Bangladesh J. Bot. **52**(2): 569-574, 2023 (June) Special DOI: https://doi.org/10.3329/bjb.v52i20.68222

BROAD BEAN WILT VIRUS 2 IN *COMMELINA COMMUNIS* L. IN CHINA

SHUANG GANG*, SHIRUI ZHANG, SHUNYAN ZHANG, JIA SUN AND CAIXIA YANG

Liaoning Key Laboratory of Urban Integrated Pest Management and Ecological Security, College of Life Science and Engineering, Shenyang University, Shenyang 110044, China

Keywords: Fabavirus, Broad bean wilt virus 2, Molecular characterization

Abstract

In the present study transcriptone sequencing and RT-PCR detection of leaf sample of *Commelina communis* L. were investigated. A diseased leaf sample was selected for transcriptome sequencing and two contigs with lengths of 5845 and 3450 nucleotides (nt) were obtained. They were confirmed by RT-PCR with seven pairs of primers, and RNA1 and RNA2 (GenBank MZ571836 and MZ571837) were determined to be 5880 nt and 3478 nt, respectively. Homology analysis and evolutionary relationship analysis showed the highest homology with BBWV2 (MN786954 and MN786955), for 97.5 and 88.4% at the nt and amino acid (aa) levels, respectively. This is the first report of BBWV2 infection in *C. communis* in the world. Specific primers R2-2F/R were used to assess the association of BBWV2 with *C. communis* disease, and positive fragments were obtained only in the diseased plants, indicating the association of BBWV2 with the disease.

Introduction

Broad bean wilt virus 2 (BBWV2) is a well-known economically harmful virus in the genus *Fabavirus*, belonging to *Secoviridae*, causing great loss to vegetables, ornamental and medicinal plants worldwide (Kwak *et al.* 2013, Svoboda *et al.* 2013, Thompson *et al.* 2017, Wei and Li 2017, Rui *et al.* 2019). Its genome consists of two single-stranded RNA molecules: RNA1 and RNA2, which are 6.0 and 3.6 kb, respectively. RNA1 encodes a polymeric protein that is further processed into protease cofactor, helicase, protease, NTP-binding proteins (NTP), and RNA-dependent RNA polymerase (RdRP). RNA2 encodes a multi-protein that is further processed to form movement protein (MP) and capsid proteins (large coat protein: LCP and small coat protein: SCP) (Thompson *et al.* 2017). In China, BBWV2 occurs in a variety of plants, such as eggplant, pepper, soybean and sesame in the past years (Wang *et al.* 2017, Wei and Li 2017, Rui *et al.* 2019, Li *et al.* 2020, He *et al.* 2021). However, there have been no reports of BBWV2 occurrence in *Commelina communis*, which is an abundant and widely distributed weed that occurs all over the world. In China, it is also well known for its edible and medicinal value (Zhang *et al.* 2018). *C. communis* was naturally infected by brome mosaic virus and cucumber mosaic virus in Fayetteville and northern Florida in the USA, respectively (Valverde 1983, Kucharek *et al.* 1998), but has not yet been reported to be infected by a viral agent in China. In June 2018, *C. communis* showed different degrees of yellow mosaic symptoms that were observed in Shenyang City, Liaoning Province (Fig. 1). To provide more molecular data about the agent of the disease, transcriptome sequencing, and RT-PCR detection were carried out. A new isolate of BBWV2 from *C. communis* plants are reported for the first time.

Materials and Methods

In June 2018, more than 80% of *C. communis* plants showed different degrees of yellow mosaic symptoms in Dadong district, Shenyang City, Liaoning Province (Fig. 1). The diseased leaves were collected and stored in an ultra-low temperature refrigerator at −80°C.

*Author for correspondence: <gang_shuang@163.com>.

A sample with diseased symptoms was selected for RNA extraction. The qualified RNA was then constructed in a transcriptome database without rRNA using NEBNext Ultra™ RNA Library Prep Kit for Illumina (NEB, USA) and sequenced using Illumina Novaseq6000 of Berry Genomics Corporation (Beijing, China). Raw reads were processed by removing low-quality reads, and then all clean reads were obtained and assembled to longer contigs with CLC Genomics Workbench 9.5 (QIAGEN, Valencia, CA, USA) and Velvet 1.2.10 (Zerbino and Birney 2008). The BLASTn or BLASTx program in the National Center for Biotechnology Information (NCBI) database was used to search for contigs that may be the source of the virus. The transcriptome data were marked for reference to design primers specific for BBWV2 RNA1 and RNA2. Seven pairs of primers were used to amplify the whole BBWV2 genome. The sequences and locations of the primers are shown in Table 1.

