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Full Length Research Paper

Isolation and characterization of a candidate gene for resistance to cereal cyst nematode from *Aegilops variabilis* in China

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Cereal cyst nematode (CCN) (*Heterodera avenae* Woll.) is one of the most economically damaging endoparasite pests of wheat worldwide. We isolated and characterized a novel cereal CCN resistance candidate gene, *CreV8*, from *Aegilops variabilis* (2n = 28, UUS^VS^V). The gene was 3,568 bp long and showed high nucleotide similarity with the known nematode resistance genes *Cre3*, *CreZ,* and *go35*. *CreV8* consisted of two exons, one intron and a complete ORF of 2,763 bp. The deduced peptide sequence consisted of 920 amino acid residues and shared 97.83% amino acid identity with *Cre3*. The sequence harbored a signal peptide domain, a nucleotide-binding ARC (NB-ARC) domain, and a leucine-rich repeat (LRR) domain, all of which are typical characteristics of resistance genes. We proposed the resistance mechanism of *CreV8* based on functional analysis and predictions from its conserved domains and tertiary structure. Real-time polymerase chain reaction (PCR) showed that *CreV8* was expressed in CCN-inoculated roots of resistant plants, and its expression levels were 2.80 and 27.45 times higher than that in the untreated control at 3 and 11 days post-inoculation, respectively. These results indicate that *CreV8* belongs to the nucleotide binding site (NBS)-LRR resistance gene family and that it is a candidate CCN-resistance gene.

Key words: *Aegilops variabilis*, *CreV8*, cereal cyst nematode (CCN), *Heterodera avenae*, tertiary structure.

INTRODUCTION

Wheat plays a key role in solving the food crisis for the increasing world population (Marathée and Pherson, 2001; Clover, 2003). The cereal cyst nematode (CCN), *Heterodera avenae*, is a major biotic constraint for crop production in many wheat and barley growing regions around the world (Bonfil et al., 2004; Holgado et al., 2006; Williamson and Kumar, 2006; Nicol and Rivoal, 2007; Sharma et al., 2007). One of the most cost-effective and environmentally friendly ways to reduce losses caused by CCN is to use genetically resistant plant varieties (Ferry and Gatehouse, 2010). Although CCN has caused significant economic losses over the last 40 years (Holgado et al., 2006), relatively few resistance genes have been cloned and characterized. To date, 16 different CCN resistance genes designated as *Cre1* to *Cre8, CreR*, *CreX*, *CreY* (Smiley and Nicol, 2009), *CreZ* (Zhai et al., 2008) and *Ha1* to *Ha4* (Bakker et al., 2006) have been genetically mapped in wheat and its relatives.

Some of these genes have been introgressed into wheat backgrounds (Barloy et al., 2007). Among the known *Cre* genes, only *Cre3* was isolated through map-based cloning (Lagudah et al., 1997), and only *Cre3* and *CreZ* have had their complete open reading frame (ORF) sequences registered in GenBank. None of these genes has been functionally characterized. The effectiveness of these *Cre* genes in conferring resistance to CCN depends on the pathotype of the nematode (Rivoal et al., 2001; Montes et al., 2008; Akar et al., 2009). Many nematode resistance genes belong to the nucleotide binding site- leucine-rich repeat (NBS-LRR) super-family, Including *Mi-1*, *HeroA, Gpa2,* and *Gro1-4* from tomato (Williamson and Kumar, 2006), *Cre3* from *Ae. tauschii* (Lagudah et al., 1997) and *Cre1* and *CreZ*

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from wheat (Zhai et al., 2008). These genes often contain highly conserved domains such as the nucleotide-binding ARC (NB-ARC) and the LRR domains and confer resistance to a diverse range of nematode species, suggesting that there are similar signaling events in plant defense (Baker et al., 1997).

Ae. variabilis No. 1 (2n = 28, $UUS^{v}S^{v}$) is resistant to root-knot nematode, *Meloidogyne naasi* and French pathotypes of CCN (Barloy et al., 2007). *Ae. variabilis* belongs to the tribe *Triticeae* and is readily crossable as a male parent to bread wheat (Coriton et al., 2009), confirming its potential as a source of resistance for wheat breeding (Rivoal et al., 2001; Kilian et al., 2011). CCN resistance in *Ae. variabilis* No. 1 is oligogenic (Barloy et al., 2007) and two genes, *CreX and CreY* are associated with this resistance (Rivoal et al., 1986; Jahier et al., 1996). *CreX* might be the same gene as *Cre2* (Jahier et al., 1996). Furthermore, the Institute of Plant Protection, Chinese Academy of Agriculture Sciences (CAAS) reported that *Ae. variabilis* No. 1 was resistant to a Chinese pathotype of *Heterodera avenae* (Deng et al., 2001). In the same bioassay, *Ae. variabilis* No. 2 was detected to be susceptible to Chinese pathotype of *H. avenae*. The first occurrence of *H. avenae* in China was reported in 1989 (Chen et al., 1992; Peng et al., 2007). Since then, various CCN pathotypes have been identified in China (Peng et al., 1996, 2003; Zheng et al., 1996, 2000; Yuan et al., 2010), but no resistance gene has been cloned. Identification and cloning *Cre* resistance genes from *Ae. variabilis* No.1 may provide new wheat germplasm for resistance to Chinese pathotypes of CCN. Few studies have been conducted on the 3D architecture, the conserved domains and the functional mechanisms of CCN resistance genes. Information about the processing of *Cre* genes might further our understanding of their resistance mechanisms. The aim of the present study was to isolate and characterize two NBS-LRR resistance gene analogs, to identify their conserved domains and to determine their sequence similarity to previously reported genes. We also discuss the tertiary structure of the gene product and its relationship with the molecular mechanisms of the CCN defense response.

