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ORIGINAL RESEARCH ARTICLE

Serological detection of important pepper viruses and characterisation of pepper mild mottle virus in Saudi Arabia

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Abstract

A total of one-hundred and fourteen *Capsicum annum* L. samples were collected from Qassim and Al-Baha regions, Saudi Arabia in the growing season of 2021–2022, showing virus like symptoms including, mottling, mosaic, chlorosis, leaf distortion, stunted growth, and necrotic lesions, dwarfing of leaves and fruits. These samples were tested against important viruses infecting pepper using ELISA kits. According to ELISA results, 80/114 (70%) samples were found to be infected with one or more of the tested viruses. However, incidence of the PMMoV was observed in 18/21 (85.7%) and 23/59 (38.98%) from Al-Baha and Qassim regions respectively. Based on highly positive ELISA samples of PMMoV three samples were selected for biological detection using mechanical inoculation with sap prepared and the results showed that systemic mosaic, mottling, necrotic spots, yellowing on *N. tabacum, N. benthamiana, N. glutinosa, Solanum nigrum and* necrotic and chlorotic spots on *Chenopodium quinoa* and *C. amranticolor*, while local lesions symptoms were produced on *Datura stramonium.* According to the ELISA results, positive samples from Qassim and Al-Baha regions were amplified by RT-PCR analysis, and 474-bp fragment was amplified using PMMoV specific primers. Two directional sequencing was done for the amplified products and the sequences of the virus isolates were submitted in the NCBI database with the following accession numbers, OP723368, OP723369, OP723370, OP723371, OP723372. The phylogenetic analysis showed the close relationship our isolates with other isolation of PMMoV and pairwise nucleotide sequence identity analysis revealed that these PMMoV isolates shared their identity between 92.5 and 100% with PMMoV isolates available in the databases. This study provides comprehensive report regarding the diagnosis of chili infecting viruses through serological and characterization of PMMoV through serological, biological, and molecular methods in the kingdom of Saudi Arabia.

Keywords Pepper · PMMoV · RT-PCR · Sequencing · Tobamovirus

Introduction

An important spice and vegetable crop, pepper (*Capsicum annuum* L.) is grown all over the world in a variety of climatic and conditions (Kumari et al. 2020). Peppers are economically important crop belonging to nightshade family Solanaceae, originated in Mexico, South Peru, and Bolivia (Greenleaf and Basset 1986; Pickersgill et al. 1979; Sreedhara et al. 2013) and significantly contributes in Saudi Arabian agriculture. Numerous diseases, including bacteria, viruses, and fungus, are hindering pepper production. A significant limiting factor for low productivity and poor fruit quality is viruses (Roberts et al. 2004). About 68 different viral species belonging to different viral groups have been found to infect pepper and cause serious crop losses (Kumari et al. 2020; Pernezny 2003).

Several Tobamovirus species have been found to infect pepper including: pepper mild mottle virus (PMMoV) in the United States (McKinney 1952) and in Italy (Wetter et al. 1984), tobacco mosaic virus (TMV) (Acuña-Fuentes et al. 2022), tomato mosaic virus (ToMV) (Arogundade et al. 2020), tobacco mild green mosaic virus (TMGMV) (Wetter 1986), bell pepper mottle virus (BPeMV) (Wetter et al. 1987), paprika mild mottle virus (PaMMV) (Yordanova and Stoimenova 2008) and obuda pepper virus (ObPV)

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(Matsumoto et al. 2009). Tomato brown rugose fruit virus (ToBRFV) is another tobamovirus which is reported to infect the pepper from different countries such as Iran (Esmaeilzadeh and Koolivand 2022) and Syria and Lebanon (Abou Kubaa et al. 2022). Tomato mottle mosaic virus (ToMMV) is an emerging virus belonging to genus Tobamovirus and which poses serious threats to this crop and cause severe economic losses worldwide (Tu et al. 2021).

PMMoV was first identified in Italy in 1984 (Wetter et al. 1984) belonging to the genus *Tobamovirus*. Since then, this virus and its pathotypes has been intercepted from number of capsicums growing countries that are capable of breaking L allele mediated resistance (Kumari et al. 2020). In Africa it has been reported from Egypt (Othman 1991), Senegal (Huguenot et al. 1996), Zambia (Ndunguru and Kapooria 1996), Tunisia (Mnari-Hattab and Ezzaier 2006), Ethiopia (Sidaros et al. 2009) and South Africa (CABI 2022). In Asia it has been reported from China (Wang et al. 2009), Taiwan (Cheng et al. 2011),Turkey (Çağlar et al. 2013), India (Rialch et al. 2015), Pakistan (Ahmad et al. 2015), Japan (Kato et al. 2018). There was a mixed infection with Tobacco mild green mosaic virus reported from United States in 2018 (Escalante et al. 2018). In Europe PMMoV has been reported from Hungary (Burgyan et al. 1978), Netherlands (Rast 1979), Denmark (Paludan 1982), United Kingdom (Fletcher 1984), Spain (Wetter et al. 1984), Greece (Avgelis 1986), Italy (Betti et al. 1992), Belgium (Verhoyen 1994), Bulgaria (Kostova and Lisa 2003), Czech Republic (Svoboda et al. 2006) and Germany (Menzel et al. 2019) and it has also been reported from United States (Secrist and Ali 2018).

PMMoV causes mild symptoms that can go overlooked in the field and only exhibit themselves during the fruiting stage (Kumari et al. 2020; Rialch et al. 2015). PMMoV is a extremely infectious virus that has the potential to spread through infected seeds, contaminated soil, infected plants, or through agricultural equipments, which increases the danger of an epidemic. PMMoV has a + ssRNA with genome size of 6.4 kb which encodes at least four proteins: a 126-kDa and 183-kDa protein needed for genome replication, as well as 30-kDa movement protein (MP) and 17.5-kDa coat protein (CP), which are produced by sub genomic processes (Yu et al. 2018; Menzel et al. 2019; Rialch et al. 2015).

