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Characterization of different isolates of *Zucchini yellow mosaic virus* from cucurbits in Saudi Arabia

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During the 2012 growing season, thirty three samples were collected from squash, pumpkin and muskmelon plants showing virus-like symptoms in Riyadh and Al-Madena regions of Saudi Arabia. Eleven of these samples were found positive for *Zucchini yellow mosaic virus* (ZYMV) by double antibody sandwich ELISA (DAS-ELISA). In the host range study for the five selected ZYMV isolates, 11 out of the 22 mechanically inoculated test plants were infected and showed variable symptoms. The amplification of viral DNA product through reverse transcription-polymerase chain reaction amplification (RT-PCR) using a primer pair specific for ZYMV, yielded fragments of approximately 1185 bp. Southern blot hybridization confirmed the results obtained through RT-PCR, using a specific DNA probe homologous to ZYMV. Nucleotide sequences for the coat protein gene from all five Saudi isolates of ZYMV indicated a similarity of 97.1-100.0% between them. Comparative analysis of the nucleotide sequences of coat protein gene from the Saudi isolates and other ZYMV isolates obtained from NCBI, showed a relatively high nucleotide sequence similarity that ranged between 92.0-98.8%. The highest similarity was found with Syria, Jordan, Iran, Hungarian, Austria, Slovenia and Germany isolates (97.1 to 98.8%). The nucleotide sequence data obtained for the five ZYMV isolates was deposited in the GenBank under the accession numbers JQ899263, JQ899264, JQ899265, JQ899266 and JQ899267.

Key words: ZYMV, DAS-ELISA, RT-PCR, Hybridization, Sequence.

INTRODUCTION

Zucchini yellow mosaic virus (ZYMV) is a species belonging to the genus *Potyvirus* in the family *Potyviridae* and characterized by a monopartite, positive-sense, single-stranded RNA genome encapsidated in flexuous, filamentous particles. It was first reported in Italy in 1981 (Lisa et al., 1981) and is responsible for major economic losses in cucurbit crops in many parts of the world causing severe mosaic, necrosis and malformation. Since

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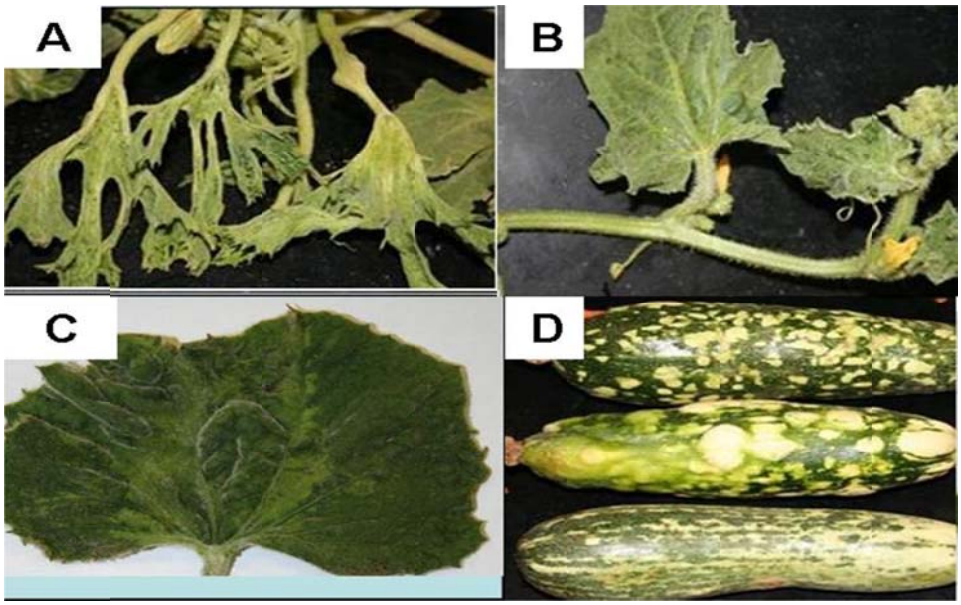

Figure 1. ZYMV symptoms on naturally infected cucurbit plants. Leaves of (A) Squash showing mosaic and malformation, (B) muskmelon showing yellow-green mottle and blistering, (C) pumpkin showing mosaic blisters and vein clearing and (D) fruits showing, warty raised yellow areas.