MATERIALS AND METHODS

Ae. variabilis No 1 and No. 2 are deposited in the Chengdu Institute of Biology, Chinese Academy of Sciences. Accession No. 1 is resistant to Chinese pathotypes of CCN (*H. avenae*) and *Ae. variabilis* No. 2 is susceptible. The plants were cultivated in ceramic plant pots (15 cm diameter, 15 cm high) in a greenhouse at approximately 20°C under a 16 h light/8 h dark photoperiod.

Genomic DNA extraction and PCR conditions

Genomic deoxyribonucleic acid (DNA) was extracted from individual young vegetative leaves of *Ae. variabilis* No 1 and No. 2 by the cetyl trimethylammonium bromide (CTAB) method. To isolate analogs of the NBS-LRR resistance gene, a pair of PCR primers (forward: 5`-ATCTGTCTGTAGTGATGGATCC-3` and reverse: 5`-AAAGCTGGGATTCCAAGCCCGCGTCATTC-3`) was designed according to conserved sequences of the previously published genes *CreZ*, *Cre3* and *go35* (GenBank accession numbers EU327996, AF052641 and EU327996, respectively). Each PCR mixture (25 µl total volume) consisted of 100 to 300 ng genomic DNA, 200 µM each dNTP, 50 µM each primer, 1.25 units high-fidelity *ExTaq* polymerase (Takara, Dalian, China), and 1 × PCR buffer (supplied with the polymerase). The reaction conditions were as follows: initial denaturation at 94°C for 4 min followed by 30 cycles of 40 s at 94°C, 40 s at 58°C and 3.5 min at 72°C; and a final extension step at 72°C for 15 min.

Cloning and DNA sequencing

The desired DNA fragment was extracted and purified using a gel extraction kit (Biomiga, San Diego, CA, USA), then cloned into the pMD19-T vector (Takara, Dalian, China). The vectors containing inserts were transformed into competent cells of *Escherichia coli* JM109. Positive clones were identified by PCR with the aforementioned primers and *Bam*HI restriction digestion assays. The amplification and cloning was repeated three times and 10 positive clones were randomly selected for sequencing (Invitrogen, Shanghai, China). The sequencing was repeated twice for accuracy. DNA sequences were analyzed using DNAMAN V6.0 and online tools. The ORFs of the sequences were identified by FGENESH (http://linux1.softberry.com/) and the amino acid sequence was obtained at the same time. We used DNAMAN V6.0 to predict the molecular mass and the isoelectric point (PI), for multiple alignments of deduced amino acid sequences and to construct the homology tree. Functional domain prediction was carried out using the Simple Modular Architecture Research Tool (SMART, http://smart.embl-heidelberg.de/). Conserved motifs were annotated by combining the results of SMART and domains alignment (http://www.ncbi.nlm.nih.gov/Structure/cdd/cdd.shtml).

Tertiary structure prediction and characterization

The protein tertiary structure image was created using CPHmodels-3.0 Server (http://www.cbs.dtu.dk/services/CPHmodels/). The motif positions were marked on protein 3D structures by 3D-Mol Viewer, a component of Vector NTI Suite 9.0.

Quantitative real-time PCR (qPCR)

The expression levels of *CreV8* were determined in the roots of *Ae. variabilis* No. 1 at seven time-points after inoculation with CCN; 1 day post-inoculation (dpi), 3, 5, 9, 11, 13 and 20 dpi. Each plant was inoculated once with 1000 second-stage juveniles (J2) of CCN. One day after inoculation, the roots were thoroughly washed with sterile water for 10 min. This was repeated three times to remove all CCNs adhering to the roots. Then, plants were transplanted into 500 ml glass containers filled with sterilized perlite to prevent further CCN penetration and to ensure synchronized development of syncytia. We also analyzed expression of *CreV8* in non-infected plant roots at the 1 day time-point as the control. We harvested roots from 10 plants at each time-point, with two replicates. Total ribonucleic acid (RNA) was extracted using a Biomiga RNA kit (Biomiga, San Diego, CA, USA) and complementary DNA was synthesized by M-MLV Reverse Transcriptase (Invitrogen, CA, USA) with oligo dT primers. The qPCR was performed on the Chromo 4^{TM} System CFB-3240 (Bio-Rad Lab. Inc., Hercules, CA, USA). According to the BLAST-n of *CreV8* in NCBI, we focused on a sequence of the LRR domain (253 bp in length) for the qPCR experiment.

To amplify the sequence, we used the primer pair 5`-GATGGTGGGCAGGCAAAATG-3` (forward) and 5`-GAGCCAAAAATACGTAAGAGAC-3` (reverse). The amplified product showed a single peak on the melting curve of the qPCR. Two housekeeping genes (*GAPDH* and *actin*) were used as interior references for accuracy, and all reactions were repeated three times (Rieu and Powers, 2009). The qPCR mixture contained 1 µl five-fold diluted cDNA, 7 µl iQ^{TM} SYBR$^{®}$ Green Supermix (Bio-Rad), and 300 nM each gene-specific primer in a final volume of 14 µl. The reaction conditions for thermal cycling were as follows: 95°C for 3 min, followed by 40 cycles of 94°C for 20 s, 60°C for 20 s and 72°C for 20 s. Target quantities of amplified product in all samples were analyzed using the manufacturer's software. The abundances of the transcripts were normalized against those of *GAPDH* and *actin* to adjust for differences in transcript levels of the target RNA in each sample.