In Saudi Arabia, pepper production and cultivation is increasing due to its demand and is affected by many pests and diseases including viruses. However, there is no abundant data available about the viruses infecting pepper crop in Saudi Arabia. Some of the viruses infecting pepper crop includes bean yellow mosaic virus (BYMV) (Al-Shahwan et al. 2017), pepper leafroll chlorosis virus (PLRCV) (Kamran et al. 2018), tomato yellow leaf curl virus(TYLCV) (Sohrab and Haq 2020), PMMoV (Al-Wabli Afaf et al. 2017) and alfalfa mosaic virus (AMV) (Moury and Verdin 2012) have been reported of Saudi Arabia. The present study encompasses the diagnosis of 15 viruses infecting pepper crop and detailed biological and molecular characterization of PMMoV, the most prevalent virus.

Materials and methods

Sample collection and serological detection

A total of 114 samples were collected in growing seasons of 2021–2022 from two regions (ninety-three from Qassim and twenty-one from Al-Baha) of Saudi Arabia. Samples from the plants showing mottling, mosaic, chlorosis, leaf distortion, deformed fruits, dwarfing of leaves and fruits. In some plants stunted growth and necrotic lesions were seen. Collected samples were tested against the respective virus and positive samples were used as the source of inoculum PMMoV was maintained in its primary host Pepper. DAS-ELISA was used for the detection of all collected samples according to Clark and Adams (1977). ELISA kits were purchased from two different commercial companies, LOEWE® and DSMZ® from Germany. Complete protocol for performing ELISA was followed as recommended by the manufacturers. Samples were tested against PMMoV, ToBRFV, PepMoV, ChiVMV, PVMV, CaCV, TCSV, PepMV, PVY, PYMV, TBRV, TSWV, ToCV, ToMV and BPeMV.

Biological detection

Healthy seeds of pepper and other hosts of PMMoV including *Nicotiana tabacum, N. banthamiana, N. glutinosa, Solanum nigrum, Chenopodium quinoa, C. amranticolor*, and *Datura stramonium* were grown in the growth chamber. After germination (seven to fourteen days) and the emergence of the first true leaves (ten to fourteen days post emergence), seedlings were transplanted and dusted with carborundum powder. One gram of PMMoV-infected tissues was crushed in 3 ml of potassium phosphate buffer and gently rubbed onto dusted healthy plant leaves. Plants were kept inside the cages to avoid any contamination and inoculated plants were maintained in the growth chamber to observe PMMoV symptoms. The extracted sap obtained from positive samples for PMMoV, homogenized and passed through a double layer of cheesecloth and gently rubbed onto dusted healthy plant leaves to selected herbaceous hosts including *N. glutinosa (*as propagative host), *C. quinoa, C. amranticolor*, *N tabaccum, N. banthamiana, S. nigrum*, and *D. stramonium*. Five plants of each host were inoculated, kept in the greenhouse (25–32 °C) for 2–3

weeks and observed continuously for symptoms expression. Several healthy plants were kept as a control. Symptoms expressions on each species were checked by ELISA, RT-PCR and by back-inoculation to healthy test-plants.

RT-PCR

Based on DAS-ELISA results, a total of 13 and 8 positive samples were selected from Qassim and Al-Baha regions, respectively and total RNA were isolated using Thermo Scientific Gene JET Plant RNA Purification Mini Kit according to the instruction of the manufacturer. Specific primers of each PMMoV-CP-F and PMMoV-CP-R were used to detect PMMoV (Symonds et al. 2018) using one step-RT-PCR kit according to manufacturer's recommendations (Thermo Fisher Scientific Invitrogen's (Invitrogen, Lithuania). Adjusting the One step RT-PCR cycles, 60°C for 10 min and 98°C for 2 min for the synthesis of cDNA followed by 35 cycles, 94°C for 1 min, 55°C for 1 min, 72°C for 1 min and 72°C for 5 min as final extension, respectively (Peng et al. 2015). The amplified PCR products were analysed by agarose gel electrophoresis stained by Acridine Orange hydrochloride in 1% TBE buffer (0.1 M Tris base, 0.5 M Boric acid, and 2 mM EDTA pH 8.0) 50 bp HyperLadder DNA; (Bioline Ltd, USA), and 100 bp DNA Ladder Gene Ruler, Thermo, USA were used to determine the size of RT-PCR products. The results were observed with Gel Documentation System (Syngene Bio Imaging-Ingenius, UK) (Russell and Sambrook 2001).

Partial nucleotide sequencing and phylogenetic analysis

The selected RT-PCR amplified products were sent to Macrogen, South Korea (Macrogen Inc. Seoul, South Korea) for two-directional Sanger sequencing. The obtained sequence results were analysed using BLAST program. Phylogenetic tree was constructed from clustal W-aligned sequences on MEGA-11, using the Maximum-Likelihood method with 1000 boots trap replications (Tamura et al. 2021).

Results

Sample collection and serological studies

Symptomatic pepper samples were collected from different fields in Qassim and Al-Baha regions in Saudi Arabia. Symptomatic leaf samples exhibited mosaic, mottling, leaf narrow, deformation, dark and light green, blistering, and stunted growth (Fig. 1), and symptomatic fruits exhibited irregular necrotic lesions, deformation, blistering and

Table 1 Total number and percentage of samples infected with the tested viruses from the two regions during serological studies. Some samples had mixed infections

Regions	Total tested Samples	Total Infected Samples	Total infection																	
			Tobamovirus				Potyvirus				Tospovirus				Begomovirus	Potexvirus	Crinivirus	Nepovirus		
			PMMoV	BPeMV	ToBRFV	ToMV	PepMoV	ChiVMV	PVMV	PVY	CaCV	TCSV	TSWV	PYMV	PepMV	ToCV	TBRV			
Qassim	93	59	23	0	3	6	12	20	5	3	3	3	0	7	16	2	1			
Al Baha	21	21	18	0	0	0	0	0	0	12	5	0	6	0	0	0	0			
Total	114	80	41	0	3	6	12	20	5	15	8	3	6	3	16	2	1			
Infection %age		70	35.96	0	2.6	5.2	4.3	17.5	4.3	13	7	2.6	5.2	2.6	14	1.7	0.8			

