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LETTER TO THE EDITOR

Characterization and complete nucleotide sequence of potato virus M isolated from tomato in China

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Potato virus M (PVM) (the genus *Carlavirus*) is one of the most widespread and economically important pathogens of potato worldwide. PVM was firstly reported by Schultz and Folsom and can be found in nearly all the potato-growing areas in the world (*1*). The PVM-infection of potato plants was associated with many symptoms, such as mottling, mosaic patterning, crinkling and rolling of the leaves and stunting of shoots, which can cause a yield reduction of about 15–45%, depending on the potato cultivars and environmental conditions (*2–4*). PVM is transmitted by aphids in a non-persistent manner, or by mechanical inoculation with sap from young leaves, but not from older leaves (*2*). Some species of plants, such as *Datura metel* and *Gomphrena globosa,* can be used as indicators of PVM, which could display local lesions in these plants (*5*). So far, only one partial nucleotide sequence is available from tomato plant in Italy. Here, we firstly reported and determined the complete nucleotide sequence of PVM isolated from tomato in Gansu province (PVM-Gansu), China.

Tomato fields were surveyed during September 2011 in different areas of China. Symptomatic leaves showed curling and yellow mosaic. Forty-six symptomatic leaf samples and symptomless samples were collected from Qingquan, Zhangyi, Yumen, Gulang and Linyi districts. The leaf samples were stored at -80°C. All fresh leaf samples were tested by DAS-ELISA (*6*) according to the manufacturer′s instructions of PVM ELISA kit (Agdia). Total RNA was extracted from 100 mg of tomato leaves using Trizol (Invitrogen) and was used as template for the amplification of first-strand cDNA. The tomato samples positive in DAS-ELISA were confirmed by RT-PCR with a pair of primers specific for CP gene (CP-F: 5′-TAAGGTAAATCTGAAATAGTG-3′, CP-R: 5′-GCCACCCTGGTTACGTGCTT-3′). In order to amplify the full-length genomic sequence of PVM, eight pairs of primers were designed (PVM-F1: 5′-TAAACAAACAT ACAATATCTGG-3′, PVM-R1: 5′- TTCCTTCCGCGCG TTAAG-3′; PVM-F2: 5′-CCTGGTGGAACATT TGGG-3′, PVM-R2: 5′-TGCCAGTATGGCGGCATG-3′; PVM-F3: 5′-TTGCATAGGTCATGAATGCT-3′, PVM-R3: 5′-ACGCTCGTTCATCCCAAC-3′; PVM-F4: 5′-GAGAAATATGCGTGAGAAATTTG-3′, PVM-R4: 5′-CTTTTCTTCACGGCCATT AG-3′; PVM- F5: 5′-GAGGTGGTGATGCAGGA-3′, PVM-R5: 5′-CACA TTCTCTCTA GCAC-3′; PVM-F6: 5′-GGTGATGAA TTCATATGCTTTG-3′, PVM-R6: 5′-GCAAGCTCAA TGCTTCCAAC-3′; PVM-F7: 5′-GCTGGTGTAGAGGA CCAAC-3′, PVM-R7: 5′-ACGGCCTGTTGTACGGATT-3′; PVM-F8: 5′-TAAGGTAAATCTGAAATAGTG-3′, PVM-R8: 3′-RACE). The 5′ untranslated region (5′-UTR) was amplified by the specific primers (5′-TAAACAAACATACAATATC

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Abbreviations: CP = coat protein; DAS-ELISA = double-antibody sandwich ELISA; PVM = potato virus M; 3′-RACE = 3′ rapid amplification of cDNA ends