Single oligonucleotide nested PCR (SON-PCR)

We used SON-PCR to identify the start site of *CreV8*. We used cDNA from plants at 1, 3, and 9 dpi as the templates, and designed two nested primers, soncre1: 5′ (+171 bp)-CATGACCCGTTCCAGCACCA-3′ (+152 bp) and soncre2: 5′-(+153 bp) CAAGATTTTTGGCTCAAGTTG-3′ (+133 bp), according to the 5′conserved sequences of *CreV8*. The SON-PCR reactions were performed with 100 to 300 ng cDNA, 200 µM each dNTP, 50 µM each primer, 1.25 units *ExTaq* polymerase and 1×PCR buffer. The reaction conditions were as described elsewhere (Antal et al., 2004). The resulting sequences were analyzed using NSITE-PL to identify regulatory motifs (http://linux1.softberry.com/).

RESULTS

Isolation and sequence analysis of CCN resistance candidate genes from *Ae. variabilis*

A band of approximately 3,600 bp was specifically amplified from the genomic DNA of *Ae. variabilis* No. 1, but no band was generated from *Ae. variabilis* No. 2 (Figure 1). The band was cloned into the pMD19-T vector and recombinant clones were confirmed by PCR and *Bam*HI digestion. Ten positive clones were randomly chosen and sequenced, and were designated as *CreV1–CreV10*. Multiple sequence alignments indicated that the 10 sequences could be classified into two groups; the first group contained sequences that were 3,571-bp long (*CreV1, CreV2*, *CreV3, CreV4, CreV5, CreV6, CreV7, CreV9* and *CreV10*), and the second group consisted of only one sequence (*CreV8*), which was 3,568 bp in length. The two groups shared 98.7% similarity. Analyses with BLAST-n at the NCBI website showed that the two groups shared more than 84% nucleotide identities with the known *Cre* genes (*Cre3*, *CreZ* and *go35*). There was a "T" insertion in the coding region of group 1 sequences at position 259 (Figure 2A). This would result in a frame shift mutation leading to an in-frame stop codon (Figure 2A). This suggested that these sequences may be pseudogenes.

According to the FGENESH analysis, *CreV8* consisted of 2 exons and 1 intron (Figure 2B) and its coding domain was 2,763 bp. It could be translated into a peptide of 920 amino acid residues with deduced molecular weight of 103,852 Da and a theoretical pI of 7.40. Sequence comparison revealed that the predicted protein sequence of *CreV8* shared more than 80% identity with those of the CCN resistance genes *Cre3* and *CreZ* (Figure 3). We conducted BLAST-p searches of the GenBank database and selected 10 genes whose similarities to *CreV8* ranged from 45 to 98% (E values = 0) for further analyses. These included *Cre3* (Genbank accession No. AACO5834.2), a putative NBS-LRR disease resistance protein (ABA90487.1), *CreZ* (ABY28270.1), *V6* (AAF19149), *RP1-DP8* (ACF22730), *NB-ARC DOMAIN-AB* (ABA95210.1), *Os11g0668000* (NP-001068424.2), *Os06g0279900* (NP-00105389.1), NBS-LRR class RGA (AAM69850) and *LR21* (AC053397.1). A homology tree was constructed based on comparison of these 11 polypeptides. In the tree, *CreV8* clustered with *Cre3* and grouped with three other nematode resistance (*Nem-R)* genes, ABA90487.1, *CreZ* and *V6*, to form a branch (Figure 3).

Expression profiling of *CreV8*

Using SON-PCR, we obtained three sequences that were 287 bp long from cDNAs from plants at 1, 3 and 9 dpi, respectively (Figure 1, lane 7 to 9). Sequencing showed that these sequences were identical. The sequence contained 171 bp of the start of *CreV8* and a 116 bp sequence that probably represented the upstream sequence of the ATG site of *CreV8*. The sequence included a binding factor motif located at -111 bp to -104 bp. We investigated the relative expression levels of *CreV8* by qPCR. Compared with its expression in control plants, the expression level of *CreV8* in CCN-inoculated plants increased slowly from 0.97 times at 1 dpi to 2.80 times at 3 dpi and then dropped slowly to 0.65 times at 5 dpi. The expression increased significantly from 9.03 times at 9 dpi to peak at 27.45 times at 11 dpi and then decreased to 0.61 times at 13 dpi and ultimately to 1.22 times at 20 dpi (Figure 4).

Conserved domains and function prediction of *CreV8*

SMART analysis indicated that the deduced polypeptide of the *CreV8* protein contained three conserved domains; a predicted signal peptide (the first 29 amino acids (aa), an NB-ARC domain (from 182 to 481 aa) and a LRR domain (from 599 to 875 aa) (Figure 5A). The NB-ARC domain harbored eight motifs, five of which (Walker A, Walker B, RNBS-B, GLPL and RNBS-D) were highly conserved relative to the well-known genes *Ced-4CED-9* (protein accession No. 2A5Y_B, a protein complex related to programmed cell death in *Caenorhabditis elegans*), *PRF* (Q96485, signal transduction component from