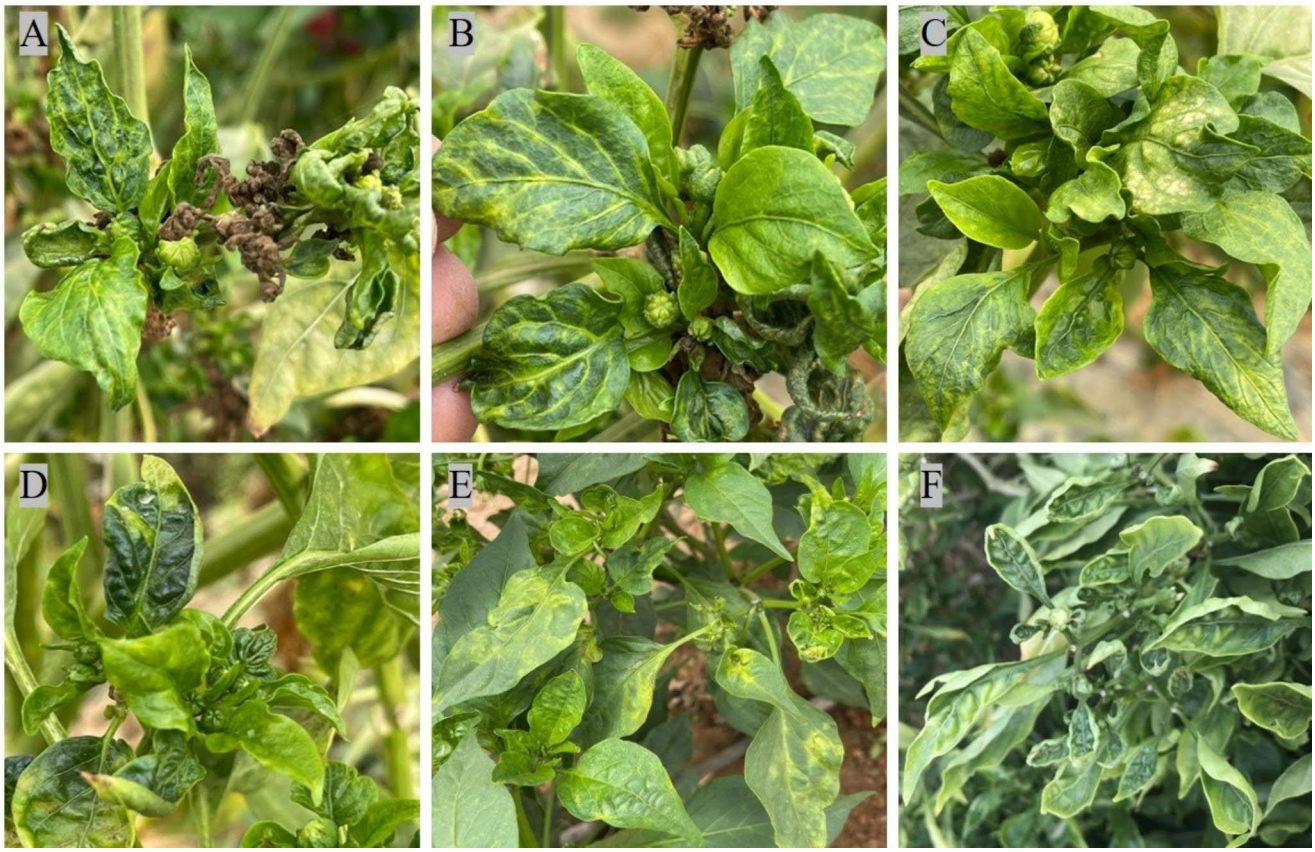

Fig. 1 The symptomatic samples of pepper plants naturally infected in the field with variety of viruses exhibiting variety of different symptoms on leaves including leaf crumpling/blistering and vein yellowing (Panel **A**), mosaic, mottling and vein swelling (Panel **B**), chlorosis and leaf narrow (Panel **C**), leaf blistering and dark and light green (Panel **D**), leaf deformation and reduced size of leaf (Panel **E**), reduced leaf size and upward leaf curling and stunted growth (Panel **F**)

yellow green spots (Fig. 2). For Qassim region, according to the ELISA results, 59/93 (63.44%) samples were infected with thirteen out of fifteen tested viruses including PMMoV, ToBRFV, ToMV, PepMoV, ChiVMV, PVMV, CaCV, TCSV, PepMV, PVY, PYMV, TBRV, ToCV. The BePMV and TSWV could not be detected in samples collected from Qassim region (Table 1). The results show that, 30 out of 59 infected samples (52.38%) were infected with single virus including PMMoV (9 samples), PepMoV (4 samples), ChiVMV (7 samples), PepMV (6 samples), CaCV, PYMV, TCSV, PVY (1 sample each). Meanwhile, 29 samples had mixed infection of at least two viruses from all the the tested viruses (data not shown). For Al-Baha region samples, 21/21 (100%) were found to be positive with only four viruses (PMMoV, CaCV, TSWV, and PVY) out of 15 tested viruses. In Baha region, PMMoV was the most prevalent virus infecting 18 out 21 (85.7%) samples including 4 samples (19%) which were singly infected by PMMoV and other 17 samples (76%) showed mixed infection with two of the tested viruses. BPeMV was the only virus which could not be detected in any sample of both the regions (Table 1).

