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Isolation and validation of a candidate *Rsv3* gene from a soybean genotype that confers strain-specific resistance to soybean mosaic virus

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ABSTRACT

Soybean mosaic virus (SMV), a member of the genus *Potyvirus*, significantly reduces soybean production worldwide. *Rsv3,* which confers strain-specific resistance to SMV, was previously mapped between the markers A519F/R and M3Satt in chromosome 14 of the soybean [*Glycine max* (L.) Merr.] genotype L29. Analysis of the soybean genome database revealed that five different NBS-LRR sequences exist between the flanking markers. Among these candidate *Rsv3* genes, the full-length cDNA of the *Glyma.14g204700* was successfully cloned from L29. Over-expression of *Glyma.14g204700* in leaves inoculated with SMV inhibited viral infection in a soybean genotype lacking *Rsv3*. In addition, the transient silencing of the candidate gene caused a high accumulation of an avirulent strain in L29 carrying *Rsv3*. Our results therefore provide additional line of evidence to support that *Glyma.14g204700* is likely *Rsv3* gene that confers strain-specific resistance to SMV.

1. Introduction

Soybean [*Glycine max* (L.) Merr.] is the world's largest source of animal protein feed and the second largest source of vegetable oil for human consumption. According to The Food and Agriculture Organization Corporate Statistical Database, the global production of soybean during the period 2005–2007 was about 217.6 million metric tons (Masuda and Goldsmith, 2009). Soybean production, however, is greatly reduced by plant pathogens; the production loss in 2006 was estimated to be about 59.9 million metric tons in the top eight soybean-producing countries (Wrather et al., 2010).

Soybean viruses are emerging threats to soybean production (Hill and Whitham, 2014), and soybean mosaic virus (SMV) is the most damaging (Ramteke et al., 2015). SMV is a member of the genus *Potyvirus* in the family *Potyviridae* and consists of an approximately 9.6-kilobase (kb) single-stranded positive-sense RNA. It was long believed that the viral genome encodes a long polyprotein that is processed into 10 mature proteins; however, Chung et al. (2008) discovered another short PIPO fusion protein as a P3-PIPO resulting from ribosomal frameshifting or transcriptional slippage at a highly conserved $G_{1-2}A_{6-7}$ sequence within the coding region of P3 protein. This seed-born and aphid-transmitted virus can induce diverse symptoms, including mosaic, mottling, chlorosis, and wrinkling (Babu et al., 2008).

To protect against virus infection, plants have evolved various types of resistance genes. Plant disease resistance genes can be classified into two groups: recessive and dominant genes. Recessive resistance is passive and results from a lack of a specific host component (or mutations in that component) required for virus replication (Diaz-Pendon et al., 2004). Dominant resistance involves an active mechanism in which R proteins recognize specific avirulent (Avr) factors from viruses and trigger resistance responses (Chisholm et al., 2006). According to the gene-for-gene concept, a plant that produces an *R* gene product is specifically resistant to a pathogen that produces a corresponding avirulent (*Avr*) gene product (Flor, 1971). Most *R* genes encode proteins containing a nucleotide-binding site (NBS) and a leucine-rich repeat (LRR) region (Ellis et al., 2000). The NBS domain includes a functional nucleotide-binding site that binds to and hydrolyzes ATP (Tameling et al., 2002) and is involved in signaling of resistant responses (Rairdan and Moffett, 2006). The LRR domain consists of conserved repeats of an 11 or 12 residue stretch, LxxLxLxxN/LxL or LxxLxLxxN/LxxL, in which "L" is Leu/Ile/Val/or Phe, "N" is Asn/Thr/Ser/or Cys, and "x" denotes any amino acid; the LRR domain is believed to be responsible for Avr recognition (Ellis et al., 2007; McHale et al., 2006). Based on the structure of the N-terminal domain, these NBS-LRR proteins are classified into two groups: the TIR-NBS-LRR proteins that contain an N-terminal domain with Toll/Interleukin-1 receptor (TIR) homology,