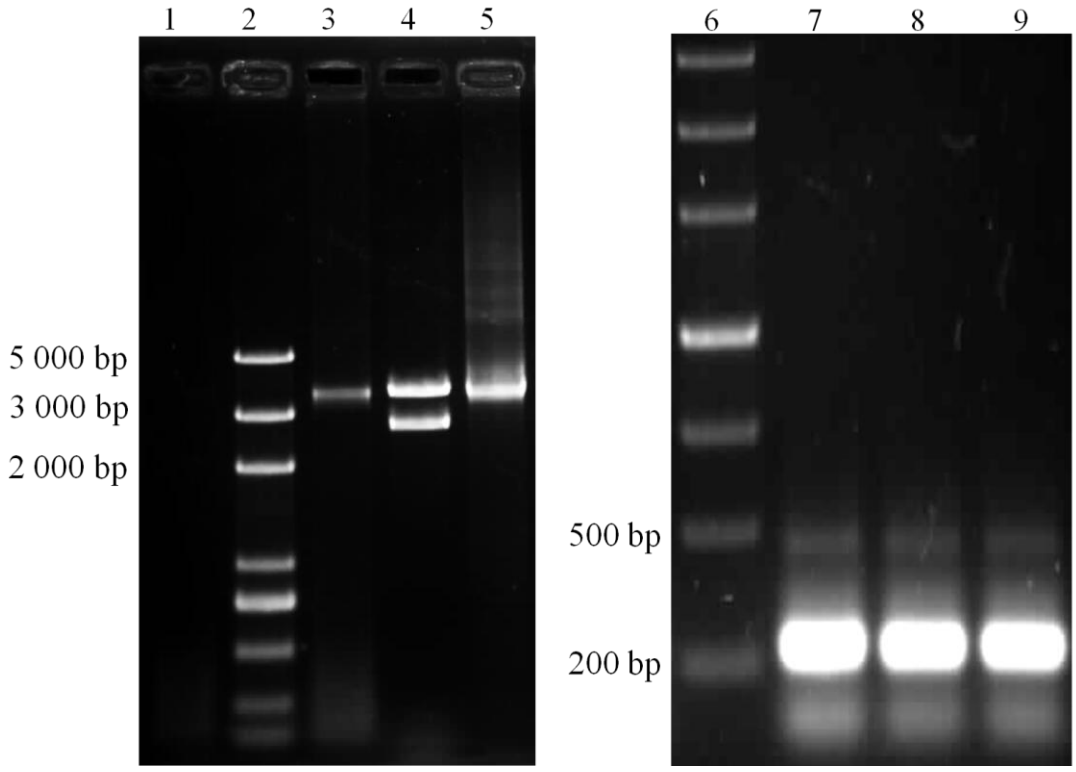

Figure 1. RGA cloning from *Ae. variabilis* by PCR approaches. PCR amplification from *Ae. variabilis* No. 2 (lane 1), *Ae. variabilis* No. 1 (lane 5), positive recombinant plasmid of pMD19T-*CreV8* (lane 3), and restriction of positive recombinant plasmid of pMD19T-*CreV8* with *Bam*HI (lane 4). Lane 7, 8 and 9 are SON-PCR amplification from cDNAs of 1 dpi, 3 dpi and 9 dpi, respectively. Lane 2 and lane 6 are DNA Marker 2K Plus and DNA Marker III of Tiangen Company, China, respectively.

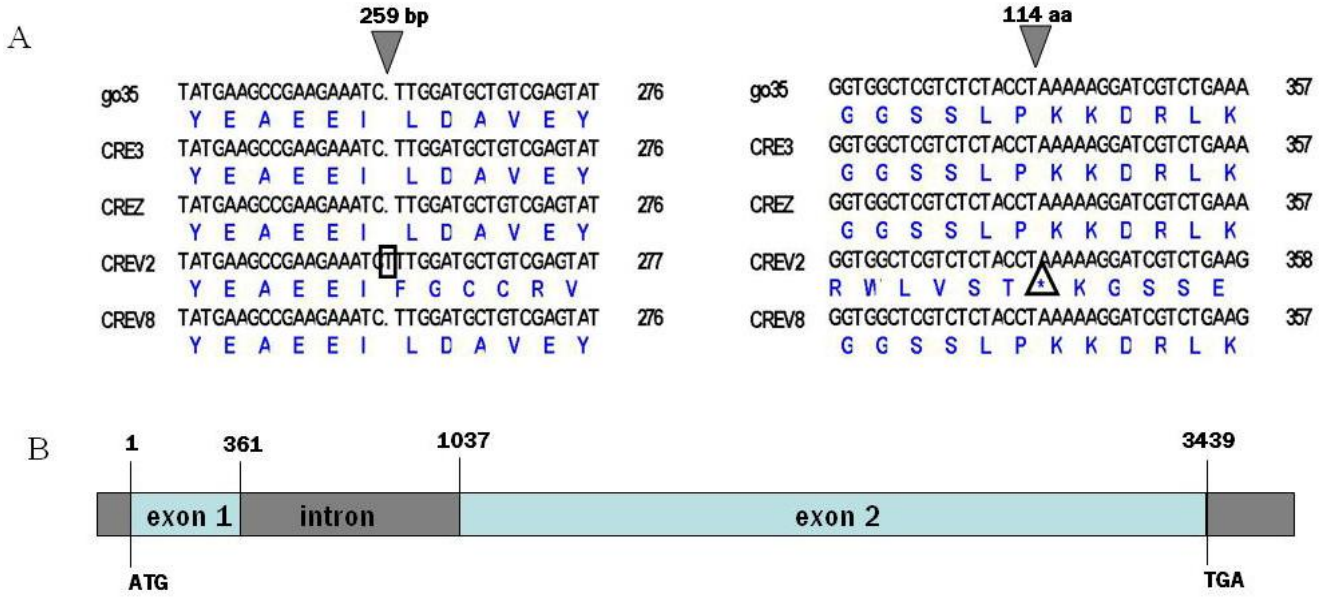

Figure 2. Frameshift mutation in *CreV2* and gene structure of *CreV8*. A, Partial alignment of *CreVs* with *go35, Cre3* and *CreZ*. *CreVs* share high homology with *go35, Cre3* and *CreZ* at the beginning of the nucleotide sequences and amino acid sequences. The T insertion (indicated by quadrangle) at position 259 bp of *CreV2* leads to an inframe stop codon (indicated by triangle) at 114 amino acid. B, Components of *CreV8* nucleotide sequence. Numbers indicate base positions.