Host range studies by mechanical inoculation

After fourteen days of inoculation, inoculated plants showed a variety of symptoms. Pepper plants (*C. annum*) showed mosaic and mottling on leaves in the beginning, and it becomes severe as time passed, in case of severe infection, plants showed stunted growth as well. *N. tabacum* showed severe mottling and necrotic spots in the end. *N. benthamiana* and *N. glutinosa* showed yellowing and mosaic pattern with collapsing plants in the end. *S. nigrum* expressed clear necrotic spots right after one week of inoculation. *C. quinoa* and *C. amranticolor* showed necrotic and chlorotic spots respectively while *D. stramonium* shows local lesions which spread, and leaves become yellow (Fig. 3). To confirm the infectivity of inoculated PMMoV, the diagnosis of the virus from systemic leaves of inoculated plants was verified by RT-PCR using PMMoV specific primers (Symonds et al. 2018). Inoculated host plants showed positive results of infectivity showing PCR amplified bands of 474 bp (Fig. 4C).

Fig. 2 Naturally infected pepper plants showing different symptoms on fruits including fruit deformation and reduced fruit size (Panel **A**), blistering and yellow patches on fruit (Panel **B**), irregular necrotic lesions, deformation, blistering and yellow green spots and reduced fruit size (**C**)

Molecular detection of PMMoV using RT-PCR

Highly positive samples obtained with ELISA results were selected and subjected to one-step RT-PCR reaction for partial genome amplification of PMMoV using specific primers. The obtained results show that DNA fragments were visible at the expected size (474 bp) which were run on Acridine Orange hydrochloride stained agarose gel electrophoresis. Thirteen samples from Qassim region and eight samples from Al-Baha region were tested positive with RT-PCR and amplified PMMoV partial genome using specific primers spanning the N-terminal region of CP gene (Fig. 4A & B).

Partial nucleotide sequence and phylogenetic tree analysis

The five selected amplified partial sequences of coat protein gene of PMMoV (2 from Qassim and 3 from Al-Baha regions) were sequenced and the obtained results were analysed using different bioinformatics tools. These sequences were analysed by BLAST in NCBI database and showed their similarity with PMMoV isolated from different countries. Both isolates from Qassim region (OP723368 and

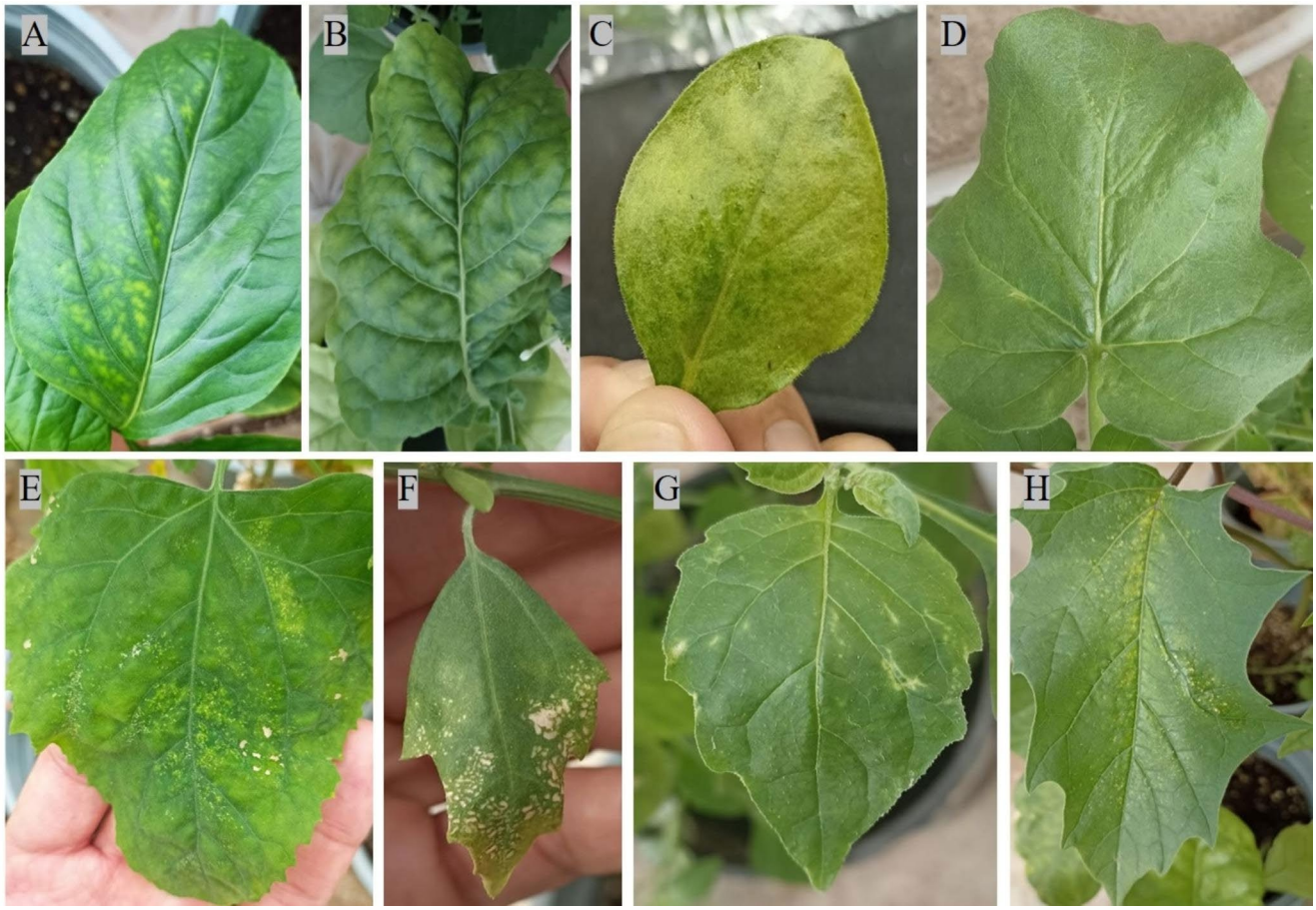

Fig. 3 Sap inoculated plants showing PMMoV symptoms. Sap from pepper plant singly infected with PMMoV was inoculated to a variety of host range plants by rubbing the sap with carborundum and the reactions of host range plants was recorded. The different plants responded differently showing a range of symptoms including yellow spots and vein yellowing in *Capsicum annum***(A)**, leaf crumpling and vein swelling in *Nicotiana tabacum***(B)**, leaf yellowing in *Nicotiana benthamiana***(C)**, asymptomatic to very mild leaf curling in *Nicotiana glutinosa***(D)**, small yellow spots and necrotic lesions in *Solanum nigrum***(E)**, severe necrotic lesions in *Chenopodium quinoa***(F)**, irregular yellow spots in *Chenopodium amranticolor***(G)**, and yellow spots and necrotic lesions in *Datura stramonium***(H)**

