, R. 1981. "Sources of Resistance to Viruses in *Lagenaria siceraria*." *Cucurbit Genetics Cooperative Report* 4: 38-9.

[20] Lecoq, H., Fabre, F., Joannon, B., Wipf-Scheibel, C., Chandeysson, C., Schoeny, A., and Desbiez, C. 2011. "Search for Factors Involved in the Rapid Shift in *Watermelon mosaic virus* (WMV) Populations in South-Eastern France." *Virus Research* 159: 115-23.

[21] Parry, J. N., and Persley, D. M. 2005. "Carrot as Natural Host of *Watermelon mosaic virus*." *Australasian Plant Pathology* 34: 283-4.

[22] Purcifull, D. E., and Hiebert, E. 1979. "Serological Distinction of *Watermelon mosaic virus* Isolates." *Phytopathology* 69: 112-6.

[23] Rahimian, H., and Izadpanah, K. 1978. "Identity and Prevalence of Mosaic-Inducing Cucurbit Viruses in Shiraz, Iran." *Journal of Phytopathology* 92: 305-12.

[24] Brault, V., Uzest, M., Monsion, B., Jacquot, E., and Blanc, S. 2010. "Aphids as Transport Devices for Plant Viruses." *Comptes Rendus Biologies* 333: 524-38.

[25] Aldryhim, Y., and Khalil, A. F. 1996. "Aphididae of Saudi Arabia." *Fauna of Saudi Arabia* 15: 161-96.

[26] Adams, M. J., Antoniw, J. F., and Beaudoin, F. 2005. "Overview and Analysis of the Polyprotein Cleavage Sites in the Family Potyviridae." *Molecular Plant*

Pathology 6: 471-87.

[27] Desbiez, C., Joannon, B., Wipf-Scheibel, C., Chandeysson, C., and Lecoq, H. 2009. "Emergence of New Strains of *Watermelon mosaic virus* in South-Eastern France: Evidence for Limited Spread but Rapid Local Population Shift." *Virus Research* 141: 201-8.

[28] Ghasemzadeh, A., Bashir, N. S., and Masoudi, N. 2012. "Sequencing Part of *Watermelon mosaic virus* Genome and Phylogenetical Comparison of 5 Isolates with Other Isolates from World." *Journal of Agriculture and Food Technology* 2: 93-101.

[29] Hsu, Y., Yeh, T., and Chang, Y. 2005. "A New Combination of RT-PCR and Reverse Dot Blot Hybridization for Rapid Detection and Identification of Potyviruses." *Journal of Virological Methods* 128: 54-60.

[30] Lee, B. Y., and Ryu, K. H. 2009. "Incidence of Virus Diseases and RT-PCR Detection of Daphne-Infecting Viruses in Korea." *European Journal of Plant Pathology* 124: 127-32.

[31] Sanchez, F., Wang, X., Jenner, C. E., Walsh, J. A., and Ponz, F. 2003. "Strains of Turnip Mosaic *Potyvirus* as Defined by the Molecular Analysis of the Coat Protein Gene of the Virus." *Virus Research* 94: 33-43.

[32] Shang, Q., Xiang, H., Han, C., Li, D., and Yu, J. 2009. "Distribution and Molecular Diversity of Three Cucurbit-Infecting Poleroviruses in China." *Virus Research* 145: 341-6.

[33] Singh, R. P. 1998. "Reverse-Transcription Polymerase Chain Reaction for the Detection of Viruses from Plants and Aphids." *Journal of Virological Methods* 74: 125-38.

[34] Moreno, I. M., Malpica, J. M., Díaz-Pendón, J. A., Moriones, E., Fraile, A., and García-Arenal, F. 2004. "Variability and Genetic Structure of the Population of *Watermelon mosaic virus* Infecting Melon in Spain." *Virology* 318: 451-60.