



Short communication

A determinant of disease symptom severity is located in RNA2 of broad bean wilt virus 2



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ABSTRACT

Broad bean wilt virus 2 (BBWV2), which belongs to the genus *Fabavirus*, is a destructive pathogen of many economically important horticultural and ornamental crops. In this study, we constructed infectious full-length cDNA clones of two distinct isolates of BBWV2 under control of the *cauliflower mosaic virus* 35S promoter. BBWV2-PAP1 isolated from paprika (*Capsicum annuum* var. *gulosum*) induces severe disease symptoms in various pepper varieties, whereas BBWV2-RP1 isolated from red pepper (*Capsicum annuum* L.) causes mild symptoms. Agrobacterium-mediated inoculation of the infectious cDNA clones of BBWV2-PAP1 and RP1 resulted in the same symptoms as the original virus isolates. The infectious cDNA clones of BBWV2-PAP1 and RP1 were used to examine the symptoms induced by pseudorecombinants between the two isolates to localize in which of the two genomic RNAs are the symptom severity determinants in BBWV2. The pseudorecombinant of RP1-RNA1 and PAP1-RNA2 induced severe symptoms, similar to those caused by the parental isolate PAP1, whereas the pseudorecombinant of PAP1-RNA1 and RP1-RNA2 induced mild symptoms, similar to those caused by the parental isolate RP1. Our results suggest that BBWV2 RNA2 contains a symptom determinant(s) capable of enhancing symptom severity.

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Broad bean wilt virus 2 (BBWV2), a member of the genus *Fabavirus* in the family *Secoviridae*, infects a wide range of plants, many of which are important agricultural and horticultural crops (Ferrer et al., 2011; Kwak et al., 2013a). BBWV2 is easily transmitted by aphids, mostly *Aphis gossypii* and *Myzus persicae*, in a non-persistent manner. The BBWV2 genome is composed of two single-stranded positive-sense RNA molecules, RNA1 and RNA2, which are encapsulated separately into icosahedral virions (Atsumi et al., 2013; Ferrer et al., 2011). BBWV2 RNA1 and RNA2 are approximately 5960 and 3600 nucleotides (nt) in length, respectively. The 5' end of each is linked to viral genome-linked protein (VPg), and the 3' end is polyadenylated. Each RNA segment contains a single open reading frame (ORF) that is translated into a single polyprotein precursor. The polyprotein precursor encoded by BBWV2 RNA1 is processed by proteolytic cleavage to yield five mature proteins: protease cofactor (Co-Pro), NTP-binding motif (NTBM), VPg, protease (Pro), and RNA-dependent RNA polymerase (RdRp). The polyprotein precursor encoded by RNA2 is processed

to yield three mature proteins: movement protein (MP), large coat protein (LCP), and small coat protein (SCP) (Ferrer et al., 2011; Kwak et al., 2013a). BBWV2 causes various symptoms, including wilt, vein clearing, mottling, necrosis, and mosaic, depending on the virus isolate and the host plant. Recently, an infectious cDNA clone of a BBWV2 isolate from gentian was constructed and its infectivity was investigated in various host plants (Atsumi et al., 2013), but the viral pathogenic determinants of BBWV2 have not yet been identified.

In our previous studies, we collected isolates of BBWV2 from six different crop plants in field surveys performed throughout Korea from 2006 to 2012, and determined the full genome sequences of 14 isolates to examine genetic diversity in the Korean BBWV2 population (Kwak et al., 2013a,b). From this BBWV2 collection, we selected two isolates, PAP1 and RP1, to construct infectious cDNA clones. The isolates PAP1 and RP1 differ clearly in inducing disease symptoms in pepper plants: BBWV2-PAP1 isolated from paprika (*Capsicum annuum* var. *gulosum*) in 2010 induces severe disease symptoms (severe mosaic, yellowish vein clearing, leaf malformation) in various pepper varieties, whereas BBWV2-RP1 isolated from red pepper (*Capsicum annuum* L.) in 2007 causes mild symptoms (mild mosaic) (Fig. 1 and Table 1).

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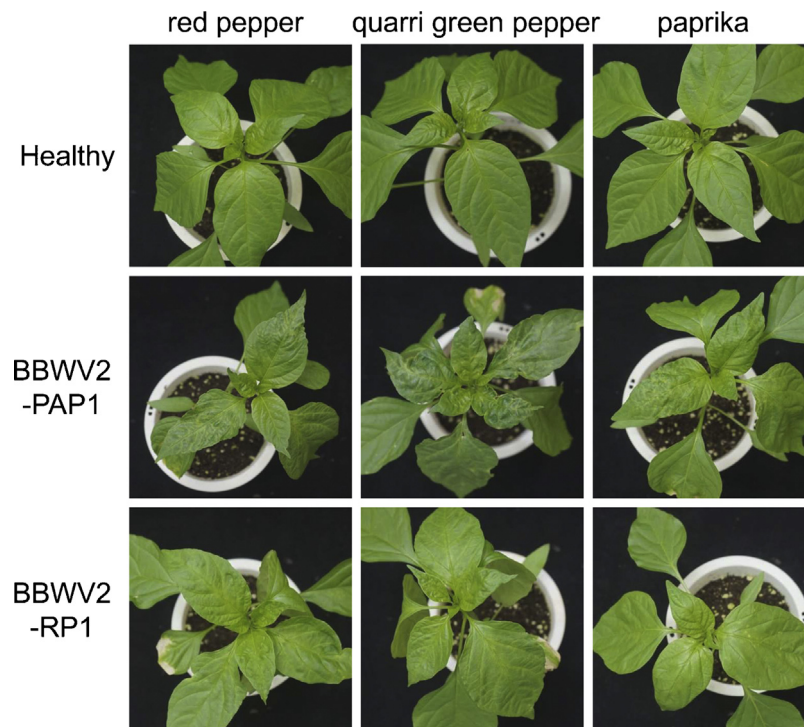