OP723369) showed 92.5% similarity with each other while shared 94–98% nucleotide identity with other isolates available in the GenBank. The nucleotide sequence identity for OP723368 was found above 99% with the most isolates found in the database while for OP723369 isolate the highest identity was 94% with Chinese isolates (MG437273, KX524521), USA (MH063882), Pakistan (KT853037), India (MN496153) and Spain (KX063611), while the lowest similarity (93.5%) observed was with four Chinses isolates (MK784568, MF076786, KX524520, KY234295) (Fig. 5). Three isolates of PMMoV were sequenced from from Al-Baha region (OP723370, OP723371 and OP723372) which shared 96–100% nucleotide sequence identity with one another and with other isolates available in the databases. The highest percentage (100%) was observed with Chinese isolates (MG437273, KX524521), USA (MH063882), Pakistan (KT853037), India (MN496153) and Spain (KX063611), while the lowest similarity (99%) observed was with four Chinses isolates (MK784568, MF076786, KX524520, KY234295). The one isolate from Al-Baha (OP723370) showed a little divergence with the highest similarity of 97% with USA isolate (MH063882), while the lowest similarity (96%) was found with all the remaining isolates except USA. The lowest similarity was observed (63.7–67.5%) between PMMoV Saudi isolates with other three different virus isolates represented in the same genus ToMV (Z98201), ToMMV (ON924176) and ToBRFV (MW284988). Phylogenetic tree for PMMoV was constructed from clustal W-aligned sequences in which all the PMMoV isolates are in the same clade while three different isolates of other viruses (ToMV, ToMMV and ToBRFV) were making a different clade (Fig. 6). The Sequence Demarcation Tool (SDT) was used to construct the SDT graph for the explanation of pairwise nucleotide identity percentage (Fig. 5).