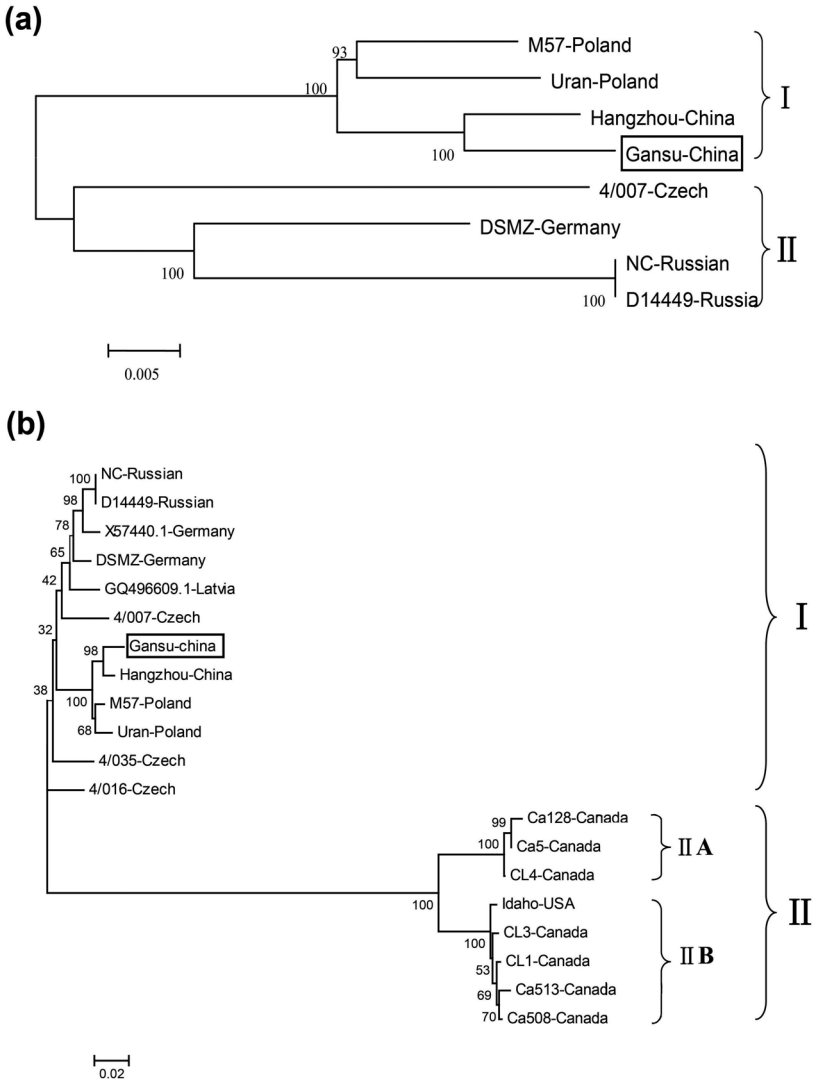

Fig. 1

TGG-3′), which were designed based on the conserved UTR region of reported PVM isolates. The sequences of the 3′-terminal region were determined using a rapid amplification of cDNA ends kit (3′-full RACE Core Set Ver.2.0) (TaKaRa). RT-PCR was performed using the procedures described previously (*7*). PCR products were purified using a PCR purification kit (Axygen), and the resulting fragments were inserted into pMD18-T vector (TaKaRa). After *Escherichia coli* DH5a was transformed with the recombinant DNA, positive clones were identified by colony PCR and sequenced. The sequences were compared with other PVM sequences published in GenBank using DNAMAN. A phylogenetic tree was derived using the neighbour-joining method (*8*) with 1,000 bootstrap replicates in the MEGA software (*8, 9*).

The detection result of DAS-ELISA showed that only one symptomless tomato plant of all plants tested was positive for PVM, which was further confirmed by RT-PCR. The result was similar to those observed by Flatken *et al.* (*4*) that tomato could be systemically infected by PVM without obvious symptoms. PVM infection of tomato was probably the result of PVM transmission from a nearby pepino plant, on which PVM can induce conspicuous mosaic symptom. According to our knowledge, this is the first report of PVM isolated from tomato in China. The complete nucleotide sequence of PVM-Gansu was determined. It consists of 8520 nts, excluding the poly (A) tail at the 3′ terminus and has been submitted to GenBank (Acc. No. JN835299). PVM-Gansu contains six ORFs, like other PVM isolates reported previously (*10*). ORF1 to ORF6 encode polypeptides of 1964,

229, 109, 63, 304, and 114 amino acids, respectively. ORF1 encodes a 223 kDa polypeptide involved in virus replication (RNA-dependent RNA polymerase) (*11–13*). ORF2, ORF3, and ORF4 encode three putative polypeptides of 25 kDa, 12 kDa, and 7 kDa respectively, named triple gene block proteins, which are known to function in spread of the virus from cell to cell (*14–16*). ORF5 and ORF6 encode putative polypeptides of 34 kDa (coat protein, CP) and 11 kDa (nucleic acid binding protein) respectively (*17*).