then it has been reported in many countries throughout the world (Lisa and Lecoq, 1984; Provvidenti et al., 1984; Davis, 1986; Dolores and Valdez,1988; Al-Shahwan, 1990; Yardimci and Korkmaz, 2004; Glasa and Pittnerova, 2006; Safaeizadeh, 2008; Coutts et al., 2011b; Vucurovic et al., 2012). Yield losses of up to 100% due to infection by this virus have been reported (Al-Shahwan et al., 1995; Ghorbani, 1988). ZYMV was reported in Saudi Arabia for the first time in the central region of the country in 1990 (Alshahwan, 1990). Because it causes abundant yield losses, studies to determine its biological, serological, and molecular characteristics were conducted to better understand the scope of diversity in its population structure (Mahgoub et al., 1998; Tóbiás and Palkovics, 2003; Bananej et al., 2008; Safaeizadeh, 2008; Yakoubi et al., 2008; Chikh Ali et al., 2009). The virus is transmitted from infected plants to healthy ones by several aphid species in a non-persistent manner (Gal-On et al., 1995). ZYMV has caused devastating epidemics in a number of commercial cucurbits worldwide, and several distinct biological strains of ZYMV have been described (Provvidenti et al., 1984; Desbiez and Lecoq, 1997). Strains of ZYMV isolated from distinct geographic origins exhibit biological diversity, especially in their host range, symptomatology and aphid transmission (Desbiez et al., 1996, 2002). Lack of studies concerning variability of ZYMV in Saudi Arabia encouraged initiation of the present investigation. The objective of this study was, therefore, to characterize five ZYMV isolates infecting three cucurbit species in Saudi Arabia using biological, serological and molecular methods, as well as sequence data analysis.

MATERIALS AND METHODS

Source of viral isolates and host range test

Eleven squash samples (*Cucurbita pepo* L.), were collected from Riyadh (Al-Hair and Wadi Eldwaser), ten muskmelon (*Cucumis melo* L.) samples were collected from Al-Kharj, and seven pumpkin (*Cucurbita maxima* L.) and five squash samples were collected from Al-Madena governorate showing malformed, yellow-green mottled and blistered leaves. Vein banding was also observed on leaves showing vein clearing, and warty raised yellow areas appeared on infected fruits (Figure 1). Samples were tested by double antibody sandwich ELISA (DAS-ELISA) as described by Clark and Adams (1977), to detect *Squash mosaic virus* (SqMV), *Cucumber mosaic virus* (CMV), *Watermelon mosaic virus* (WMV), *Alfalfa mosaic virus* (AMV) and ZYMV. Based on ELISA results, five ZYMV isolates which were singly detected in their hosts were selected to represent the plant species from which they were detected and the locations from which they were collected. Inoculums of these five ZYMV isolates were prepared from freshly collected leaf samples of squash from Riyadh (Al-Hair; ZYMV-SA-1, Wadi Eldwaser, (ZYMV-SA-4), Al-Madena (ZYMV-SA-2), muskmelon from Riyadh (Al-Kharj; ZYMV-SA-3), and pumpkin from Al-Madena (ZYMV-SA-5). Inoculums were prepared in 0.01 M potassium phosphate buffer containing 0.1% sodium sulphite (Na_2SO_3), pH 7.2 using an extraction ratio of 1:4 (w/v), and were applied on leaves of selected host plants that were previously dusted with 600-mesh carborundum. Plants utilized for the host range test are indicated in Table 1. After inoculation, the plants were maintained in a greenhouse at 25-30°C. For biological purification, single local lesion assay was performed according to Khan and Monroe (1963) using *Chenopodium amaranticolor* Coste & Reyn as a local lesion host. A single local lesion out of the several observed was used for back inoculation on squash which was used as propagative host for the ZYMV isolates. Viral symptoms on plants were recorded two weeks after inoculation, and then at regular intervals during the next 4 weeks. All plants showing no symptoms were assayed for virus infections by back inoculation to *C. pepo, C. melo, Chenopdium*

Table 1. Reaction of different plant species to inoculation with the five Saudi isolates of ZYMV and to the DAS-ELISA assay.