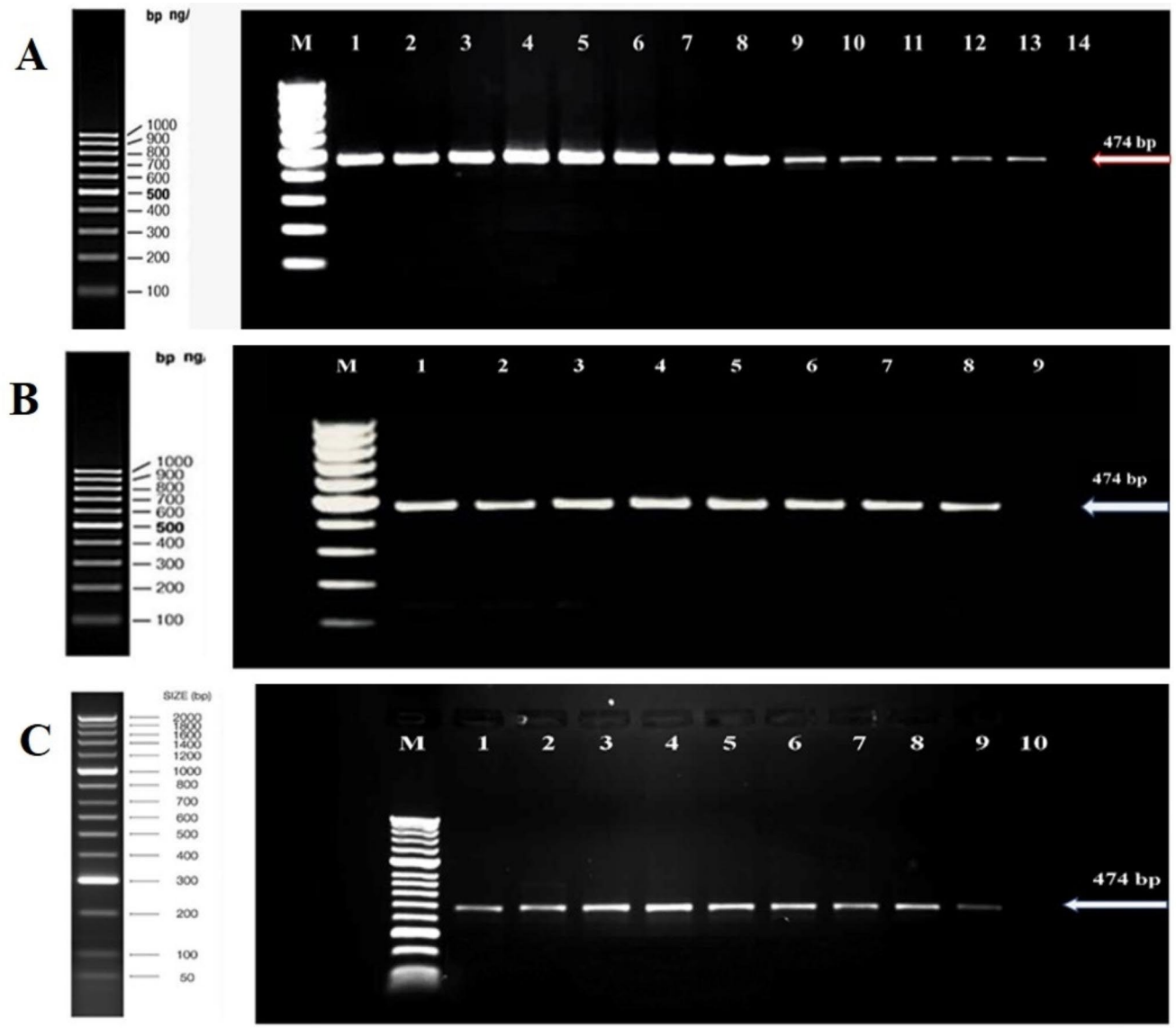

Fig. 4 Agarose gel electrophoresis (1%) showing the RT-PCR amplification of PMMoV using specific primers (474 bp) of selected samples from Qassim region (Panel **A**) lanes 1–13 showing positive samples, lane 14 was a negative control and lane M represent a DNA Ladder (100 bp; Gene ruler marker), selected samples from Al-Baha region (Panel **B**) lanes 1–8 represent positive samples, lane 9 is a negative control and lane M represent a DNA Ladder (100 bp; Gene ruler marker) and Panel **C** is showing the RT-PCR amplification of PMMoV using specific primers (474 bp) from host range plants *C. annum* (lane 1, 2), *N. tabacum* (lane 3), *N. benthamiana* (lane 4), *N. glutinosa* (lane 5), *S. nigrum* (lane 6), *C. quinoa* (lane 7), *C. amranticolor* (lane 8), *D. stramonium* (lane 9), negative control (lane 10) and lane M represent a DNA Ladder (100 bp; Gene ruler marker)

Discussion

Pepper is an economically important crop contributing substantially in Saudi Arabian agriculture due to its higher consumption and demands. Viruses are major threatening and limiting factor for the crop productivity and causing serious losses in pepper production every year in the Kingdom (Kamran et al. 2018) as well as throughout the World. About 68 viruses are known to cause infection in pepper crops worldwide and 20 species are most destructive leading to devastating losses to pepper crop (Kumari et al. 2020; Moury and Verdin 2012). Along with all other viruses, tobamovirus group is threatening the crop due to its rapid spread which can occur through mechanical and seed transmission. Due to its fast spread and sometimes mixed infection with other viruses, it poses major problems towards pepper production. In commercial production, contaminated seeds are the main source of virus outbreaks. For example, stable viruses like tobamoviruses only stay in the seed coat without infecting the embryo and are then transmitted to the seedling after germination (Kaur et al. 2020).

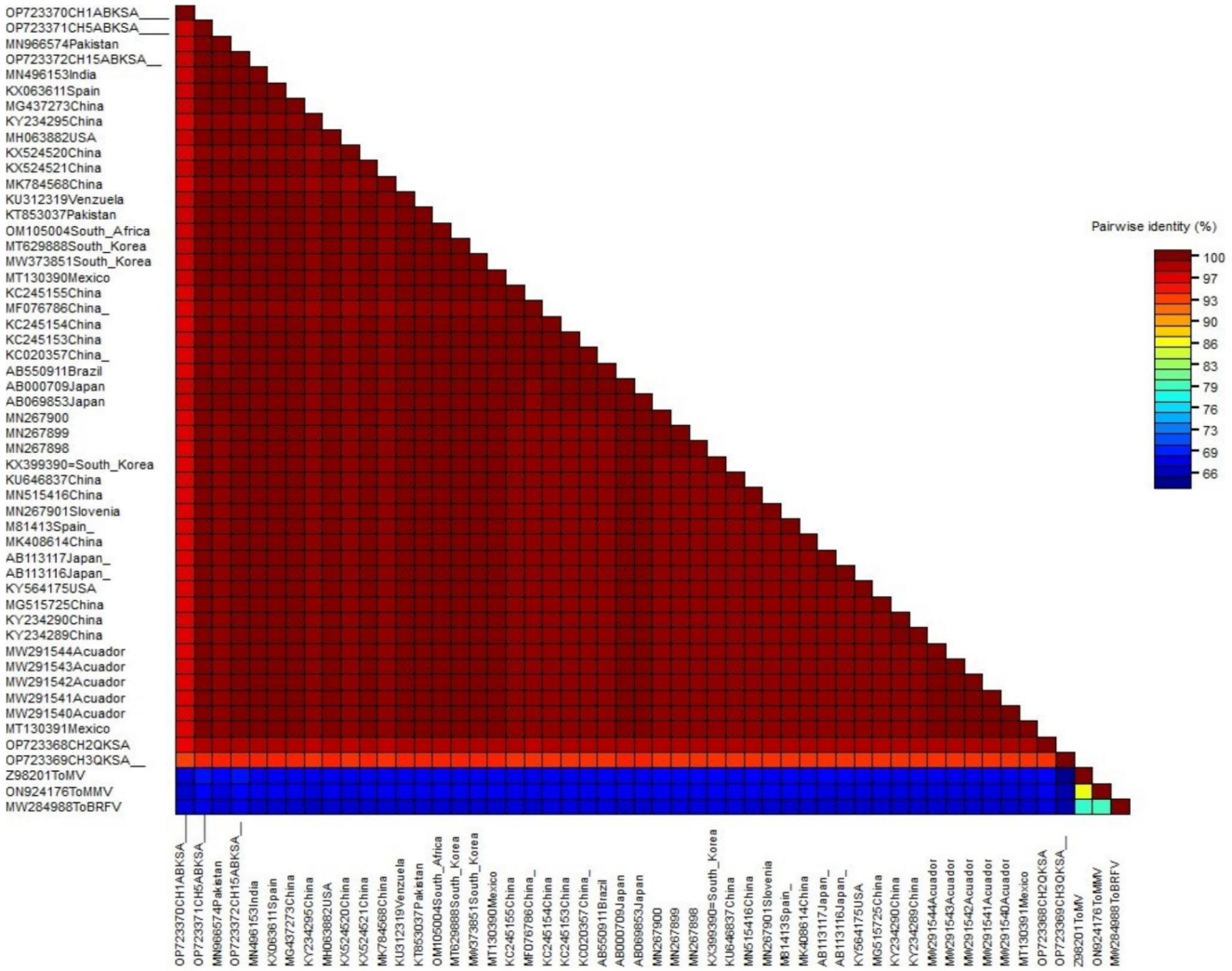

Fig. 5 Distance matrix illustrating the pairwise nucleotide identity percentage of PMMoV Saudi isolates (Two from Qassim (OP723368 and OP723369) and three from Al-Baha (OP723371, OP723372, OP723370) as compared with other most similar sequences. A higher nucleotides sequence identity percentage of our isolate of PMMoV with other isolates of PMMoV are highlighted with red box color. The lower sequence identity percentage of the other unrelated viruses like ToMMV, ToMV and ToBRFV genome were used as an outgroup and highlighted in blue box color

To our knowledge, only five viruses have been reported to infect pepper crop in Saudi Arabia which includes, PLRCV (Kamran et al. 2018), TYLCV (Sohrab and Haq 2020), PMMoV and AMV (Abdalla et al. 2020) and BYMV (Al-Shahwan et al. 2017).

