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LETTER TO THE EDITOR

Characterization of tomato aspermy virus isolated from chrysanthemum and elucidation of its complete nucleotide sequence

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Tomato aspermy virus (TAV) (the genus *Cucumovirus*, the family *Bromoviridae*) is of great economical importance worldwide. The first isolation of TAV was reported by Blencowe and Caldwell in 1949 (*1*). In China, TAV was first isolated by Ma *et al.* from beans in 1983 (*2*). TAV can infect more than 100 crop varieties of 24 dicotyledonous families and three monocotyledonous families (*3*). TAV-infected plants develop a number of symptoms, such as mottled leaves, flower distortion and depression of ray florets and chlorotic ring mosaics on leaves (*4–6*). TAV is spread by aphids in a non-persistent manner and it is transmissible also by mechanical inoculation (*7*). Some species of plants develop local lesions indicative of TAV infection, such as *Nicotiana glutinosa* and *Lycopersicum esculentum* (*8*). TAV could be found in nearly all chrysanthemum-growing areas in the world (*9–10*). TAV infects different varieties of chrysanthemum (*Chrysanthemum morifolium*) and causes flower dwarfism and deformations in cultivated chrysanthemum. For these reasons, surveillance of TAV infection in chrysanthemum is necessary to control TAV-related diseases. So far, only one partial nucleotide sequence is available from chrysanthemum in Korea (GenBank Acc. Nos. AJ320273, AJ320274, AJ237849). Here, we report and determine the complete nucleotide sequence of TAV isolated from chrysanthemum within the Henan province (TAV-HN) of China.

Chrysanthemum fields were surveyed during June to October 2012 in different areas of China. Symptomatic leaves showed chlorotic ring mosaic. One hundred and twenty-two symptomatic and symptomless leaf samples were collected from Jiaozuo city in Henan, Kaifeng city in Henan, Harbin city in Heilongjiang, Guangzhou city in Guangdong, Kunming city in Yunnan, Chengdu city in Sichuan, Wuxi city in Jiangsu, Xi'an in Shannxi and Beijing suburbs. The leaf samples were stored at -80°C. All of the fresh leaf samples were tested by DAS-ELISA, according to the manufacturer's instructions (Tomato aspermy virus ELISA kit, Agdia**,** USA). Total RNA was extracted from 100 mg of chrysanthemum leaves using TRIzol (Life Technologies, USA) and was used as a template for first strand cDNA synthesis. The positive chrysanthemum samples detected by DAS-ELISA were confirmed by RT-PCR with a pair of specific primers for the TAV CP gene (TAV-CP-F 5'-ATGGCCCAAAACGGTACG-3'; TAV-CP-R 5'-TCACMCSGGRRGCGTTGAAG -3'). TAV is a positive-sense single-stranded RNA (+ssRNA) virus with a tripartite genome, designated RNA1, 2, and 3 in decreasing order of their sizes. In order to amplify the complete genomic sequence of TAV-HN, five primer pairs were designed according to the conservative regions of TAV RNA1, 2, 3

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Abbreviations: TAV = tomato aspermy virus; CP = coat protein; ORF(s) = open reading frame(es)

sequence of TAV-Beijing (Acc. Nos. HQ424163, HQ424164, HQ424165) and TAV-KC (Acc. Nos. AJ320273, AJ320274, AJ237849) available in the GenBank database. The primer sets for RNA1 amplification are: TAV-F-1-1 (5'-GTTTGTCTAT CAAGAGC -3') and TAV-R-1-1 (5'-AAAAGTGACAAAT GTTGATAGTT-3', with the annealing temperature 56°C); TAV-F-1-2 (5'-TTTTGCTTCATTAACGCGCC-3') and TAV-R-1-2 (5'-TGGGACCCCTAGG-3', with the annealing temperature 56°C). The primer sets for RNA2 amplification are: TAV-F-2-1 (5'-GTTTGTCTATCAAGAGC-3') and TAV-R-2-1 (5'-CACTGAAGTCTTTGATCTCTA GAG-3', with the annealing temperature 50°C), TAV-F-2-2 (5'-CGCTGTTTCAACGCTTTCAACG-3') and TAV-R-2-2 (5'-TGGGACCCCTAGG-3', with the annealing temperature 60°C). The primer sets for RNA3 amplification are: TAV-F-3 (5'-GTTTACCAACCAACC-3') and TAV-R-3 (5'-TGGGACCCCTAGG-3', with the annealing temperature 56°C). RT-PCR was performed using the procedures described previously (*11*). The PCR reaction conditions were as follows: 94°C for 5 min, followed by 35 cycles of 30 sec at 94°C, 45 sec at 50–60°C (specific annealing temperature for each primer), 90 sec at 72°C, and a final extension for 10 min at 72°C. PCR products were purified using a PCR purification kit (Axygen, USA), and the resulting fragments were inserted into the pMD18-T vector (TaKaRa, Japan). After *Escherichia coli* JM109 cells were transformed with the recombinant DNA and positive clones were identified by colony PCR and sequenced using an automated DNA sequencer (ABIPRISMTM 3730XL DNA Analyzer). The nucleotide (nt) and deduced amino acid (aa) sequences were compared with other TAV sequences published in GenBank using DNAMAN6.0 (Lynnon Biosoft, Canada). A nucleotide-based phylogenetic tree was derived using the neighbor-joining method (*12*) with 1,000 bootstrap replicates found in the Molecular Evolutionary Genetics Analysis (MEGA) software (version 6.0) (*13*).