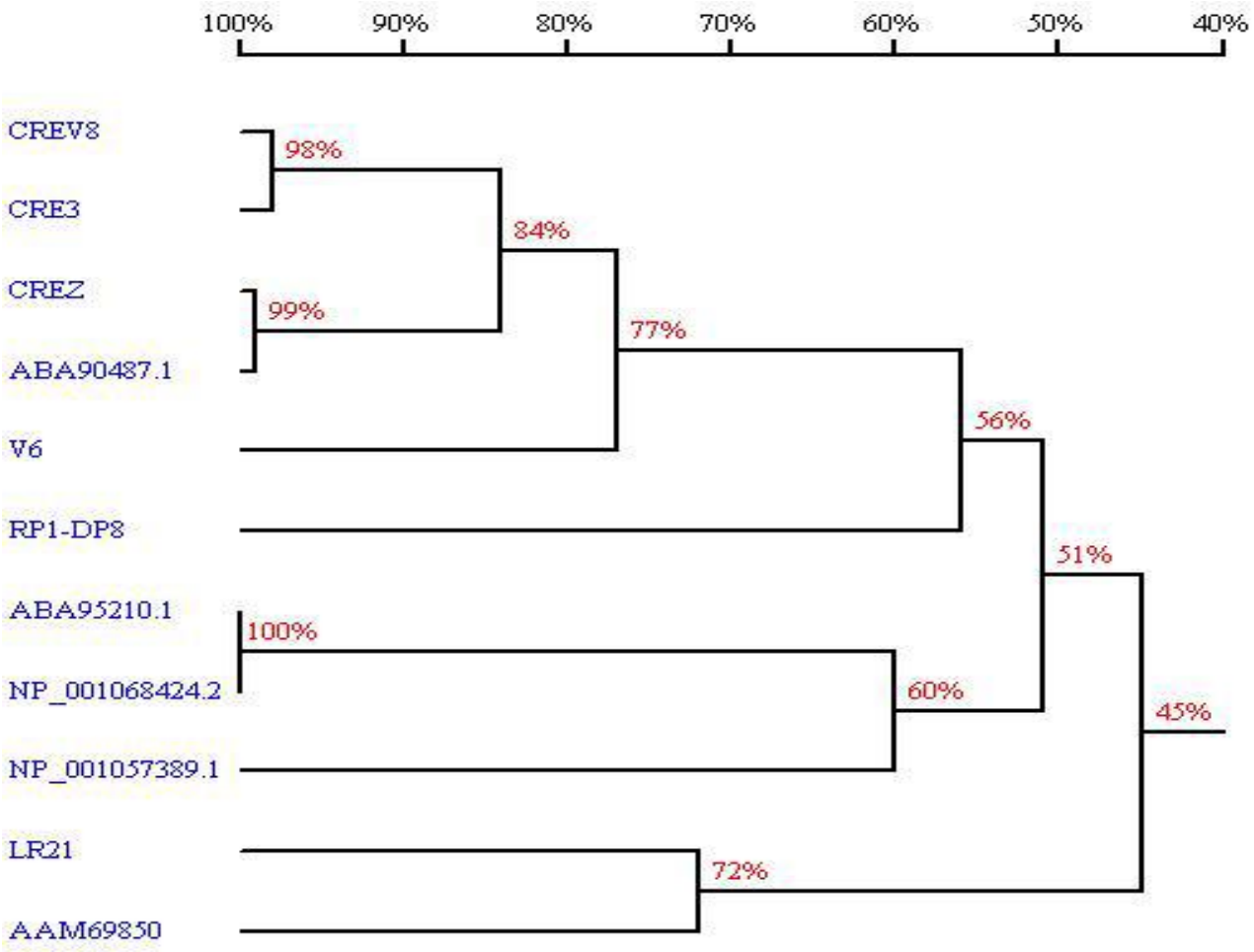

Figure 3. Homology tree based on alignment of amino acids of *CreV8*, *Cre3*, putative NBS-LRR disease resistance protein (ABA90487.1), *CreZ, V6*, *RP1-DP8*, NB-ARC_DOMAIN-AB (ABA95210.1), *Os11g0668000* (NP-001068424.2), *Os06g0279900* (NP-00105389.1), NBS-LRR class RGA (AAM69850) and *Lr21*. Percentage values indicate the level of homology.

tomato related to plant resistance and Fenthion sensitivity), *APAF-1* (O14727, apoptotic protease-activating factor 1 from human) and *I2C-1* (O24015, a partial resistance gene from tomato for resistance to *Fusarium oxysporum f. sp. lycopersici*) (Figure 5B).

The other three motifs of *CreV8*, RNBS-A, RNBS-C, and MHD, were highly conserved with *Cre3*, *CreZ*, and *V6,* and slightly less conserved with *Lr21* (Figure 5C). The three-dimensional structure of the *CreV8* protein was predicted *via* CPHmodels-3.0 Online Server. It resembled a basket with an unsealed base (Figure 6D). The signal peptide domain resembled a handle protruding from the basket, whereas the LRR domain was located at the bottom. The NB-ARC domain formed the main body of the basket, in which the Walker A, RNBS-A, Walker B and RNBS-B motifs were located close together (Figure 6A); the GLPL and RNBS-C motifs were close together (Figure 6B), and the MHD motif was close to the RNBS-D motif (Figure 6C).

