Capsicum annuum, a new host of watermelon mosaic virus

Mohammad Hajizadeh¹ · Kazhal Mohammadi¹

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Abstract The occurrence of Watermelon mosaic virus (WMV) in sweet pepper (*Capsicum annuum* L.) in Kurdistan province, Iran was confirmed by reverse transcriptase-polymerase chain reaction (RT-PCR) and partial characterization of coat protein. To the best of our knowledge, this is the first report of WMV infecting *C. annuum*, adding a new host to list of more than 170 species infected by this virus.

Keywords Sweet pepper · Coat protein · RT-PCR · Recombination

Sweet pepper (*Capsicum annuum* L.) is an edible plant belongs to the family Solanaceae that is a valuable vegetable in Iran. Aphid transmitted viruses, Pepper mottle virus (PeMV), Cucumber mosaic virus (CMV), Zucchini yellow mosaic virus (ZYMV) and Potato virus Y (PVY) are known to affect sweet pepper. However, watermelon mosaic virus (WMV), a aphid transmitted virus that occurs worldwide is not known to infect sweet pepper. WMV has a wide host range and is able to infect cucurbits, some legumes, orchids and many weeds [2]. In the summer 2014, peppers with virus-like symptoms including mosaic, leaf-distortion, mottling, stunting and yellowing were observed in the Kurdistan province of Iran. Twelve symptomatic

pepper samples were collected and tested for the presence of three viruses including CMV, WMV and ZYMV.

Total RNA was extracted from symptomatic leaves as described by [4] and subjected to random primed cDNA synthesis using the HyperScriptTM kit (GeneAll, Seoul, Korea) according to the manufacturer's protocol. PCR was done using specific primer pair CMV (CPf/CPr) [5] and a ~867 bp expected fragment was amplified from one sample (Pe1). Attempts to detect the ZYMV using a primer pair ZYMV-F(+)/ZYMV-R(−) [6] was unsuccessful. Whereas, a ~657 bp expected amplification for WMV with another primer pair WMV-F(+)/WMV-R(−) [6] was detected from three samples (Pe1, Pe8 and Pe18). No DNA fragment was earned from non-symptomatic plant (Fig. 1b). WMV was detected of samples with leaf-distortion and mosaic (Fig. 1a). Nine samples did not result in amplification of any DNA fragment and probably these plants were infected with the other viruses, which incite similar symptoms. Since the WMV was not previously reported from *Capsicum annuum* in worldwide, to confirm the presence of WMV, PCR product from one isolate of WMV (Pe18) was cloned using pTG19 Vector (SinaClon, Iran) and sequenced. The initial BLAST between new isolate Pe18 (GenBank accession no. KP751208) and other Iranian isolates showed the range of nucleotide sequence identity was between 93 and 98 % and amino acid identity range was between 97 and 99 %. Nucleotide sequence identity between our isolate with non-Iranian isolates retrieved from GenBank ranged between 92 and 98 %. In addition, amino acid identity range was between 96 and 99 %.

Previous studies have shown that WMV isolates are phylogenetically classified into three main genogroups called classical (Cl), group-2 (G-2) and emerging group (Em) with four subclades, Em1, Em2, Em3 and Em4 [2].

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✉ Mohammad Hajizadeh
m.hajizadeh@uok.ac.ir

¹ Department of Plant Protection, Faculty of Agriculture, University of Kurdistan, Sanandaj, Iran

Fig. 1 **a** Leaf symptoms of *Watermelon mosaic virus* (WMV) infection in sweet pepper (*Capsicum annuum*) along with non-symptomatic healthy leaf. **b** Electrophoresis of representative RT-PCR detection of WMV using CP specific primers; M = 1 kb DNA ladder; lane 1 template from Pe18 leaf sample; lane 2 template from Pe1 leaf sample; lane 3 template from Pe10 leaf sample; lane 4 template from Pe8 leaf sample; lane 5 template from Pe9 leaf sample; lane 6 template from non-symptomatic leaf sample.

c Neighbor-joining tree obtained on a 657 bp, partial CP fragment of WMV isolates/strains. *Soybean mosaic virus* (SMV) isolate was used as out group member. A bootstrap analysis was applied using 1000 replicates. Bootstrap values higher than 50 are indicated on nodes and the nodes less than 50 were condensed. Each sequence is labelled with isolate/strain name and new isolate Pe18 is indicated by marker (black circle)

Therefore, to investigate the phylogenetic relationship of newly obtained WMV isolates from sweet pepper, the partial CP sequence was compared with 44 WMV isolates representing all genogroups of WMV (Supplementary Table 1). Phylogenetic tree was constructed using MEGA6 (www.megasoftware.net) on the matrices of aligned sequences with 1000 bootstrap replicates following neighbor-joining method.

The tree comprised of six clades, consistent with previously reported WMV genogroups (Fig. 1c). Our WMV isolate (Pe18) from sweet pepper placed between two main clade, classical and G-2, suggested that recombination event may has been occurred in this isolate. Therefore,

recombination analyses were performed in RDP4 with different recombination detection methods [3] with default settings and results agreed with the phylogenetic analysis. Recombination analysis based on three automated methods, Maxchi, SiScan and 3Seq revealed that new isolate Pe18 was parented by local isolate HOR.HA.1 (EU667628) and unknown isolate.

In Iran, since the WMV has been reported from various crops [1]. To the best of our knowledge, this is the first report of WMV infecting *C. annuum* in worldwide, adding a new natural host to the list of more than 170 plant species have been infected naturally and experimentally by this virus.

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