

## LETTER TO THE EDITOR

### Genetic diversity and biological characterization of watermelon mosaic virus isolates from Iran and Iraq

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Approximately 35 viral diseases are associated with cucurbits, at least 10 of which are caused by viruses from the genus *Potyvirus* (the family *Potyviridae*) (1). One of the most important and destructive potyviruses in cucurbits is the watermelon mosaic virus (WMV). With a broad-spectrum of host range - up to 170 the plant species belonging to 27 botanical families (2). WMV was first reported by Webb and Scott in 1965 (3). In 1974, WMV was reported for the first time from Iran (4). No data was available for this virus from Iraq.

The current study aimed to investigate the partial spreading, genetic diversity, and phylogenetic analysis of nine different isolates of WMV from Iran and Iraq and compare them with other WMV isolates available in the GenBank. We used two degenerate primer pairs designed within the conserved amino acids of partial nuclear inclusion protein b (NIB) coding region and a part of the cytoplasmic inclusion protein gene (5, 6). To have a clear view of the biological properties of Iranian and Iraqi WMV isolates, we investigated the partial host range of the virus.

To uncover mixed infection, we used three specific primer pairs to detect other common cucurbits viruses

[e.g. cucumber mosaic virus (CMV), green mottle mosaic virus (GMMV), squash mosaic virus (SqMV)].

The results of RT-PCR amplification with two degenerate primer pairs [cylindrical inclusion (CI), (NIB)] confirmed the infection of 9 (45%) out of 20 samples with WMV deposited in the GenBank database under the accession numbers of MN464239-MN464256. The infection rate with other viruses was 35% for ZYMV and 20% for CMV. Neither squash mosaic virus nor green mottle mosaic virus was detected in the studied samples.

The sequence identity of partial CI gene among four Iraqi isolates was between 92.11 to 99.71% at the nucleic acid level and from 97.41 to 99.14% at amino acid level respectively, whereas the identity among Iranian isolates ranged from 94.55 to 99.14% at nt level and from 98.71 to 100% at aa level, respectively. On the other hand, the identity between Iranian and Iraqi isolates ranged from 91.54 to 99.71% at nt level, whereas it was from 96.98 to 100% at aa levels. These results showed that there was a high variability in the partial sequence of the CI gene at the nt level, even though most of the mutations were silent, indicating a high selection for aa conservation. Having compared the studied isolates with other sources of WMV from the GenBank, we found the highest (99.71%) nt identity in the partial CI gene between Iranian (WMV6) and a French isolate (JF273460). Also, the lowest nt (89.48%) identity was between Iranian (WMV8) and another French isolate (JF273467). Isolates including France JF273460 and Spain (MH469650 and MH469651)

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**Abbreviations:** CI = cylindrical inclusion; NIB = nuclear inclusion protein b; WMV = watermelon mosaic virus