Host range	Host reaction					ELISA assay
	ZYMV-SA-1	ZYMV-SA-2	ZYMV-SA-3	ZYMV-SA-4	ZYMV-SA-5	
Cucurbita pepo, Vegetable Marrow	YM	M, Ma	M, Vb	M,	M	+
Cucumis melo, Russian	M	M, b	M, Vb	Mm, b	M	+
Cucumis sativus L. cv.Beit Alpha	Mm	Mm	M, Vb	Mm	M	+
Citrullus lanatus (Thunb.) Matsum & Nakai), cv.Sugar Baby	Mo	Mo	M	Mo	M	+
Cucurbita maxima, Duch. E Lam cv. Connecticut Field	Mm	M	M, Vb	M, Vb	M, Vb	+
Luffa acutangula L.	M	M	M	M	M	+
Cucurbita okeechobeensis (Small) L. H. Bailey	Mm	M	M	Mm	M	+
Chenopodium quinoa Willd	CL	CL	CL	CL	CL	+
Chenopodium amaranticolor Coste & Reyn	CL	CL	CL	CL	CL	+
Phaseolus vulgaris L. cv. Black Turtel 2	CL	CL	-	CL	-	+
Pisum sativum L. cv. *Alsk*	SL	SL	-	SL	-	+

CL = Chlorotic local lesion, M = mosaic, Vb = vein banding, b = blisting, Ma = malformation, Mm = mild mottle, YM = yellow mosaic, Mo = mottle, SL = symptomless, - = no reaction, + = positive reaction.

quinoa Willd, *C. amaranticolor*, and by DAS-ELISA (Clark and Adams, 1977).

ELISA detection

Detection of cucurbit viruses in the leaf samples collected from different locations was carried out using the DAS-ELISA method. ELISA kits were purchased from Agdia (Agdia Inc., 30380, Country Road, Elkhart, Indiana, 46514, USA), and used to test for the presence of ZYMV and other cucurbit viruses in the collected plant samples. The plates were read at 405 nm using a microtiter plate reader (Bio-Tek ELx808). Samples were considered positive when the absorbance values at 405 nm (A_{405}) exceeded the mean of the negative controls (healthy) by at least a factor of two (Al-Shahwan et al., 1995; Sammons et al., 1989). All samples were assayed in three repeats. The presence of ZYMV isolates was also confirmed by reverse transcription-polymerase chain reaction amplification (RT-PCR) as detailed below.

Extraction of viral RNA and RT-PCR

To confirm the presence of ZYMV isolates by RT-PCR, total RNA was extracted from eleven samples [six squash samples collected from Riyadh region (Al-Hair and Wadi Eldwaser), Al-Madena, three muskmelon samples from Riyadh region (Al-Kharj) and two pumpkin samples collected from Al-Madena region] which tested positive for ZYMV by DAS-ELISA using the SV–Total RNA Isolation System according to the manufacturer's protocol (Promega).

A pair of ZYMV specific forward (ZYU: 5'-gct cca tac ata gct gag aca gc-3`) and reverse primer (ZYD1186: 5`- tag gct tgc aaa cgg agt cta atc -3`) which amplify part of the cytoplasmic inclusion gene, the coat protein gene and the 3' untranslated region (UTR) of the ZYMV genome, as described by Choi et al. (2002) and Yoon (1999) were used. RT-PCR was performed using the One Step RT-PCR Kit (Qiagen). RT-PCR reactions were amplified using the following cycling parameters, hold at 50°C for 30 min (RT step), hold at 95°C for 15 min (hot start to PCR), then subjected to one cycle of amplification: 94°C for 1 min, 35 cycles of 94°C for 30 s, 65°C for 30 s, 72°C for 1 min, followed by 72°C for 5 min. Amplified DNA fragments of the expected size (1185 bp) were electrophoresed in 1% TAE buffer (40 mM Tris, 40 mM acetate, and 1 mM EDTA). DNA was stained with ethidium bromide added to the gel at a concentration of 0.5 µg/ ml. DNA was visualized on a UV transilluminator and photographed using DNA documentation gel analysis. A 1 kb DNA marker (Promega) was used to determine the size of RT-PCR amplified cDNA products (Sambrook et al., 1989).

