Transient Expression of Homologous Hairpin RNA Interferes with Broad bean wilt virus 2 Infection in Nicotiana benthamiana

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(Received on October 30, 2012; Revised on November 15, 2012; Accepted on November 26, 2012)

Broad bean wilt virus 2 (BBWV2), genus Fabavirus, subfamily Comovirinae, family Secoviridae, causes damage in many economically important horticultural and ornamental crops. Sequence alignments showed several conserved sequences in 5’ non-coding regions (5’ NCRs) of RNA 1 and RNA 2 in all BBWV2 strains characterized so far. Based on this observation, we generated a hpRNA construct (pIR-BBWV2) harboring an inverted repeat containing a 210 bp cDNA fragment homologous to 5’ NCR portion of BBWV2 RNA 1 to investigate the silencing potential for its ability to interfere with a rapidly replicating BBWV2. Agrobacterium-mediated transient expression of the IR-BBWV2 had a detrimental effect on BBWV2 infection, showing no distinct symptoms in non-inoculated leaves of the agroinfiltrated Nicotiana benthamiana plants. BBWV2 genomic RNAs were not detected by RT-PCR from tissues of both the inoculated leaves and upper leaves of the agroinfiltrated plants. Accumulation of virus-derived small interfering RNAs was detected in the inoculated leaf tissues of N. benthamiana plants elicited by transient expression of IR-BBWV2 indicating that RNA silencing is responsible for the resistance to BBWV2.

Keywords: Agroinfiltration, Broad bean wilt virus 2, Resistance, RT-PCR, Small-interfering RNA, Transient expression

Introduction

Pathogen-derived resistance (PDR) is a specific resistance of plants to pathogens by introducing a pathogen into the plant genome. It is widely shown that the PDR to virus infection are relevant to RNA silencing known well for homology-dependent selectively degradation of RNA (Baulcombe, 2005; Hull, 2002). The RNA silencing machinery recognizes several features of viral infections involving the formation of double-stranded (ds) RNA and initiates a response that degrades viral RNA and eventually enables the plant to recover from virus infection. The dsRNA triggers degradation of homologous RNAs and is diced into small interfering (si) RNAs of 21–25 nts in length. The siRNAs then act as guide sequences to recognize complementary RNAs for their degradation (Voinnet, 2005; Waterhouse et al., 2001). RNA silencing is also activated by transgenes expressing inverted-repeat (IR) structures that produce dsRNA (Chuang and Meyeriwitz, 2000; Waterhouse et al., 2001) or aberrant transcripts that could be templates for a cellular RNA-dependent RNA polymerase activity (Lipardi et al., 2001). In addition, RNA silencing can be induced by expression of hairpin (hp) RNA in plants, and a variant of this construction which also encodes a spliceosomal intron inserted between the hpRNA arms (so called intron-hpRNA) induced RNA silencing with almost 100% efficiency when directed against RNA virus or endogenous plant genes (Pandolfini et al., 2003; Smith et al., 2000). Transient expression triggered by infiltration of Agrobacterium tumefaciens
High Resistance to BBWV2 Infection By Transient Expression of a Homologous Hairpin RNA

Broad bean wilt virus 2 (BBWV2), genus Fabavirivirus, subfamily Comovirinae, family Secoviridae, causes damage in many economically important horticultural and ornamental crops (Koh et al., 2001; Lisa and Boccardo, 1996; Qi et al., 2000; Wang et al., 2008; Xu et al., 1988). Since some BBWV2 isolates identified cause severe damages in pepper production, BBWV2 is one of harmful viruses for pepper production in Korea (Lee et al., 2000; Cho et al., 2007; Choi et al., 2001; Choi et al., 2005). BBWV2 has a wide host range and is transmitted by aphids in a non-persistent manner. BBWV2 virion is icosahedral particles, composed of two proteins (the large and small coat proteins; LCP and SCP) and a genome composed of two single-stranded positive-sense RNA molecules of about 6 and 4 kb (Lisa and Boccardo, 1996). Both RNAs are translated into single polyproteins from which functional proteins are divided by proteolytic cleavage. RNA 1 encodes proteins involved in genome replication and expression, and RNA2 encodes the movement protein and the two CPs (Lisa and Boccardo, 1996). RNA silencing has been efficiently used to generate resistance against plant viruses in many ornamental plants (Bucher et al., 2006; Hammond et al., 2006; Tenllado et al., 2004) and in different host systems to obtain resistance against several other viruses (Abhary et al., 2006; Di Nicola-Negri et al., 2005; Lennefors et al., 2006; Pooggin et al., 2003; Tenllado et al., 2003; Vanitharani et al., 2003). Particularly, transgenic expression of pathogen-derived sequences encoding hpRNAs that undergo to an efficient RNA silencing is a new and agricultural sustainable strategy to obtain virus-resistant plants (Smith et al., 2000). However, it is not known if transient expression of a hpRNA could block multiplication and spread of a widely replicating BBWV2 in non-transgenic plants.

In this study, we show that transient expression of a hpRNA construct using agroinfiltration allows high resistance to BBWV2 in Nicotiana benthamiana. RNA silencing is responsible for this resistance to BBWV2 and this approach makes it possible to construct transgenic crops conferring resistance against BBWV2.

Materials and Methods

Plasmid construction

A cDNA fragment homologous to 210 bases of 5' NCR positions in 5' NCRs are shown above BBWV2 genome. Name and RNA source of each strain are shown on the right. Nucleotide positions were indicated on the left. The nt identical to the consensus are indicated by dots within the alignment and the nt different from the consensus are indicated by dashes within the alignment. Accession numbers deposited to GenBank are as follows: BBWV2-P RNA 1 (in this study), BBWV2-IP RNA 1 (AB2023484), BBWV2-ME RNA 1 (NC_003003), BBWV1-PV132 RNA 1 (AB084450), and BBWV1-Ben RNA 1 (AY781171). (B) A construct (pIR-BBW2) containing hpRNA sequences contained the CaMV 35S promoter (a black arrow) and the 3'-termination sequences of octopine synthase (black square). The sense (+) and antisense (−) cDNA fragments homologous to the 5' NCR sequences of BBWV2 RNA 1 are represented by blue arrows. The cDNA fragments encoding sense or antisense 5' NCR sequences are separated by a charcone synthase (CHSA) intron derived from P. hybrida. Restriction enzyme sites used for cloning are shown above the construct.