The DAS-ELISA revealed that fourteen symptomatic samples from Kaifeng, nine symptomatic samples from Jiaozuo, and seven symptomatic samples from the Beijing suburbs displayed a positive signal. These results were further confirmed by RT-PCR. According to our knowledge, this is the first report to isolate TAV from chrysanthemum in China. The isolated TAV strain from chrysanthemum within Henan province was named TAV-HN. The complete nucleotide sequence of TAV-HN was also determined in this study *via* the race method. TAV-HN RNA1 contains 3410 nts, TAV-HN RNA2 contains 3096 nts, TAV-HN RNA3 contains 2222 nts, and each of these sequences have been

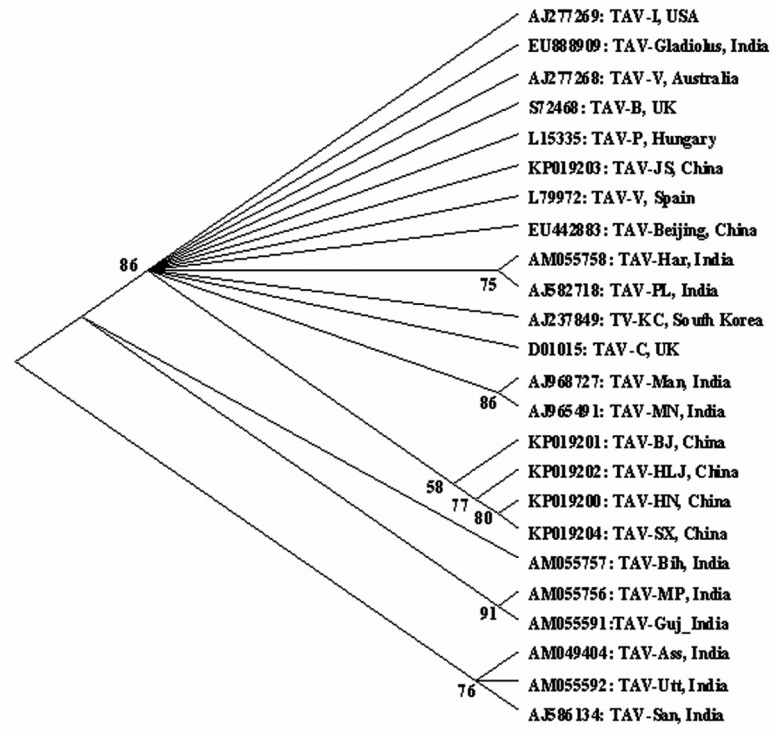

Fig. 1
Phylogenetic analysis based on CP gene nucleotide sequences of TAV-HN (Acc. No. KP019200) isolate, other four TAV isolates from different regions of China and nineteen TAV isolates available in the GenBank database
The dendrogram was generated by the MEGA tool v 6.0. using the Neighbor-joining method with 1,000 replicate bootstraps.

submitted to GenBank (Acc. Nos. KF432413, KF432414, KF432415, respectively). TAV-HN contains five open reading frames (ORFs) like other previously reported TAV isolates. ORF1 to ORF5 encode polypeptides of 994, 759, 105, 247 and 218 amino acids, respectively. ORF1 in TAV-HN RNA1 encodes a RNA helicase and methyltransferase with a putative polypeptide of 111 K (*14–15*). ORF2 in TAV-HN RNA2 encodes a 99 K RNA-dependent RNA polymerase (*15–16*). ORF3 in TAV-HN RNA2 encodes the amino acid sequence that is involved in targeting to the nucleus within the host plant (*17*). This sequence may also allow for adaptation to various host plants *via* suppression of host gene silencing factors that are involved in pathogenic resistance (*18*). ORF4 and ORF5 in TAV-HN RNA3 encode the 3a protein and coat protein, respectively, that are involved in viral movement and in synthesis of subgenomic RNA (*19–21*).