Preparation of the cDNA probe of ZYMV and nucleic acid hybridization

The purified DNA fragment (1185 bp) which was amplified from ZYMV using the ZYU and ZYD1186 primer pair was used as template to synthesize a cDNA Dig labeled probe. The probe was labeled by PCR using the Genius™ System (Boehringer Mannheim Corp.) according to the manufacturer's instructions. The capillary transfer of DNA from the gel to nitrocellulose membrane support was done as previously described (Southern, 1975). The DNA was fixed on the membranes by ultraviolet cross-linking for 30 s. Hybridization and immunological detection were carried out using the Genius II DNA Labeling and Detection Kit (Boehringer Mannheim, IN) according to the manufacturer's instructions.

DNA sequencing and data analysis

Bands of the expected size from five selected Saudi isolates of ZYMV (ZYMV-SA-1, ZYMV-SA-2, ZYMV-SA-3, ZYMV-SA-4 and ZYMV-SA-5) were excised and recovered with a Wizard PCR clean up kit (Promega). The nucleotide sequence of the isolated ZYMV gene was carried out in two directions with the specific complementary and homologous primer at King Faisal Specialist Hospital & Research Center, Riyadh, Saudi Arabia using an AB3730xl DNA Analyzer model Hitachi. Sequence analyses were performed and the homology tree analyses were done using the DNAMAN trial version 5.2.10 program. The GenBank accession numbers for the different ZYMV isolates obtained from NCBI were used in the comparison (Table 2).

RESULTS

Isolation, detection and host reaction of the causal virus

In the present study, some ZYMV isolates occurring in

Table 2. Comparison of nucleotide identities of the CP gene sequences for Saudi Arabia isolates with those of ZYMV isolates originating in different countries.

ZYMV isolates	Country	Isolate	JQ899263	JQ899264	JQ899265	JQ899266	JQ899267
JQ899263	KSA	ZYMV-SA-1	100%	100.0%	99.2%	99.2%	98.6%
JQ899264	KSA	ZYMV-SA-2	100.0%	100%	99.2%	98.6%	98.6%
JQ899265	KSA	ZYMV-SA-3	99.2%	99.2%	100%	98.6%	97.7%
JQ899266	KSA	ZYMV-SA-4	99.2%	98.6%	98.6%	100%	97.1%
JQ899267	KSA	ZYMV-SA-5	98.6%	98.6%	97.7%	97.1%	100%
EU999757	Syria	SYR-B2	98.5%	98.5%	97.7%	98.0%	98.0%
AJ420015	Austria	Austria 10	98.1%	98.1%	97.3%	97.4%	97.4%
AJ420018	Slovenia	Slovenia 1	98.0%	98.0%	97.1%	97.3%	97.3%
AJ251527	Hungarian	10	98.0%	98.0%	97.1%	97.3%	97.3%
FJ705272	Iran	Azr.Mak.W	98.7%	98.7%	97.9%	98.0%	98.0%
EU999758	Jordan	JOR-B3	98.1%	98.1%	97.3%	97.4%	97.4%
AJ420019	Germany	Berlin 1	98.8%	98.8%	98.0%	98.1%	98.1%
AY074810	China	Ningbo	95.0%	95.0%	94.1%	93.9%	93.5%
AY074809	China	Beijing	95.0%	95.0%	94.2%	94.2%	94.2%
AJ420020	Italy	Italy 1	95.2%	95.2%	94.4%	94.4%	94.4%
AY278998	Vietnam	KR-PA	93.1%	93.1%	92.3%	92.0%	92.0%
AF486823	China	Hainan	93.9%	93.9%	93.1%	93.1%	93.1%
AF127931	Taiwan	TW-TC1	93.5%	93.5%	92.6%	92.6%	92.6%
AJ316228	China	SG	92.9%	92.9%	92.1%	92.1%	91.5%
AF127934	Taiwan	TW-PT5	92.9%	92.9%	92.1%	92.3%	91.5%
AF435425	China	Hangzhou	93.8%	93.8%	92.9%	92.9%	92.2%
AJ429071	Korea	A	93.3%	93.3%	92.7%	92.5%	92.5%
D13914	USA	Florida	93.2%	93.2%	92.4%	92.4%	92.4%
DQ645729	Spain	ZYMV C16	92.9%	92.9%	92.0%	92.0%	92.0%
AF014811	Singapore	Singapore	84.8%	84.8%	84.1%	84.1%	83.5%
AJ515907	China	SXS	82.5%	82.5%	81.8%	82.1%	81.1%
AJ515908	China	MM	82.2%	82.2%	81.5%	81.7%	80.7%
AJ515911	China	WM	82.4%	82.4%	81.7%	81.9%	81.0%
AY611025	China	BJ-03	83.0%	83.0%	82.3%	82.4%	81.6%