Sequence analysis demonstrated that the identity of the whole genomic sequence of PVM-Gansu and the seven other PVM isolates downloaded from GenBank ranged from 91.65% to 98.10%. The PVM-Gansu shared the highest nucleotide identity (98.10%) with the PVM-Hangzhou (Acc. No. AJ43748) isolated from pepino (*Solanum muricatum*) in China and the lowest identity (91.65%) with the PVM-4/007 (Acc. No. HM854296) isolated from potato in the Czech Republic. The low identity may be the result of about 20nts deletion in both terminal regions of the genome of the PVM-4/007 isolate.

Phylogenetic analysis of the full-length PVM genomic sequences suggested that eight PVM isolates cluster into two groups (Fig.1a). In order to further understand the genetic relationship between the PVM-Gansu and the other PVM isolates, nineteen PVM isolates from different regions were selected to construct a phylogenetic tree based on CP gene sequences. The results showed that the twenty PVM isolates could be divided into two different groups, group I and II (Fig. 1b). PVM-Gansu formed the group I together with some isolates from Russia, Germany, China, Latvia, the Czech Republic and Poland, which indicated that isolates belonging to this group were distributed in some areas of Europe and Asia. In contrast, group II contained eight isolates from Canada and USA distributed across North America. Group II could be further divided into two subgroups, IIA and IIB. The PVM isolates that clustered into group II were all from Canada and USA, it was therefore suggested that the PVM isolates in group II might have the same origin.

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References

1. Schultz E, Folsom D, J. Agric. Res. 25, 43–118, 1923.
2. Xu HM, Aubin JD, Nie JB, Virol. J. 7, 25, 2010. http://dx.doi.org/10.1186/1743-422X-7-25
3. Ruiz de Galarreta JI, Carrasco A, Salazar A, Barrena I, Iturritxa E, Marquinez R, Legorburu FJ, Ritter E, Potato Res. 41, 57–68, 1998. http://dx.doi.org/10.1007/BF02360262
4. Flatken S, Ungewickell V, Menzel W, Maiss E, Arch. Virol. 153, 1385–1389, 2008. http://dx.doi.org/10.1007/s00705-008-0127-5
5. Hiruki C, Phytopathology 60, 739–740, 1970. http://dx.doi.org/10.1094/Phyto-60-739
6. Clark MF, Adams AN, J. Gen. Virol. 34, 475–83, 1977. http://dx.doi.org/10.1099/0022-1317-34-3-475
7. He Z, Jiang DM, Liu AQ, Sang LW, Li WF, Li SF, Virus Genes 42, 440–443, 2011. http://dx.doi.org/10.1007/s11262-011-0592-x
8. Saitou N, Nei M, Mol. Biol. Evol. 4, 406–425, 1987.
9. Tamura K, Dudley J, Nei M, Kumar, Mol. Biol. Evol. 24, 1596–1599, 2007. http://dx.doi.org/10.1093/molbev/msm092
10. Zavriev SK, Kanyuka KV, Levay KE, J. Gen. Virol. 72, 9–14, 1991. http://dx.doi.org/10.1099/0022-1317-72-1-9
11. Ju HJ, Samuels TD, Wang YS, Blancaflor E, Payton M, Mitra R, Krishnamurthy K, Nelson RS, Plant Physiol. 138, 1877–1895, 2005. http://dx.doi.org/10.1104/pp.105.066019
12. Koonin EV, Dolja VV, Crit. Rev. Biochem. Mol. Biol. 28, 375–430, 1993. http://dx.doi.org/10.3109/10409239309078440
13. Rozanov MN, Koonin EV, Gorbalenya, AE, J. Gen. Virol. 73, 2129–2134, 1992. http://dx.doi.org/10.1099/0022-1317-73-8-2129
14. Hiroko S, Yasuyuki Y, Nami M, Takuya S, Kensaku M, Masayoshi H, Chihiro M, Yutato N, Shigetou N, J. Virol. 85, 10269–10278, 2011. http://dx.doi.org/10.1128/JVI.05273-11
15. Kryldakov R, Akbergenov R, Hohn T, Iskakov B, J. Cell. Mol. Biol. 9, 15–20, 2011.
16. Morozov SY, Miroshnichenko NA, Solovyev AG, Zelenina DA, Fedorkin ON, Lukasheva LI, Grachev SA, Chernov BK, Virology 183, 782–785, 1991. http://dx.doi.org/10.1016/0042-6822(91)91011-5
17. Gramstat A, Courtpzanis A, Rhode W, FEBS Lett. 276, 34–38, 1990. http://dx.doi.org/10.1016/0014-5793(90)80500-I