DISCUSSION

The use of resistance genes to plant-parasitic nematodes offers prospects for creating new, insect-resistant lines. This can reduce the need for environmentally damaging nematicides. It can also reduce the requirement for crop rotation, which requires time and expense (Ferry and Gatehouse, 2010). As well as being an ideal candidate as a resistance gene against CCN, *CreV8* may also have potential for developing wheat lines with resistance to Chinese pathotypes of CCN. Furthermore, the results obtained by *in-silico* analysis may also be helpful to understand the structures of CCN-resistance genes and the molecular mechanisms of CCN resistance (Boyle, 2003; Worley, 2003). In this study, we obtained two groups of CCN resistance-like genes from *Ae. variabilis*

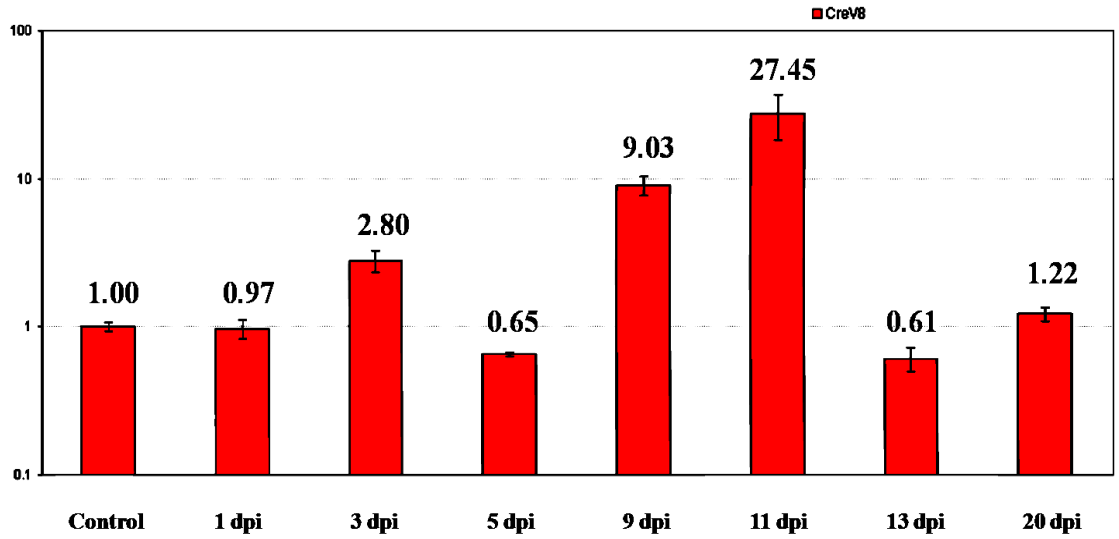

Figure 4. Expression of *CreV8* in CCN-infected roots of *Ae. variabilis* No. 1 showing a two-peaked mode.