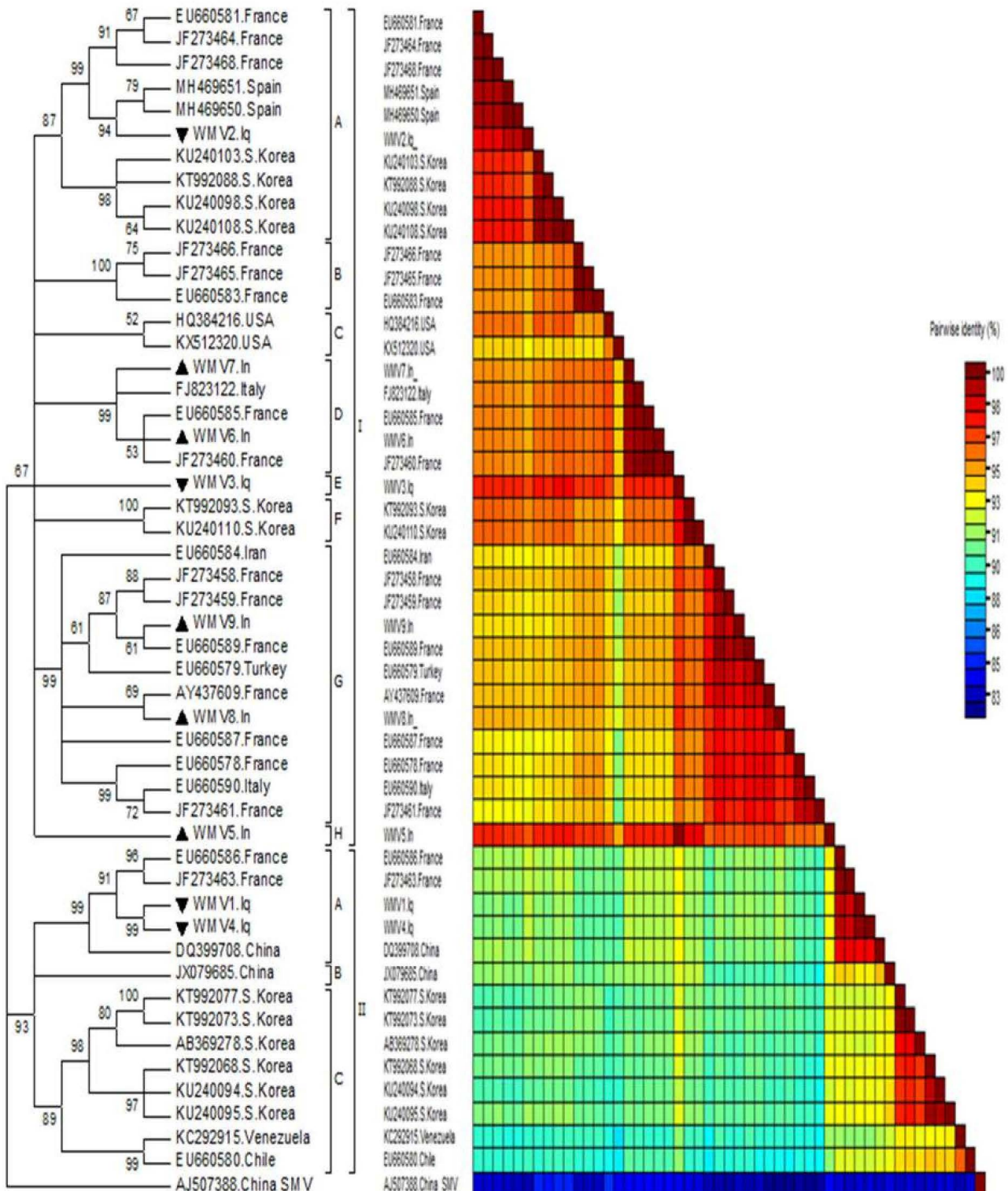


Fig. 1

**The phylogenetic tree of 50 WMV isolates**

Maximum-likelihood phylogenetic tree of 50 WMV isolates constructed based on nucleotide sequences of partial cylindrical inclusion (CI) gene, the Iraqi isolates was marked with ▼ and Iranian isolates with ▲. The tree was constructed using MEGA6 software and bootstrapped with 1000 replicates. Bootstrap values  $\geq 50\%$  are shown at the branch internodes. Two dimensional nucleotide diversity plots constructed based on SDT MUSCLE alignment.

demonstrated the highest level (100%) of identity at aa level with WMV7, WMV3, WMV5, and WMV9 isolates. Isolates WMV9 and KT992077 from South Korea had the lowest level of aa identity (96.81%). The pairwise sequence identity of the partial NIB gene of Iranian and Iraqi isolates showed more identity than the corresponding data from the CI gene.

The phylogenetic analyses based on the partial CI gene with soybean mosaic virus (SMV) isolate (AJ507388) as outgroup showed clustering of the 50 WMV isolates (9 from this study and 41 from the GenBank) into two different evolutionary groups, nominated I and II (Fig. 1). The partial CI gene of Iranian isolates was located in group I, whereas Iraqi isolates were clustered in group I and group II. The phylogenetic analyses of 9 partial sequences of NIB gene plus 41 of their counterparts from the world clustered all of these isolates (Iran and Iraq) in one group (I).

According to the virus isolate and plant species, the interreaction between experimental host plants and the nine studied isolates showed various degrees of symptom intensity. The initial systemic symptoms in most isolates appeared 14 days after inoculation on squash plants, which included varying degrees of mosaic, blistering, vascular clearance, and leaf deformity, while localized chlorotic lesions appeared on *Chenopodium amaranticolor*. Phylogenetic analysis based on partial CI gene clustered Iranian isolates with low genetic diversity in group I, which is in agreement with previous studies (7). The identity between two WMV8 and WMV9 isolates from Mazandaran province (North of Iran) was 97.42% whereas it was 99.14% between two isolates from Jimabad (WMV6) and Torbat Heydariyeh (WMV7) (Khorasan Razavi, Northeast of Iran). The identity of the isolates from two Iranian provinces (Mazandaran and Khorasan Razavi) (WMV6 and WMV8) was 94.55%, indicating the adaptation of Iranian isolates. The lowest identity of 92.11% was detected between two Iraqi isolates WMV1 (Wasit) and WMV2 (Najaf), while the two other Iraqi isolates from Baghdad (WMV1 and WMV2) showed 93.11% identity suggesting the potential exposure of propagated materials with different geographical origins improved through genetic drift (8, 9). Furthermore, the abundance of overwintering weeds and other WMV hosts may play an important role in the efficiency of virus-vector inter-

action. It may also impose genetic barriers to gene flow or recombination between isolates that would explain the separation of Iraqi isolates between two CI-based phylogenetic groups. In addition, the highest and lowest identities between the studied and the GenBank isolates showed that the two genes (CI, NIB) were more similar to specific and geographically scattered isolates. A mixed infection among different world isolates in the studied areas may be an explanation for the appearance of variable symptoms in laboratory test plants. The main reason for developing this high level of diversity was probably the new open trade system for goods and crops between Iraq and the rest of the world associated with the lack of appropriate quarantine measures. On the other hand, all of the studied isolates showed a low level of diversity in the partial NIB gene and obviously appeared in the NIB-based phylogenetic tree in one group (I), alongside with other isolates. The most probable explanation for this is the functional or selective constraint of the NIB gene, which acts as a replication-associated protein.

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