Fig. 1. Yellow mosaic symptoms on *Commelina communis*

Another diseased sample was used for RNA extraction using the RNASimple Total RNA Kit (DP419), and then cDNA was synthesized using the TIANScript II RT Kit (KR107-01) from Tiangen Biotech (Beijing) Co., Ltd. Seven pairs of primers were used to amplify the whole genome of the virus, and the PCR products were detected on 1% agarose gel. Seven expected fragments were obtained and purified using a TIANgel Purification Kit (DP219-02) from Tiangen Biotech (Beijing) Co., Ltd. The recovered products were connected to the pMD-18T clone vector with a DNA ligation Kit Ver.2.1 (6022) from Takara Biomedical Technology (Beijing) Co., Ltd and then transferred into competent cells of the DH5α strain (CB101-02) from Tiangen Biotech (Beijing) Co., Ltd. The positive fragments were sent to Sangon Bioengineering (Shanghai) Co., Ltd for sequencing. Viral genomic organization was analyzed using the ORF Finder program. A homology comparison was performed on the amino acid sequence analysis of the viral *cp* gene. A Neighbor-joining phylogenetic tree based on the RNA 2 polyprotein was constructed using Mega

X (Kumar *et al*. 2018). To assess the correlation between BBWV2 and *C. communis* disease, another nine diseased plants and two healthy plants were detected with the specific primers R2-2F/R.

Table 1. Primers for the amplification of BBWV2 RNA 1 and RNA 2.

Clone	Primer name	Nucleotide sequence 5'→3'	Position/nt	Product size/bp
RNA1	R1-1F	GGATCCAAATATTAAAACAAACAGCT	1-20	1700
	R1-1R	GAAGCTCCCGTAAAGAATATC	1672-1692	
	R1-2F	GATATTCTTTACGGGAGCTTC	1672-1692	1700
	R1-2R	CATGTCACACTCCCAAGCTCAG	3405-3426	
	R1-3F	CTGAGCTTGGGAGTGTGACATG	3405-3426	1700
	R1-3R	TGTATTGCAATTATTGATGTAAG	5085-5107	
	R1-4F	CTTACATCAATAATTGCAATACA	5085-5107	800
	M4	GTTTTCCCAGTCACGAC		
RNA2	R2-1F	GGATCC CAGTCACTAAACAGCTTTC	1-19	1600
	R2-1R	GAATGCTTGCATAGCCACTGAAC	1636-1658	
	R2-2F	GTTCAGTGGCTATGCAAGCATTC	1636-1658	1600
	R2-2R	GAATCTTGTAGAACTTCTTGCTC	3187-3209	
	R2-3F	GAGCAAGAAGTTCTACAAGATTC	3187-3209	350
	M4	GTTTTCCCAGTCACGAC		
Reverse	M4(18T)	GTTTTCCCAGTCACGAC(T)18		

Results and Discussion

Qualified RNA with an $OD_{260/280}$ of 2.14, $OD_{260/230}$ of 2.01, and RIN of 8.20 was obtained, and then a transcriptome database without rRNA was constructed. Raw reads were filtered to remove low-quality reads, contaminated joints and those with high unknown base N content, to obtain 55,585,454 clean reads. All reads were assembled to obtain two contigs with lengths of 5845 and 3450 nt. BLASTN or BLASTX searching based on the contigs showed that they were close to BBWV2 (MN786954 and MN786955), the genus *Fabavirus* of the family *Secoviridae*.