Sequence analysis demonstrated that the nucleotide similarity of the whole genomic sequence between TAV-HN (RNA1, RNA2 and RNA3) and the other three TAV (RNA1, RNA2 and RNA3) sequences previously reported ranged from 98.1% to 98.6%, 95.2% to 95.8% and 94.0% to 98.4%. TAV-HN shared the highest nucleotide identity with TAV-KC isolates (Acc. Nos. AJ320273, AJ320274 and AJ237849) from chrysanthemum in Korea and shared the lowest nucleotide identities with TAV-Beijing isolates from tomato (GenBank Acc. Nos. HQ424163, HQ424164 and HQ424165) in China. In order to further understand the genetic relationship between TAV-HN and other TAV isolates, CP gene nucleotide sequences of five TAV isolates from different regions of China were cloned, and each of these sequences have been submitted to GenBank (GenBank Acc. Nos. TAV-HN, KP019200; TAV-BJ, KP019201; TAV-HLJ, KP019202; TAV-JS, KP019203; TAV-SX, KP019204). Sequence analysis demonstrated that the nucleotide similarity of the CP gene between TAV-HN and the other TAV CP isolates from different regions of China ranged from 91.0% to 99.2%. TAV-HN, other four TAV isolates from different regions of China and nineteen TAV isolates available in the GenBank database were selected to construct a phylogenetic tree based on CP gene nucleotide sequences (Fig. 1). Nucleotide sequences of TAV-HN CP were found to be closely related with TAV-SX (Acc. No. KP019204), TAV-HLJ (Acc. No. KP019202), and TAV-BJ (Acc. No. KP019201) isolates from China, TAV-MN (Acc. No. AJ965491) and TAV-Man (Acc. No. AJ968727) isolates from India, but far related with TAV-JS (Acc. No. KP019203) from China, and another isolates from somewhere else of India (GenBank Acc. Nos. AM055757, AM055756, AM055591, and etc.), USA (Acc. No. AJ277269), Australia (Acc. No. AJ277268), UK (Acc. No. S72468), Hungary (Acc. No. L15335), and Spain (Acc. No. L79972).

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References

1. Blencowe JW, Caldwell J, Ann. Appl. Biol. 36, 320–326, 1949. http://dx.doi.org/10.1111/j.1744-7348.1949.tb06928.x
2. Ma DF, Qiu BS, Tian B, Hu WZ, Zhang ZF, Zhang CL, Song SM, J. Plant Prot. 10, 215–217, 1983.
3. Shi W, Jin ST, Zhang HF, Wang T, Chen JS, Liao QS, J. Agri. Biotechnol. 6, 1120–1126, 2011.
4. Gupta RP, Singh BP, Indian J. Mycol. Pl. Pathol. 11, 158–160, 1981.
5. Raj SK, Kumar S, Choudhary S, Australas. Plant Dis. Notes. 2, 1–2, 2007. http://dx.doi.org/10.1071/DN07001
6. Verma N, Kumar K, Kulshrestha S, Raikhy G, Hallan V, Ram R, Zaidi AA, Garg I D, Arch. Phytopathol. Plant Prot. 42, 99–111, 2009. http://dx.doi.org/10.1080/03235400600951779
7. Lawson R, Virology 32, 357–362, 1967. http://dx.doi.org/10.1016/0042-6822(67)90286-3
8. Shu XZ, Zhu SF, Hu WZ, Lu JY, Shen SL, Wang SQ, Chen YF, Virol. Sin. 2, 186–192, 1990.
9. Blencowe J W, Caldwell J, Ann. Appl. Biol. 36, 320–326, 1949. http://dx.doi.org/10.1111/j.1744-7348.1949.tb06928.x
10. Shi BJ, Li YY, Zhyang CL, Hua RM, Acta Phtypathol. Scin. 23, 231–241, 1993.
11. He Z, Jiang DM, Liu AQ, Sang LW, Li WF, Li SF, Virus Gene 42, 440-443, 2011. http://dx.doi.org/10.1007/s11262-011-0592-x
12. Saitou N, Nei M, Mol. Biol. Evol. 4, 406–425, 1987.
13. Tamura K, Stecher G, Peterson D, Filipski A, Kumar S, Mol. Biol. Evol. 30, 2725–2729, 2013. http://dx.doi.org/10.1093/molbev/mst197
14. Koonin EV, Dolja, Jack Morris, Crit. Rev. Biochem. Mol. Biol. 28, 375–430, 1993. http://dx.doi.org/10.3109/10409239309078440
15. Choi SK, Choi JK, Ryu KH, Park WM, Mol. Cells 13, 52–60, 2002.
16. Moriones E, Roossinck MJ, Gracia AF, J. Gen. Virol. 72, 779–783, 1991. http://dx.doi.org/10.1099/0022-1317-72-4-779
17. Lucy AP, Guo HS, Li WX, Ding SW, EMBO. J. 19, 1672–1680, 2000. http://dx.doi.org/10.1093/emboj/19.7.1672
18. Ding S, Li W, Symons RH, EMBO. J. 14, 5762–5772, 1995.
19. Boccard F, Baulcombe D, Virology 193, 563–578, 1993. http://dx.doi.org/10.1006/viro.1993.1165
20. Smirnyagina E, Hsu YH, Chau N, Ahliquist P, Virology 198, 427–436, 1994. http://dx.doi.org/10.1006/viro.1994.1054
21. Moreno IM, Bernal JJ, Garcia-de Blas B, Rodriguez-Cerezo E, García-Arenal F, Mol. Plant. Microb. Int. 10, 171–179, 1997. http://dx.doi.org/10.1094/MPMI.1997.10.2.171