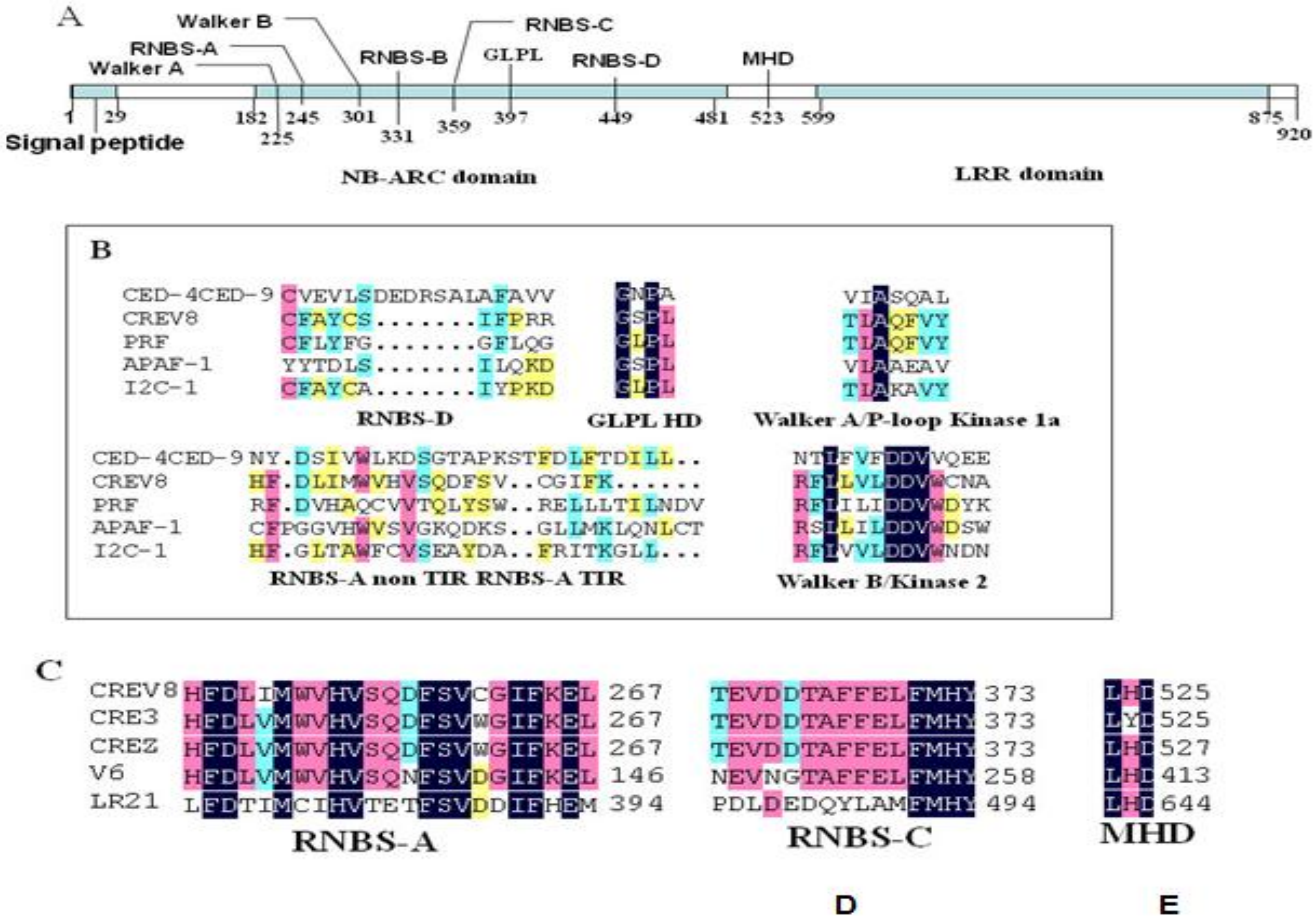

Figure 5. A. Conserved motifs of the NB-ARC domain in *CreV8. Ced-4CED-9*, *PRF*, *APAF-1* and *I2C-1* are *R* genes that contain NB-ARC domains. Seven conserved motifs are labeled with their names under the corresponding positions (marked by black lines). Numbers indicate the distance from the protein termini to the proximal and distal aligned blocks, and the distances between the blocks. **B.** Gene structure of *CreV8*, showing the position of each motif. **C.** Alignments of RNBS-A motifs of *CreV8*, *Cre3*, *CreZ*, *V6* and *Lr21*. **D.** Alignment of RNBS-C motif of *CreV8, Cre3, CreZ, V6* and *Lr21*. **E.** Alignment of the MHD motifs of *CreV8, Cre3, CreZ, V6* and *Lr21*.

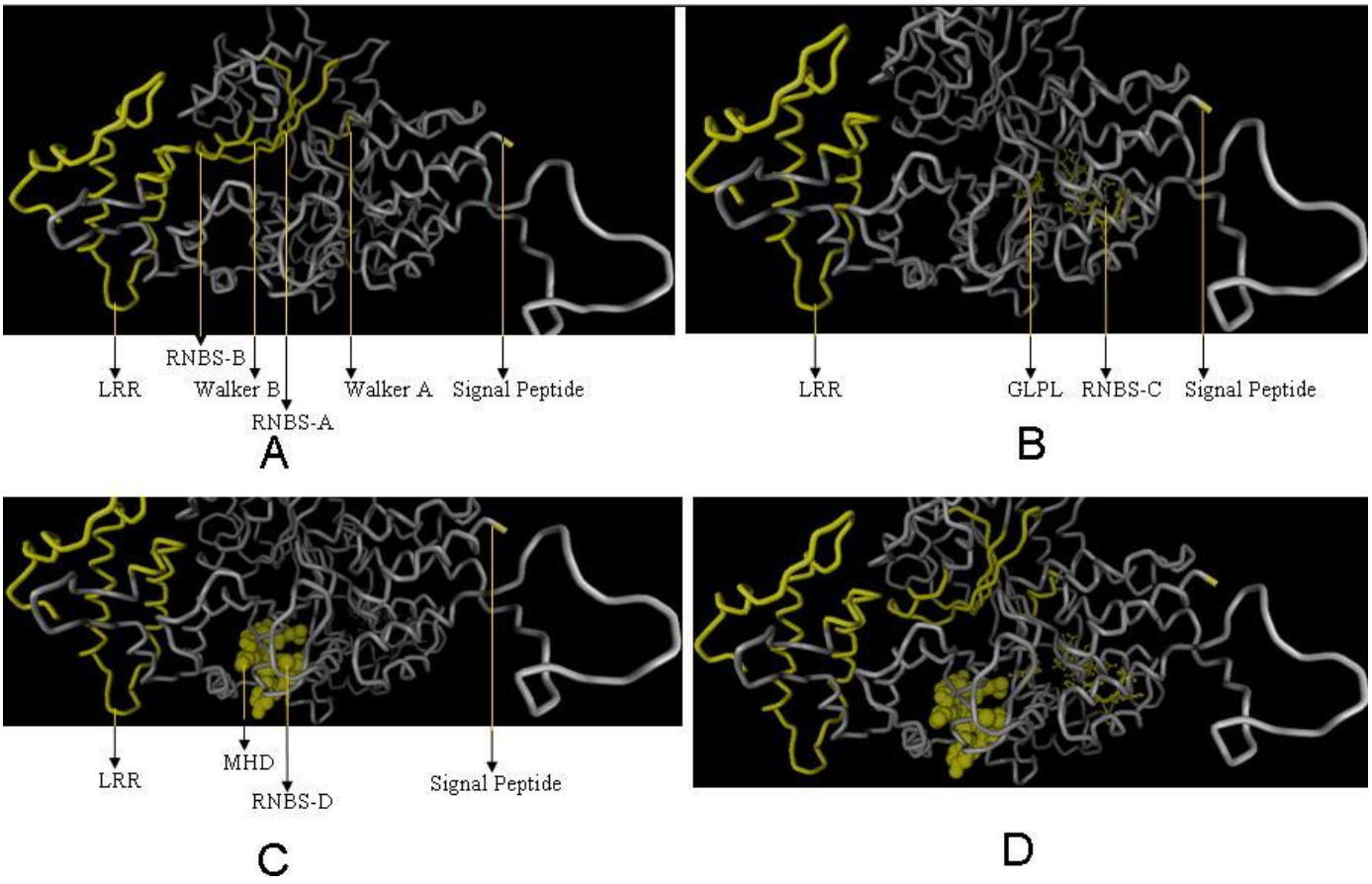

Figure 6. Positions of different motifs in the tube model tertiary structure of *CreV8* protein. **A.** Catalytic pocket of the NB-ARC domain formed by the Walker A, RNBS-A, Walker B and RNBS-B motifs. **B.** GLPL and RNBS-C make up the four-helix bundle. **C.** A winged-helix fold comprised by motifs of RNBS-D and MHD. **D.** 3D structure of putative *CreV8* protein with all domains (motifs).

No. 1, one of which group 1 was predicted to be pseudogenes.