Four primers for RNA1 and three primers for RNA2 were used to amplify specific fragments of BBWV2, and seven expected fragments were obtained (Table 1). Positive fragments were all cloned, sequenced, and assembled, which were finally determined to be 5880 bp (GenBank MZ571836) and 3478 bp (GenBank MZ571837). RNA1 encoded a polyprotein located at nt 150–5756 (1868 aa) and contained a conserved catalytic core domain (nt 4238–5173, cl40470, E-value 3.06e-172) of RdRp, an RNA_helicase domain (nt 1673–1981, pfam00910, E-value 1.56e-32), and a Peptidase_C3 superfamily (nt 3473–3595, cl02893, E-value 4.14e-06). RNA2 encoded a polyprotein located at nt 161–3355 of RNA2 (1064 aa) containing the Como_LCP superfamily (nt 1562–2686, cl03497, E-vale 0e+00) and Como_SCP superfamily (nt 2912–3289, cl03498, E-value 1.27e-06). A homology comparison was performed on the amino acid sequence analysis of the viral *cp* gene, and it had the highest homology (97.5%) with BBWV2 (MN786955) from *Chenopodium album* L in Liaoning, China. It showed 96.5% homology with BBWV2-[SK:AD:Leonurus] (KM076649) and 95.8% homology with Xinjiang Pepper isolate BBWV2-[CN:XJ:Pepper] (HQ283390) (Table 2). The Neighbor-joining tree based on the RNA2

polyprotein aa sequence identity showed that it was also closest to BBWV2 (MN786955) (Fig.2). In the assessment, all nine diseased plants obtained positive fragments, while the two healthy plants were negative. The results indicated an association between BBWV2 and the disease.

Table 2. Pairwise percent identities sequences between *Commelina communis* BBWV2 isolate and the most closely related viruses.

Accession number	Virus	Acronym	Identity *cp*[a]	Identity *cp*[b]
HQ283390	*Broad bean wilt virus* 2-[China: Xinjiang: pepper]	BBWV2-[CN:XJ:pepper]	95.8	84.9
GQ202215	*Broad bean wilt virus* 2-[China:Rehmannia glutinosa]	BBWV2-[CN:Rehmannia]	94.3	83.0
JF704084	*Broad bean wilt virus* 2-[South Korea: paprika]	BBWV2-[SK:paprika]	95.3	80.7
JQ855708	*Broad bean wilt virus* 2-[China: Xinjiang: tomato]	BBWV2-[CN:XJ:tom]	94.0	80.9
KC110085	*Broad bean wilt virus* 2-[China: Shanxi: Atractylodes macrocephala Koidz]	BBWV2-[CN:SX:Atractylodes]	93.3	84.2
KC625507	*Broad bean wilt virus* 2-[South Korea: Vicia faba]	BBWV2-[SK:Vicia faba]	95.2	81.1
KC625511	*Broad bean wilt virus* 2-[South Korea: paprika]	BBWV2-[SK: paprika]	95.3	81.1
KC625513	*Broad bean wilt virus* 2-[South Korea: Pisum sativum]	BBWV2-[SK:Pisum]	95.5	81.4
KC625515	*Broad bean wilt virus* 2-[South Korea: paprika]	BBWV2-[SK: paprika]	95.7	81.8
KC625518	*Broad bean wilt virus* 2-[South Korea: Spinacia oleracea]	BBWV2-[SK:Spinacia]	95.5	81.4
KF498697	*Broad bean wilt virus* 2-[China: Shanxi: Capsicum annuum]	BBWV2-[CN:Sx:Capsicum]	95.7	85.9
KJ789137	*Broad bean wilt virus* 2-[China: Shandong: Dioscorea opposita]	BBWV2-[CN:SD:Dioscorea]	93.3	81.7
KJ825857	*Broad bean wilt virus* 2-[China: Hunan: chilli]	BBWV2-[CN:Hu:Chi]	94.8	81.4
KM076649	*Broad bean wilt virus* 2-[South Korea: Andong: Leonurus sibiricus]	BBWV2-[SK:AD:Leonurus]	96.5	84.6
KT246496	*Broad bean wilt virus* 2-[South Korea: Dioscorea opposita]	BBWV2-[SK:Dioscorea]	93.5	82.1
KT380021	*Broad bean wilt virus* 2-[South Korea: paprika]	BBWV2-[SK:paprika]	95.0	81.4
KT380023	*Broad bean wilt virus* 2-[South Korea: Capsicum annuum]	BBWV2-[SK:Capsicum]	94.7	81.2
KU309314	*Broad bean wilt virus* 2-[South Korea: Andong: Dioscorea oppositifolia]	BBWV2-[SK:AD:Dioscorea]	94.1	81.8
KX234810	*Broad bean wilt virus* 2-[South Korea: Andong: Dioscorea]	BBWV2-[SK:AD:Dioscorea]	94.1	81.8
KX686590	*Broad bean wilt virus* 2-[South Korea: Gynura procumbens]	BBWV2-[SK: Gynura]	93.8	80.6
KY606993	*Broad bean wilt virus* 2-[China: Anhui: Vicia faba L.]	BBWV2-[CN:AH:Via]	95.5	80.5
LC497425	*Broad bean wilt virus* 2-[South Korea: Gangwon-do: Gynura procumbens]	BBWV2-[SK:GD: Gynura]	93.8	81.0
MH447989	*Broad bean wilt virus* 2-[South Korea: Gangwon-do: Achyranthes bidentata]	BBWV2-[SK:GD:Achyranthes]	93.5	81.9
MH645160	*Broad bean wilt virus* 2-[United Kingdom: Ullucus tuberosus]	BBWV2-[UK:Ullucus]	94.7	81.4
MK118749	*Broad bean wilt virus* 2-[China: Liaoning: Sesamum indicum]	BBWV2-[CN:LN:Sesamum]	95.2	84.4
MN786955	*Broad bean wilt virus* 2-[China: Liaoning: *Chenopodium album* L]	BBWV2-[CN:LN:*Chenopodium album*]	97.5	88.4