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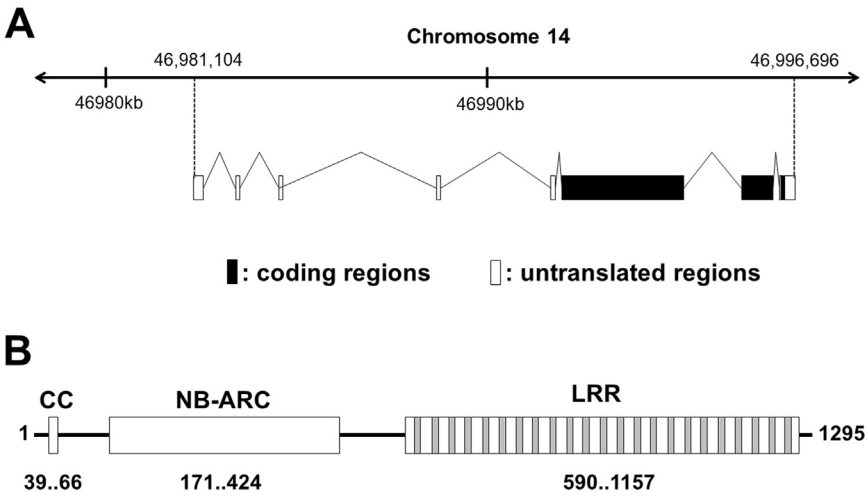

Fig. 1. The representative structures of the *Glyma.14g204700* coding sequence and putative protein. **A**, chromosomal position and exon distribution of *Glyma.14g204700* on soybean genome; numbers indicate nucleotide position on chromosome 14. **B**, representative diagram indicated the Glyma.14g204700 putative protein containing a possible coiled-coil domain (CC), a nucleotide-binding domain (NB-ARC), and a leucine-rich repeat domain (LRR); the number indicates the amino acid position from the N terminal.

while the CC-NBS-LRR proteins that are characterized by an N-terminal coiled-coil (CC) motif (Dangl and Jones, 2001).

SMV was classified into several distinct strains based on disease reactions on different soybean genotypes (Cho and Goodman, 1979). Four dominant loci containing *Rsv1*, *Rsv3*, *Rsv4*, or *Rsv5* have been genetically mapped in soybean. The *Rsv1* locus, which confers extreme resistance (ER) to SMV strain G1 through G6 but not to strain G7, is in chromosome 13 of soybean genotype PI 96983 (Chen et al., 1991; Hajimorad and Hill, 2001; Yu et al., 1994); *Rsv1* resistance was predicted to result from at least one of three NBS-LRR sequences (3gG2, 5gG3, and 6gG9) (Zhang et al., 2012). The *Rsv4* locus, which mediates resistance to all seven SMV strains (Chen et al., 1993; Ma et al., 1995), was mapped between the markers Rat2 and S6a in chromosome 2 of soybean genotype VP-5152; no NBS-LRR type *R* sequence was found in this region, suggesting that the *Rsv4* gene belongs to a new class of resistance genes (Ilut et al., 2016). Recently, the *Rsv5* was reassigned from the *Rsv1-y* that is from soybean cultivar York and highly linked to the *Rsv1* locus on chromosome 13 with a genetic distance 2.2 cM (Klepadlo et al., 2017). The *Rsv3* gene that confers ER against G5 to G7 but not G1 to G4 was previously identified between the markers A519F/R and M3Satt in chromosome 14 of soybean genotype L29 (Jeong et al., 2002). Sequence analysis of the 154-kbp region between these two markers revealed five candidate *Rsv3* genes that contain NBS-LRR domains (Suh et al., 2011). According to the recent annotation of the soybean genome database derived from the soybean cultivar William 82 (Glyma. Wm82.a2.v1, phytozome.net), the five candidate *Rsv3* genes were named *Glyma.14g204500*, *Glyma.14g204600*, *Glyma.14g204700*, *Glyma.14g205000*, and *Glyma.14g205300*. A comparative sequence analysis of various susceptible and resistant soybean cultivars showed that *Glyma.14g204700* (*Glyma.14g38533* in previous annotation) has highest transcript abundance and highest number of genotype-phenotype correlations; most of the polymorphisms of this gene were identified in LRR domain (Redekar et al., 2016). This suggested that the *Glyma.14g204700* is most likely responsible for the *Rsv3* resistance.