Fig. 1. Symptoms on pepper plants infected with BBWV2 isolates PAP1 or RP1. BBWV2-PAP1 induces severe disease symptoms (severe mosaic, yellowish vein clearing, leaf malformation) in three pepper varieties, including red pepper, quarri green pepper, and paprika, whereas BBWV2-RP1 causes mild symptoms (mild mosaic).

Table 1
Symptoms on host plants inoculated with two distinct BBWV2 isolates and their pseudorecombinants.

Host plants	Inoculum ^a					
	vBBWV2-PAP1	vBBWV2-RP1	pBBWV2-PAP1-R1 + pBBWV2-PAP1-R2	pBBWV2-RP1-R1 + pBBWV2-RP1-R2	pBBWV2-PAP1-R1 + pBBWV2-RP1-R2	pBBWV2-RP1-R1 + pBBWV2-PAP1-R2
<i>Nicotiana benthamiana</i>	SM, St ^b	MM	SM, St	MM	MM	SM, St
<i>Capsicum annuum</i> L. (red pepper)	SM, YVC, LM	MM	SM, YVC, LM	MM	MM	SM, YVC, LM
<i>C. annuum</i> (quarri green pepper)	SM, YVC, LM	MM	SM, YVC, LM	MM	MM	SM, YVC, LM
<i>C. annuum</i> var. <i>angulosum</i> (paprika)	SM, YVC, LM	MM	SM, YVC, LM	MM	MM	SM, YVC, LM

^a vBBWV2-PAP1 and -RP1, saps from the leaves of infected red pepper were inoculated. pBBWV2-PAP1 and -RP1, agrobacteria carrying full-length cDNA clones of BBWV2 RNA1 and RNA2 were inoculated.

^b Symptoms: SM, severe mosaic; St, stunting; MM, mild mosaic; YVC, yellowish vein clearing; LM, leaf malformation. Virus replication was confirmed by RT-PCR.

The BBWV2 isolates were propagated in *C. annuum* L. in a greenhouse. Total RNA was extracted from leaves infected with the isolate PAP1 or RP1 using the TRI Reagent (MRC, USA) according to the protocols provided by the manufacturer. The extracted total RNAs were used for cDNA synthesis of RNA1 and

RNA2 of the BBWV2 isolates. Specific primers (BBWV2-5E-Fw, 5'-GTTTAAATAAAATATTAACAAACAGCTTTCGTT-3'; R1-3E-KpnI-Rv, 5'-GGGGTACCTTTTTTTTTTTTTTTTTTCCCTCACTACTGA-AATTTACTT-3'; and R2-3E-KpnI-Rv, 5'-GGGGTACCTTTTTTTTTTTTTTTTAAATACTATTAAGCCTATACATATA-3') were designed

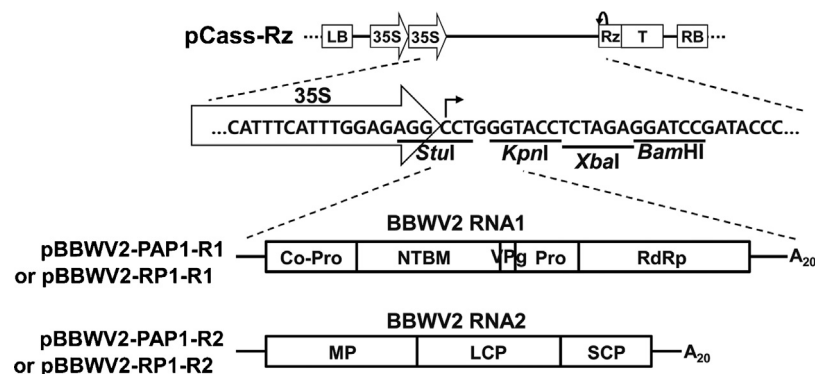


Fig. 2. Schematic representation of infectious full-length cDNA clones of BBWV2. Full-length cDNAs of RNA1 and RNA2 of BBWV2-PAP1 and -RP1 were inserted between the *Stul* and *KpnI* sites in the pCassRz vector. The pCassRz vector contains, in sequential order, a T-DNA left border (LB), a double 35S promoter, multiple cloning sites (*Stul*, *KpnI*, *XbaI*, and *BamHI*), a *cis*-cleaving ribozyme sequence (Rz), a 35S terminator (T), and a T-DNA right border (RB). Transcription begins at the first nucleotide of the BBWV cDNAs.

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