[a] Nucleotide sequence identity, [b] Amino acid sequence identity.

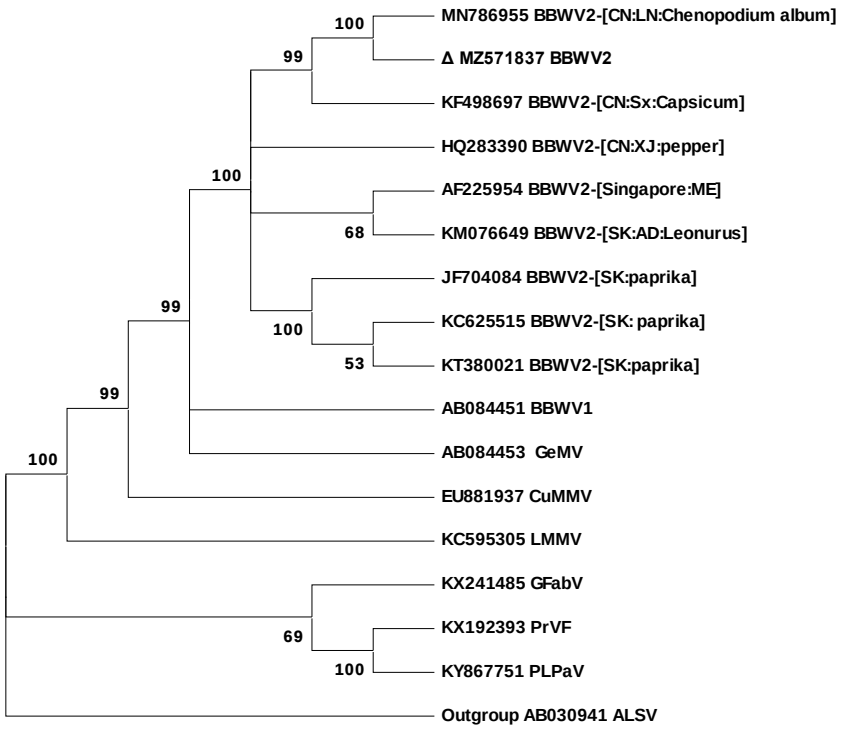

Fig. 2 Phylogenetic tree based on the RNA2 polyprotein amino acid sequences of 16 fabaviruses. The viruses from eight species of the genus *Fabavirus* are as follows: broad bean wilt virus 2 (BBWV2, AF225954, MN786955, KF498697, HQ283390, AF225954, KM076649, JF704084, KC625515, KT380021) , broad bean wilt virus 1 (BBWV1, AB084451), Grapevine fabavirus (GFabV, KX241485), gentian mosaic virus (GeMV, AB084453), cucurbit mild mosaic virus (CuMMV, EU881937), Lamium mild mosaic virus (LMMV, KC595305), peach leaf pitting-associated virus (PLPaV, KY867751), Prunus virus F (PrVF, KX192393), apple latent spherical virus (ASLV, AB030941).