symptomatic squash, muskmelon and pumpkin plants in different locations in Saudi Arabia were detected and characterized. Out of thirty three samples, eleven samples [six squash samples collected from Riyadh region (Al-Hair and Wadi Eldwaser), Al-Madena, three muskmelon samples from Riyadh region (Al-Kharj) and two pumpkin samples collected from Al-Madena region] were positive for the presence of ZYMV by DAS-ELISA. Five of these samples were singly infected whereas the other six showed mixed infection with one or more of the following viruses: SqMV, CMV and WMV. Ten of the tested samples were found to be infected with one or more of the previously expected viruses other than ZYMV, whereas the rest of the samples were negative to all viruses. The Five samples that were selected for further studies of ZYMV were all singly infected and designated ZYMV-SA-1, ZYMV-SA-2, ZYMV-SA-3, ZYMV-SA-4 and ZYMV-SA-5 as indicated before. These five ZYMV isolates were characterized by host reactions, RT-PCR and nucleotide sequence. Eleven out of the 22 inoculated plant species in the host range test were infected with at least one of the five tested ZYMV isolates. However nine of the eleven positive plants were infected with all five ZYMV isolates (Table 1).

It is clear that these five ZYMV isolates differ in their host range. Whereas ZYMV-SA-1, ZYMV-SA-2 and ZYMV-SA-4 isolated from squash infected *Pisum sativum* L. *cv.* Alsk, and *Phaseolus vulgaris* L. cv. Black Turtel 2 showing symptomless infection on the former and chlorotic local lesions on the later, the two other ZYMV isolates (ZYMV-SA-3 and ZYMV-SA-5) isolated from pumpkin and muskmelon did not infect any of these two plant species at all. All six cucurbit species tested in this investigation were infected with the five ZYMV isolates and showed the following symptoms on these plant species: mild mottle, mottle, mosaic, yellow mosaic, vein banding, formation of blisters and malformation. All five ZYMV isolates induced mosaic symptoms on *Luffa acutangula* L. and chlorotic local lesions on each of *C. quinoa* and *C. amaranticolor* (Table 1). The other plant