In Korea, the repeated cultivation of soybean cultivars with resistance against the most prevalent strain, SMV-G5H, caused the emergence of a new SMV strain, G7H. In the late 1990s, G7H caused systemic mosaic symptoms or a lethal systemic hypersensitive response (HR) in certain soybean cultivars that are resistant to G5H (Kim, 2000; Kim et al., 2003). We previously constructed infectious cDNA clones of SMV strains G5H (SMV-G5H) and G7H (SMV-G7H) under the control of the 35 S promoter of cauliflower mosaic virus (CaMV), and demonstrated that the cylindrical inclusion (CI) gene is the elicitor of *Rsv3*-mediated ER and is also a pathogenic determinant that caused the emergence of the resistance-breaking SMV-G7H strain (Seo et al., 2009a, 2009b, 2009c). In the present study, we isolated the cDNA sequence of the *Rsv3* candidate *Glyma.14g204700* from soybean cultivar L29. Co-inoculation of soybean genotype Lee74, which lacks *Rsv3*, with a gene delivery vector expressing *Glyma.14g204700* and an SMV infectious cDNA clone resulted in inhibition of the local infection and a reduction in the systemic accumulation of SMV strain G5H (SMV-G5H, avirulent in *Rsv3*-mediated resistance) but not of SMV strain G7H (SMV-G7H, virulent in *Rsv3*-mediated resistance). In addition, loss-of-function effects of *Glyma.14g204700* on SMV infection in genotype L29, which has *Rsv3*, were examined using a bean pod mottle virus (BPMV)-based virus-induced gene silencing (VIGS) tool (Zhang et al., 2010). The results showed that transient silencing of this candidate gene caused an increase in the accumulation of SMV-G5H in L29; the viral accumulation then decreased during the recovery of the *Glyma.14g204700* in the transiently silenced plant. Our results therefore suggest that the cloned candidate gene *Glyma.14g204700* is likely the resistance gene *Rsv3*, which confers strain-specific resistance to SMV.

2. Results

2.1. *Sequence analysis of Glyma.14g204700, an Rsv3 candidate gene from soybean genotype L29*

Because L29 carrying *Rsv3* is resistant to SMV-G5H, we sought to isolate the cDNA sequences of *Rsv3* candidates from the genotype's total RNA. We obtained a full-length, double-stranded cDNA clone of the candidate *Glyma.14g204700*. Based upon the gene annotation from soybean genome database Glyma. W82.a2.v1, a 15593 bp genomic sequence of *Glyma.14g204700* was found which includes 8 exons and 7 introns (Fig. 1A). The cloned coding gene, which contains 3888 base pairs (bp), encodes a putative NBS-LRR protein that contains 1295 amino acid (aa) residues (Fig. 1A and S1). As shown in Fig. 1B and S1, the positions of domains CC, NB-ARC, and LRR were predicted by bioinformatics tools. Firstly, a coiled-coil prediction method (Combet et al., 2000) indicated that the region ranging from the 39th to the 66th aa was likely to have (with a probability of $P > 0.99$) a coiled-coil domain containing heptad repeats, hxxhcxc, of hydrophobic (h) and charged (c) amino-acid residues. Secondly, an NCBI-based domain and motif search (Marchler-Bauer et al., 2016) revealed an NB-ARC domain in the region ranging from the 171st to the 424th aa, which includes the conserved motifs of P-loop (with motif GxxxxGKS/T, in which x indicates any residue), kinase 2 (with motif hhhhDD/E, in which h is mostly a hydrophobic residue), and kinase 3a (motif hhhhToR, in which o is an alcoholic residue). Finally, twenty-three LRR motifs (named LRR1 to LRR23) were detected from the 590th to the 1157th aa by using an LRR searching tool (Bej et al., 2014); four LRRs (number 3, 6,