In plants, most functionally defined *R* genes exist as large multigene families (Pan et al., 2000). It is predicted that the outcomes of recombination events give plants a selective advantage against the rapidly evolving pathogen populations (Baker et al., 1997). Gene evolution could result from nucleotide insertion, deletion or substitution, leading to new genes and pseudogenes (Han et al., 2010). However, many pseudogenes are still functional. Clarke and Appels (1999) reported that sequences with premature stop codons within the coding regions were still translated, producing truncated polypeptides. Therefore, although only CreV8 has the complete ORF, the other nine sequences might also be functional to some extent.

Analysis of the coding region of *CreV8* showed that it has high similarity with the previously reported *Nem-R* genes; *Cre3*, *CreZ*, *go35* and *V6*. In a previous study, the cloned *Nem-R* genes showed the strongest resemblance to other plant resistance genes (Williamson and Kumar, 2006). There are structurally similar host *R* genes for resistance to parasitic nematodes in evolutionarily diverse plant species, suggesting that resistance mechanisms may be strongly conserved (Baker et al., 1997). In this study, *CreV8* showed high homology with the CCN resistance gene analogs (RGAs) *Cre3*, *CreZ*, ABA 90487.1, and *V6.* Among those genes, *Cre3* was cloned from *Ae. tauschii, CreZ* from bread wheat, ABA 90487.1 from *Ae. peregrine* and *V6* from *Ae. ventricosa*. Our results suggest that *CreV8* is probably a novel CCN resistance gene derived from *Ae. variabilis*. The similarity between *Cre3* and *CreV8* was almost 98%, even though they were cloned from different species. Further research is required to determine whether these two similar sequences represent orthologs.

In this research, the expression of *CreV8* did not show a linear increase after inoculation with the pathogen. Compared with its pre-inoculation level, its expression increased to 2.80 times at 3 dpi, then decreased to 0.65 times at 5 dpi, then dramatically increased to 9.03 times at 9 dpi and 27.45 times at 11 dpi, after which it decreased at 13 and 20 dpi (Figure 4). Previous reports show that expressions of resistance-related genes led to histologically visible differences between incompatible and compatible interactions at 3 dpi (Das et al., 2010) and that 9 dpi was a critical time point at which the first

differences between incompatible and compatible interactions became visible (Das et al., 2008). Simonetti et al. (2010) reported that the activity of ascorbate peroxidase (APX) reached its maximum at 11 dpi with CCN, and then decreased. APX is one of the key enzymes in the plant antioxidant system, playing a central role in H_2O_2 scavenging. As described by Mittler et al. (1998), APX activity can be induced by the stress imposed by pathogen infection. Therefore, it is reasonable to infer that *CreV8* plays a role in the CCN-resistance process of *Ae. variabilis* No.1.

NB-ARC was first reported as a signaling domain (Tameling et al., 2006). This domain is present in the products of many NBS-LRR-type resistance genes, and in regulators of cell death in animals. It functions as a molecular switch whose state (on/off) depends on which nucleotide binds to it. Seven motifs of the NB-ARC domain are closely related to plant resistance. Among them, the Walker A motif, also known as the phosphate-binding loop (P-loop) or kinase 1a, plays a role in binding phosphates to the nucleotide; the Walker B or kinase-2 motif coordinates the divalent metal ion required for phosphotransfer reactions; kinase-3a is involved in binding the purine base or the pentose of the nucleotide (Tameling et al., 2002); RNBS-A directly influences the rate of ATP hydrolysis (Tameling et al., 2006); the RNBS-B motif regulates the location of its adjacent parts; and the MHD motif directly contacts ADP and allows nucleotide exchange and activation of the R protein (van Ooijen et al., 2008).

Besides the seven parts of the NB-ARC domain, *CreV8* contains an “LDH” amino acid sequence behind the RNBS-D motif at position 523 to 525 aa (Figure 5B). This may play the same role as the “MHD” motifs in the other four genes. The LRR domain, which might play a role in ensuring apoptosome activation, is recognition-dependent and plays a role in recognizing diverse pathogen-derived ligands (Tameling et al., 2002). Comparisons of the NB-ARC domain structure among *Cre* genes (*CreV8*, *Cre3*, *CreZ*) and *V6* revealed some typical and some unique characteristics. These results imply that members of the CCN resistance gene family may be regulated differently from other *R* genes, which is consistent with the results of the homology analyses.

Based on the well-conserved domains and previous studies, we can construct a hypothetical functional model of *CreV8* (Figure 6). The Walker A, RNBS-A, Walker B, and RNBS-B motifs make up a pocket for catalytic nucleotide-binding hydrolysis of the nucleotide (Figure 6A). RNBS-C and GLPL form a four-helix bundle (Figure 6B), which serves as the scaffold for the intramolecular interaction with the LRR domain. The RNBS-D and MHD motifs form a winged-helix fold as a regulatory element that transduces pathogen perception into *R*-protein activation *via* its interaction with the LRR (Figure 6C) (Rairdan and Moffett, 2006; van Ooijen et al., 2008). Although a crystal structure is required to confirm the 3D model of *CreV8*, the model may help us to understand the function of the conserved elements within the context of the entire protein. In addition, the putative tertiary structure provides a framework to predict how mutations in *CreV8* exert their effects (van Ooijen et al., 2008).

Conclusion

In summary, *CreV8* could be a good candidate as a resistance gene to *H. avenae*. This study provides useful information for the functional analysis of *Cre* genes and their potential use for protecting host plants against invasion of the nematode. Further studies should be conducted to confirm the function of the gene product and to analyze the regulation of the gene. Such studies increase our knowledge of the biological and functional roles of *Cre* genes at different developmental stages and in the plant response to various biotic pests, such as parasitic nematodes.

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