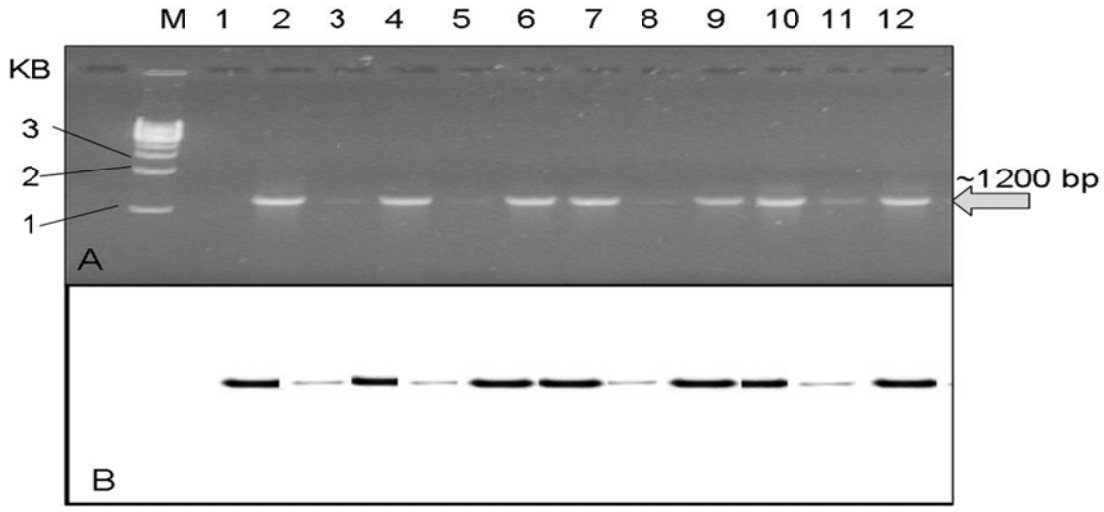

Figure 2. (A): 1% agarose gel electrophoresis of RT-PCR amplified products (1185 bp fragment) containing the complete coat protein gene using specific primers. Electrophoresis analysis of the RT-PCR products showed that single fragments of ~1.2 kb were amplified from symptomatic squash, muskmelon and pumpkin leaves collected from different locations. Lanes from left to right correspond to: squash samples collected from Riyadh region (Al-Hair: lanes 2, 3, Wadi Eldwaser: Lanes 4, 5), and Al-Madena (lanes 6, 7). Muskmelon samples from Riyadh region (Al-Kharj; lanes 8, 9, 10), and pumpkin samples collected from Al-Madena region (lanes 11, 12). Lane M: 1 kb DNA Ladder (Promega). (B) Southern blot hybridization analysis using a non-radioactive DIG–labelled cDNA probe, showing corresponding hybridization signals with the nucleic acid amplified products (1185 bp fragment). No RT-PCR and hybridization reaction was observed with uninfected squash tissues (lane 1).

species that were not infected by these isolates were *Gomphrena globosa* L.*, Datura stramonium,* L.*, Solanum lycopersicon* L.*, Nicotiana tabacum* L., cv. Samsun, Xanti-nc and White Burley*, Nicotiana rustica* L., *Nicotiana benthamiana* L.*, Capsicum annuum* L., *Beta vulgaris* L.*, Brassica oleracea* L.*, Raphanus sativs* L*,* and *Solaum nigrum* L.

Detection of ZYMV by RT-PCR and Southern blot hybridization

RT-PCR amplification of viral nucleic acid was carried out on the total RNA purified from the eleven infected samples which tested positive for ZYMV by DAS-ELISA and uninfected plant materials using an oligonucleotide primer pair specific for the complete coat protein gene of ZYMV (Figure 2A). Electrophoresis analysis of the resulting RT-PCR products showed single fragments of ~1.2 kb from RNA extracted from squash samples collected from Riyadh region (Al-Hair: lanes 2, 3, Wadi Eldwaser: lanes 4, 5), and Al-Madena (lanes 6 and 7). Muskmelon samples from Riyadh region (Al-Kharj; lanes 8, 9, 10), and pumpkin samples collected from Al-Madena region (lanes 11 and 12). No RT-PCR amplified product was observed with uninfected squash leaves (lane 1). Figure 2B illustrates Southern blot hybridization analysis using a non-radioactive DIG-labeled cDNA ZYMV probe which hybridized to the RT-PCR products amplified from symptomatic squash, muskmelon and pumpkin leaves collected from different locations (lanes 2 to 11). No hybridization reaction was observed with uninfected squash tissues (lane 1).

Sequence analysis of the ZYMV-CP gene

Following RT-PCR amplification, DNA sequences for the coat protein (CP) gene were determined for the five Saudi isolates of ZYMV based on the host plant and the location as mentioned earlier. Phylogenetic analysis using the 836 bp fragment comprising the complete CP gene fragment of the five Saudi Arabia isolates of ZYMV, along with sequences of 24 different isolates from GenBank which had been utilized in previous studies as reference sequences showed that ZYMV isolates clustered in seven groups. Saudi Arabian isolates belonged to one group to which Syria isolate also belonged. Comparing the nucleotide sequences for the five Saudi isolates indicated similarity between them that ranged between 97.1 and 100.0%. Similarity was also found between the five Saudi isolates and the remainder of the ZYMV isolates that were obtained from GenBank that ranged between 92.0 and 98.8%. The results also showed that the highest similarity was found with the following isolates: SYR-B2 isolate from Syria (97.7 to 98.5%); JOR-B3 isolate from Jordan (97.3 to 98.1%); Azr.Mak.W isolate from Iran (97.9 to 98.7%); isolate 10 from Hungary (97.1 to 98.0%); Austria 10 isolate from Austria (97.3 to 98.1%); Slovenia 1 isolate from Slovenia