Literature review suggested that, this is the first report of BBWV2 naturally occurring on *C. communis* in the world. The field test indicated an association of BBWV2 with the disease, but it could not be concluded that BBWV2 was the pathogen causing the disease without verification by Koch's postulates. In addition to vegetables and ornamental plants, a growing number of more widely distributed weeds have also been found to be natural hosts of BBWV2, indicating that weeds play an important role in the epidemic process of this disease. More than 80% of weeds showing typical symptoms of viral infection indicate a possible widespread occurrence and prevalence of BBWV2. Further studies are needed to investigate the influence of *C. communis* as an intermediate host of the BBWV2.

Acknowledgments

This work was financially supported by the Support Program for the Enterprise Project postdoctoral support of Liaoning Province. The authors would like to thank Dr. Mengji Cao of Southwest University for the transcriptome analysis.

References

He Z, Dong Z, Qin L and Gan H 2021. Phylodynamics and Codon Usage Pattern Analysis of broad bean wilt virus 2. Viruses **13**(2): 198.

Li L, Yang CX, Hou QS, Wang JZ and Yu MC 2020. Identification of broad bean wilt virus 2 on *Chenopodium album* L. in Liaoning. J. Shenyang Agricul. Univ. **51**(04): 446-453.

Kucharek TA, Purcifull DE, Christie RG and Perkins KD 1998. The association of severe epidemics of cucumber mosaic in commercial fields of pepper and tobacco in North Florida with inoculum in *Commelina benghalensis* and *C. communis*. Plant Dis. **82**(10): 1172.

Kwak HR, Kim MK, Nam M, Kim JS, Kim KH, Cha B and Choi HS 2013. Genetic compositions of broad bean wilt virus 2 infecting red pepper in Korea. Plant Pathol. J. **29**(3): 274-284.

Rui PH, Jiang L, Li S, Jiang XZ, Zhao QQ, Feng JY and Jiang T 2019. First report of broad bean wilt virus 2 infection in eggplant in China. J. Plant Pathol. **102**: 543.

Kumar S, Stecher G, Li M, Knyaz C and Tamura K 2018. MEGA X: Molecular evolutionary genetics analysis across computing platforms. Mol. Biol. Evol. **35**(6): 1547-1549.

Svoboda J and Leisova-Svobodova L 2013. First report of broad bean wilt virus2 in pepper in the Czech Republic. Plant Dis. **97**(9): 1261.

Thompson JR, Dasgupta I, Fuchs M, Iwanami T, Karasev AV, Petrzik K, Sanfaçon H, Tzanetakis I, van der Vlugt R, Wetzel T and Yoshikawa N, Ictv report consortium 2017. ICTV Virus Taxonomy Profile: *Secoviridae*. J. Gen. Viro. **98**(4): 529-531.

Valverde RA 1983. Brome mosaic virus isolates naturally infecting *Commelina diffusa* and *C. communis*. Plant Dis. **67**(11): 1194-1196.

Wei MS and Li GF 2017. First report of broad bean wilt virus 2 and turnip mosaic virus in *Tropaeolum majus* in China. Plant Dis. **101**(7): 1332

Wang HY, Zhao M, Li YQ, Yan ZY, Tang W, Gong HH and Li XD 2017. First report of broad bean wilt virus*2* infecting sesame in China. Plant Dis. **101**(8): 1556

Zerbino DR and Birney E 2008. Velvet: algorithms for de novo short read assembly using de Bruijn graphs. Genome Res. **18**: 821-829.

Zhang X, Liang CJ, Li CL, Bu MD, Bu LY, Xiao YD, Sun HG and Zhang LT 2018. Simultaneous Qualitative and quantitative study of main compounds in *Commelina communis* Linn. by UHPLC–Q-TOF-MS-MS and HPLC–ESI-MS-MS. J. Chromato. Sci. **56**(7): 582-594.

(Manuscript received on 03 March, 2023; revised on 12 May, 2023)