During the study presented here confirmed the infection of fourteen different viruses in pepper crop by serological diagnosis. These viruses include PMMoV, ToBRFV, ToMV, PepMoV, ChiVMV, PVMV, CaCV, TCSV, PepMV, PVY, PYMV, TBRV, ToCV, TBRV belonging to different genera of viruses. The samples were collected from Qassim and Al-Baha regions of Saudi Arabia. Among diagnosed viruses Potyviruses and Tobamoviruses were the most prevailing group of viruses. Some samples showed the infection of Tospoviruses, Potexvirus, Begomovirus, Crinivirus and Nepovirus. Among individual viruses PMMoV was most prevalent infecting 41/114 (35.96%) tested samples and more than 50% positive samples confirmed the presence of PMMoV. TBRV was the least diagnosed virus and was found in only one sample form Qassim region. Concerning diagnosis results of two different regions, the samples collected from Qassim region were 63.44% positive whereas samples from Al-Baha region were 100% positive. Among diagnosed viruses in the regions, it was interesting that Qassim regions samples showed infection of 14 out of 15 tested viruses while Al-Baha regions samples were positive with only 4 out of 15 tested viruses. Those four viruses were PMMoV (Tobamovirus), PVY (Potyvirus), CaCV and TSWV (Tospoviruses).

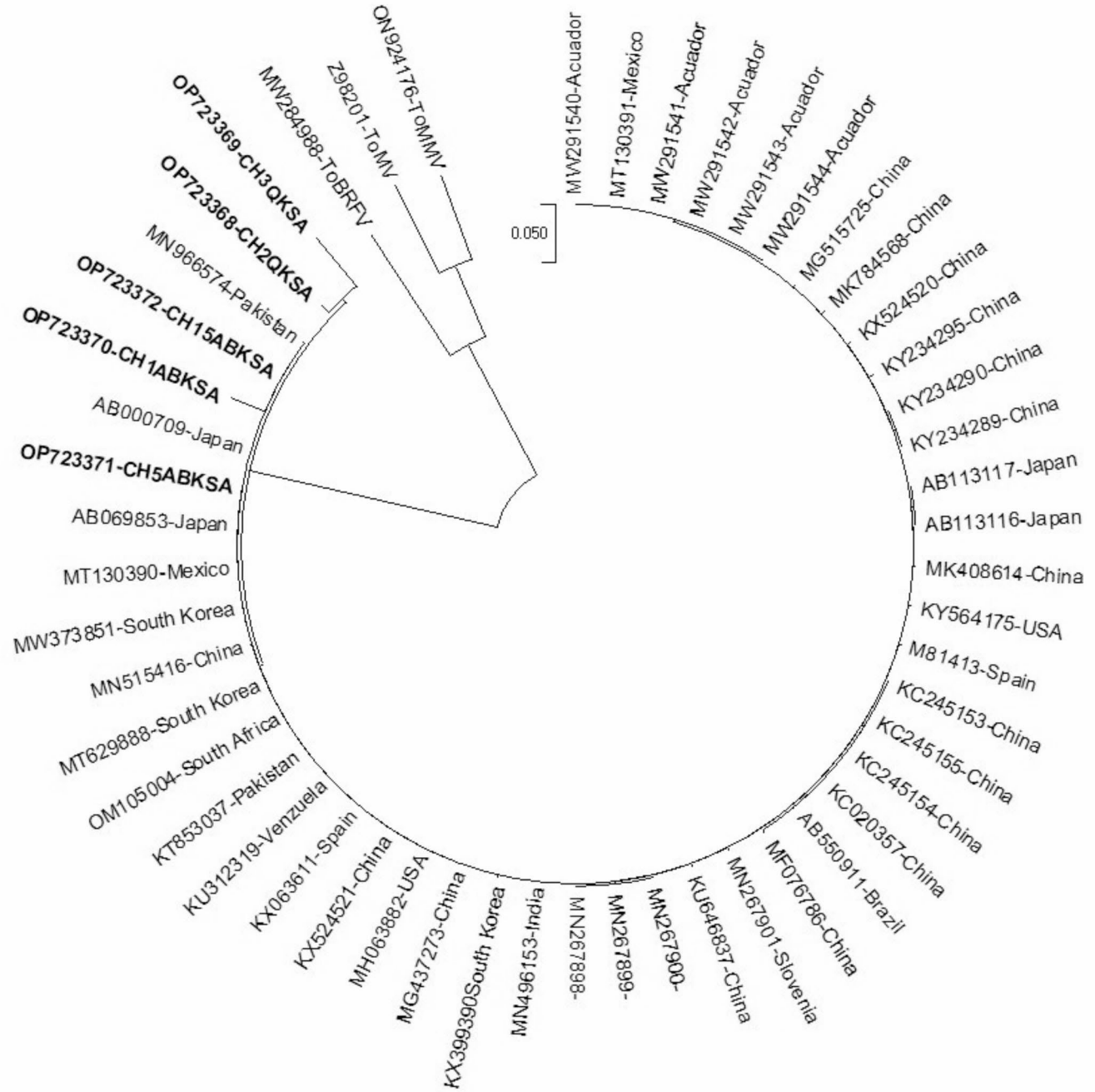

Fig. 6 Phylogenetic tree of Saudi Arabian isolates of PMMoV with other identified isolates worldwide showing grouping KSA isolates with other isolates of PMMoV from Asian countries including Pakistan, China and Japan. This indicates close relationship of our isolates with other isolates of Pepper mild mottle virus