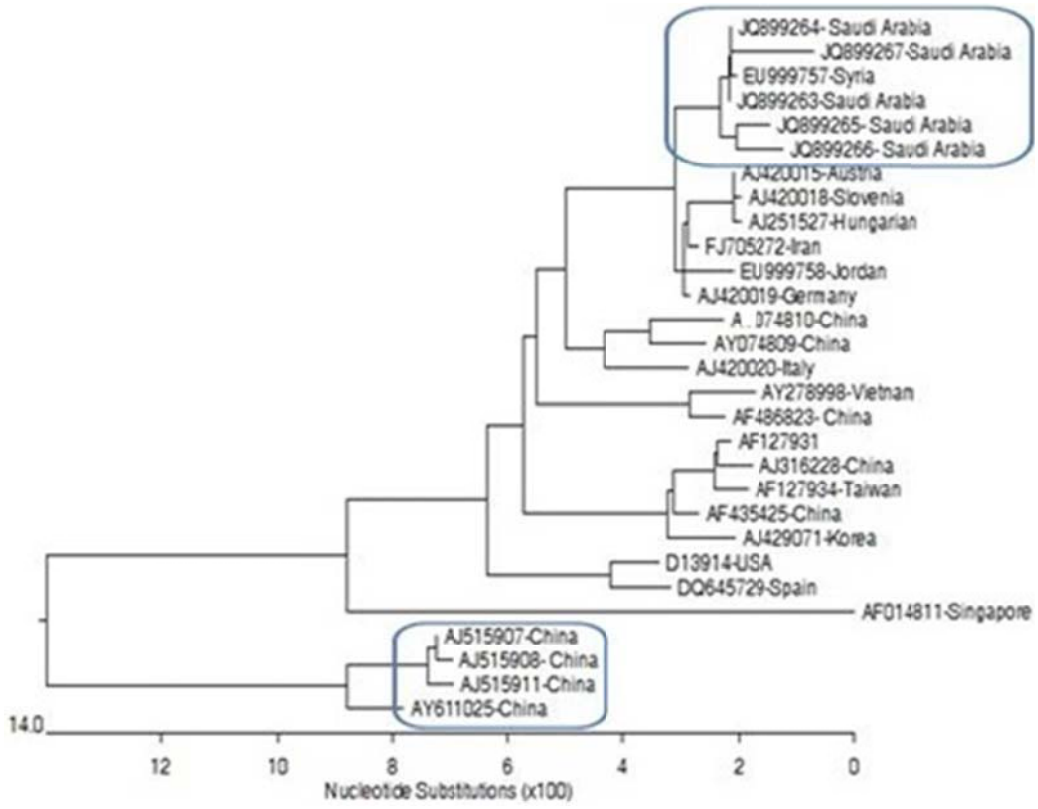

Figure 3. The homology tree based on multiple sequence alignments between five Saudi Arabia isolates of ZYMV and 24 different isolates from GenBank.

(97.1 to 98.0%); Berlin 1 isolate from Germany (98.0 to 98.8%) when compared with the five Saudi Arabia isolates. The lowest similarity was found with the SXS, WM, MM, SG, BJ-03, Hainan, Hangzhou, Ningbo and Beijing isolates from China (80.7 to 95.0%); isolate from Singapore (83.5 to 84.4%); ZYMV C16 isolate from Spain (92.0 to 92.9%); KR-PA isolate from Vietnam, KR-PA (92.0 to 93.1%), isolate A from South Korea (92.5 to 93.3%), Florida isolate from USA (92.4 to 93.2%), TW-PT5 and TW-TC1 isolates from Taiwan (91.5.6 to 93.5%%); Italy 1 isolate from Italy (94.4 to 95.2%) when compared with the five isolates of ZYMV from Saudi Arabia (Figure 3 and Table 2). The nucleotide sequences of the coat protein gene (841 bp) which were determined for all five Saudi isolates have been deposited in GenBank under the accession numbers JQ899263, JQ899264, JQ899265, JQ899266, JQ899267 (corresponding to ZYMV-SA-1, ZYMV-SA-2, ZYMV-SA-3, ZYMV-SA-4 and ZYMV-SA-5, respectively).

DISCUSSION

Out of the 22 plant species inoculated with the five Saudi isolates of ZYMV, symptoms were observed on 11 of them. All cucurbit species were infected by all five tested ZYMV isolates, however the symptoms were somewhat variable (Table 1). Among the five Saudi isolates, ZYMV-SA-2 isolated from squash in Al–Medina showed stronger symptoms on the infected hosts. These symptoms included mild mottle, mottling, mild mosaic, vein banding, mosaic, yellow mosaic and malformation. The ZYMV isolates showed variable reactions on the non-cucurbit species which included chlorotic local lesion and mosaic, whereas some of these plants did not show symptoms. Plant species exhibiting symptoms in the host range test were confirmed positive for ZYMV by ELISA. Some isolates of ZYMV have been reported to produce systemic infection without visible symptoms in *P. sativum* and tested positive by ELISA and was also reported for similar response in previous studies (Lecoq et al., 1981; Provvideenti et al., 1984; Singh et al., 2003; Safaeizadeh, 2008). Our study revealed minor differences in the host range and symptom expression of the Saudi isolates we studied. However, ZYMV-SA-2 from El-Madena was more virulent than the other four isolates. Distinct ZYMV isolates are assumed to give distinctive symptoms on infected plants. Since this is not always true in nature, incorporation of indicator plants in host range studies is generally useful, as they may help in differentiation of viral isolates. An example of this is the responses of *G. globosa, P. vulgaris* and *P. sativum* to the Saudi isolates

in this investigation. The minor differences in the host reactions observed with these isolates may be attributed to their different infection histories, which could have resulted in different host range adaptability, as suggested earlier (Chikh Ali et al., 2009).

The present study demonstrates the successful use of RT-PCR and sequencing to directly detect ZYMV in infected squash, pumpkin and muskmelon plants for the first time in various regions of Saudi Arabia. It is also worth mentioning that our DAS-ELISA and RT-PCR results were in complete agreement. More than 35 viruses have been isolated from different cucurbits so far and some of them present a serious threat to successful cucurbit production worldwide (Provvidenti, 1996), causing, in some cases, yield losses as high as 100% (Al-Shahwan et al., 1995, Abou-Jawdah et al., 2000; Coutts et al., 2011a). CMV, ZYMV, WMV and *Papaya ringspot virus* (PRSV) are transmitted from diseased plants to healthy plants by aphids in a non-persistent manner. This means that they acquire the virus from an infected plant almost immediately, but are able to infect healthy plants for only a short time, usually several hours to few days. Only a small number of aphids are needed to spread the virus throughout the field.

The nucleotide sequences were obtained for the CP gene in the five Saudi ZYMV isolates, and were used to investigate their genetic diversity and to establish their relationships with ZYMV isolates reported elsewhere in the world. Our results showed limited variability among the Saudi isolates and high similarity with other isolates from neighboring countries (Iran, Jordan and Syria), with the Iranian isolates showing the highest similarity with the Saudi isolates. These isolates and the Saudi isolates are not only similar in their nucleotide sequence, but they also share infection of the same cucurbit plants with somewhat minor differences in the symptoms induced on these plants (Table 1). The occurrence of similar ZYMV isolates in these neighboring countries may suggest spread of isolates of a common origin. High similarity was also observed between the Saudi ZYMV isolates and isolates from distant countries (Germany, Hungary, Austria, and Slovenia). The occurrence of ZYMV isolates that have nucleotide sequences similar to the Saudi isolates in these countries may suggest the common origin of distantly distributed isolates, which can be attributed to the widespread seed transmission of this viral isolate as has been reported in previous studies (Tóbiás and Palkovics, 2003; Safaeizadeh, 2008).

In conclusion, this study provided new information regarding the genetic make-up of the natural population of ZYMV isolates infecting cucurbits in Saudi Arabia. It also indicated the relative importance of ZYMV as compared to other viruses that affect cucurbit plants in the country. No substantial biological or molecular differences were observed between the characterized Saudi isolates in spite of the large area in which the study was conducted and the different plant species from which the samples were collected. These isolates did not only show high similarity among themselves but they also showed significant similarity with isolates of ZYMV in some neighboring countries, as well as in distantly located European countries too, suggesting probable transmission of this virus between these countries through transmission methods such as seeds and aphid vectors. Knowledge of the scope of variability in the population of ZYMV isolates intended in this research is not only essential for better understanding of the complexity and epidemiology of the pathogen, but also for designing of potentially effective, better adapted and durable control strategies such as determining resistance gene deployment strategies, as natural resistance genes can be rapidly overcome by adapted virus strains.

Conflict of Interests

The author(s) have not declared any conflict of interests.

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