Similar study was conducted by Ayo-John and Odedara (2017) to detect PVMV, ToMV and Cucumber mosaic virus (CMV) in 77 pepper samples from Nigeria using DAS-ELISA. According to their results, infection rate was 67.5%, 3.9% and 19.5% was observed with PVMV, ToMV and CMV respectively (Ayo-John and Odedara 2017). During 2009–2010, another similar study was conducted by Milošević et al. from Serbia to diagnose PMMoV in pepper samples through DAS-ELISA. Out of 239 samples only five samples were positive for PMMoV. In 2009 samples PMMoV was detected in 3 out of 123 samples (2.44%), single infection was reported in only 1 sample while there was mixed infection in 2 samples with CMV and PVY. In samples of 2010 pepper season, in 116 symptomatic samples only 2 were positive (1.72%) with PMMoV (Milošević et al. 2015). Fulton (1984) used immunodiffusion test to

detect four viruses belonging to genus tobamovirus including PMMoV, TMV, PTMV and ToMV from pepper plants (Fulton 1984). Similarly, in Turkey from 421 tested samples of pepper plants by DAS-ELISA the results revealed that CMV (42.2%) was highly distributed followed by TSWV and AMV (17.5%) and PMMoV (10.6%) (Topkaya 2022). Damayanti and Kurniawati (2022) detected PMMoV by dot-immunobinding assay (DIBA) infecting naturally pepper plants from Indonesia using specific antisera of PMMoV (Damayanti and Kurniawati 2022).The focus of our study was Qassim region but there was more infection found of PMMoV in Al-Baha region, where the percentage of total infection was 85.71% while single and mixed infections were 19% and 66.6% respectively, which is highly problematic. Therefore, for the further characterization of PMMoV through molecular and biological methods Al-Baha region was also selected along with Qassim region due to more infection of PMMoV.

For molecular studies thirteen samples were selected on the basis of ELISA results and subjected to RT-PCR detection and 1% agarose gel electrophoresis resulted the presence of amplification of PMMoV bands at specific size (474 bp) according to specific primers amplification. Furthermore, RT-PCR and host range experiment has been used by many scientists for rapid and easy detection of PMMoV from pepper crop, seeds, and water (Haramoto et al. 2013; Milošević et al. 2015; Patel et al. 2023). Patel et al. (2023) detected PMMoV using RT-PCR in three cultivars of pepper including Yolo Wonder, Doux des Landes, and California Wonder with infection percentages of 28.96%, 40.64%, 55.56% respectively (Patel et al. 2023). Similarly, Peng et al. (2015) detected PMMoV from pepper sauce in China using electron microscope. PMMoV was confirmed by RT-PCR using specific primers PMMoV-CP-F and PMMoV-CP-R (Peng et al. 2015). Haramoto et al. (2013) used RT-PCR for the detection of PMMoV from human feces and wastewater from Japan (Haramoto et al. 2013). Because PMMoV has a very high level of resistance against environmental circumstances, it is probably one of the causes for the more frequent occurrence of PMMoV in the water samples examined in their study. Immunocapture reverse transcriptase polymerase chain reaction (IC-RT-PCR) has been used by Mnari-Hattab and Ezzaier (2006) to detect PMMoV from Tunisia (Mnari-Hattab and Ezzaier 2006).

Afterwards the highly positive singly infected samples with PMMoV infection were selected and used in mechanical inoculation for biological studies. It has been reported earlier that this virus is mechanically transmissible (Kumari et al. 2020; Rialch et al. 2015). Our greenhouse studies confirm the presence of this virus in mechanically inoculated hosts through RT-PCR using specific primers of PMMoV.

This work provides a comprehensive and detailed study about the detection of PMMoV infecting pepper crop, a virus belonging the group Tobamovirus through serological, molecular, and biological methods in Saudi Arabia. Primers used in this study successfully amplified CP region fragment of PMMoV which were confirmed by Sanger sequencing. Phylogenetic analysis shows that Saudi Arabian isolates of PMMoV are closely related to Pakistani and Chinese isolates. Complete genome sequencing of PMMoV could offer further information about the evolutionary roots of the virus existence in Saudi Arabia. The difference between both isolates might be the result of natural genetic variation of PMMoV or mutation in CP gene. Phylogenetic tree constructed grouped all isolates of PMMoV from database and both our isolates in one group whereas, other different viruses from the same genus in group second. In host range experiment we come to know after thorough investigation that *Solanum nigrum* is reported to infect pepper plants and becomes the host for PMMoV for the first time in Saudi Arabia. Because PMMoV is extremely stable outside of its host including seeds and soil also along with its primary hosts for longer periods of time, it can be very challenging for growers to control infection when it is established in the field. Growers can remain informed about what pathogen is causing most of the disease from year to year by understanding the spread of PMMoV in symptomatic pepper fields.

Conclusions

This study revealed that PMMoV infection is very severe in both regions which needs to be managed to stop the crop losses. The host range experiment confirmed its presence in different host plants used in this study through RT-PCR. *Solanum nigrum* is found to be a host of PMMoV.

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Authors contribution Conceived the project and designed the studies: M.A.A. and M.A.A.-S.; Sample collection and execution of experiments in the lab: Z.K; M.A.A.; K.H.; and M.A.A.-S.; Analysis of data and drafting of the manuscript: M.A.; M.A A; Critical revision of the manuscript for important intellectual content: M.A.A.; K.H.; M.A.A.-S. and I.M.A.-S. All authors have read and agreed to the published version of the manuscript.

Data Availability Partial genome sequences of two isolates from Qassim and three isolates from Al-Baha using specific primers for PMMoV were submitted to the GenBank under the accession numbers OP723368, OP723369, OP723370, OP723371, and OP723372 respectively.

Declarations

Conflict of interest The authors declare no conflict of interest, and all authors approved the manuscript and agreed to submit for publication.

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