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RESEARCH ARTICLE

Cloning and RNA interference analysis of the salivary protein C002 gene in *Schizaphis graminum*

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Abstract

The full-length cDNA of functionally-unknown salivary protein C002 in *Schizaphis graminum* was cloned using rapid amplification of cDNA ends (RACE) and designated as *SgC002* (GenBank accession no. KC977563). It is 767 bp long and encodes a protein of 190 amino acid residues with a predicted mass of 21.5 kDa and a predicted cleavage site of N-terminal signal peptide between the 24th and the 25th residues. *SgC002* is specifically expressed in salivary gland with the highest level at the 2nd instar. Introducing *SgC002*-specific 476-siRNA, but not 546-siRNA to aphids through artificial diet significantly suppressed *SgC002* expression. Silencing *SgC002* gene led to lethality of the aphid on wheat plants, but not on pure artificial diet. Our study demonstrated that artificial diet-mediated RNAi can be a useful tool for research on the roles of genes in aphid salivary gland, and also provided new insights into the characteristics of *C002* in wheat aphids.

Keywords: *Schizaphis graminum*, salivary protein C002, cDNA clone, siRNA

1. Introduction

Aphids are sap-sucking insects of Hemipetera, and considered as important pests (Blackman *et al.* 2000). The greenbug, *Schizaphis graminum*, a serious pest of cereal crops, can cause serious economic losses by both direct feeding and transmitting viruses (Ryan *et al.* 1990). Aphid saliva plays an important role in aphid-host plant interactions. During the progress of probing and feeding, aphids secreted two types of saliva: gelling saliva which is solidified into tube-like sheath to protect aphid stylets from mechanical damage and chemical attacks, and watery saliva, which is secreted into plant cells, intercellular substance and phloem to assist aphids to digest nutrients (Miles 1987, 1999; Prado *et al.* 2007). Some chemicals in the saliva may elicit the host plant defense response while others can suppress it. As a big group of such chemicals, saliva proteins, such as β-glucosidases, glucose oxidase, and calcium-binding proteins, of some aphid species were recently studied, and the sequence and structure have been identified using proteomic technology and mass spectrometry.

C002 is an aphid-specific watery saliva protein and predominantly expresses in the salivary glands (Mutti 2006; Pitino *et al.* 2011). In spite of little information for this protein at molecular level, some studies showed that it is related to aphid feeding behavior and subsequent survival and fecundity. Knockdown of C002 transcript of *Acyrthosiphon pisum* led to the death of *A. pisum* possibly due to the lack of feeding (Mutti *et al.* 2006, 2008) and the reproduction rate

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of *Myzus persicae* can be increased by feeding on the host plants over-expressing *MpC002* and decreased by feeding on those producing double-strand RNA (dsRNA) against C002 (Bos *et al.* 2010; Pitino *et al.* 2011). At proteomic level, C002 protein has been identified in *A. pisum* and *M. persicae* saliva (Harmel *et al.* 2008; Carolan *et al.* 2009). Moreover, C002 protein has been detected in host plant fava bean after *A. pisum* feeding, suggesting that C002 protein was secreted into host plant during feeding (Mutti *et al.* 2008). Together, the above results indicated that C002 protein is crucial for aphid feeding and colonization on host plants and may play important roles in aphid-host plant interactions. At present, the researches on C002 have mainly focused on *A. pisum* and *M. persicae*. There is no report on C002 in *S. graminum*.

In 1998, RNA interference (RNAi) was firstly demonstrated when dsRNA was injected into *Caenorhabditis elegans* leading to silence of the homolog endogenous mRNA (Fire *et al.* 1998). Since then RNAi has become a powerful reverse-genetic tool to analyze the function of genes. Moreover, successful application of RNAi technology in insects through feeding assay especially the plant delivered dsRNA experiments demonstrated the potential of RNAi for pest control (Huvenne and Smagghe 2010). However, to our knowledge, RNAi has not been documented in *S. graminum.*

In this study, an ortholog of *C002* gene was cloned from *S. graminum*, the expression of *C002* in different tissues and instars of aphids was investigated, and the effects of siRNAs on *C002* gene expression and survival of aphid were examined. Our results demonstrated that siRNA could be taken up through the normal dietary path to silence the target gene in aphid salivary gland and also provided valuable insight into the function of *C002* and its role in aphid-host plant interactions.

2. Results

2.1. cDNA cloning and sequence analysis of salivary protein C002 gene of *S. graminum*

A single cDNA fragment of *C002* gene with the length of about 176 bp was obtained using degenerate primers and RT-PCR method. The fragment was gel purified and used in Sanger sequencing, 5′ and 3′ ends of the fragment were amplified using 5′ RACE and 3′ RACE respectively. The deduced full length cDNA (767 bp) was obtained using DNAMAN software (Fig. 1) and named as *SgC002* with GenBank accession number of KC977563. By using the ORF Finder software, we found that the cDNA sequence

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1      AGGAACAAATTTATCGTCGTGTCGCACAGGATAGTGATTATTTACAGCATGGGAAGTTAT
                                                      M  G  S  Y
61     CAATTATATGTGGCCGTCATGGCAATAGTTAGCTTAGCCGCAGTACAGAAAGCTAGTGCT
        Q  L  Y  V  A  V  M  A  I  V  S  L  A  A  V  Q  K  A  S  A
121    GACGTGTATGCCGACTATTCCGCTGAAGAAGAATACGATGAATCGAAAGAGATGTACGGA
        D  V  Y  A  D  Y  S  A  E  E  E  Y  D  E  S  K  E  M  Y  G
181    TCAGAAATAAAGCCCCATAAGTGTGAAGAATACAAATCGAAGATTTGGAACCAAGCATTT
        S  E  I  K  P  H  K  C  E  E  Y  K  S  K  I  W  N  Q  A  F
241    AGCAATCCAGCGGCTATGCAGCTGATGGAAGTAGTGTTTCAAACAGGTAAAGAATTGGGC
        S  N  P  A  A  M  Q  L  M  E  V  V  F  Q  T  G  K  E  L  G
301    ACCGATAAAGTGTGCTCAGATACAATTCGAGTCCTTTCTAACTTCGTCAATGTGATGGCC
        T  D  K  V  C  S  D  T  I  R  V  L  S  N  F  V  N  V  M  A
361    ACTAACCAGAACGCTCACTACTCGATGGGTATGTTGGGCAAGATGCTGACGTTCATTTTG
        T  N  Q  N  A  H  Y  S  M  G  M  L  G  K  M  L  T  F  I  L
421    AGAGAGATGGACATGACGTCGGATAAGTTCGTAGAGACAAAGGGCGTGTTTGAACGCATC
        R  E  M  D  M  T  S  D  K  F  V  E  T  K  G  V  F  E  R  I
481    GCGAAAAATGTTAATATCCGTGACTATATCAAGAATTCCACCTCCCGAGTCATCGACTTG
        A  K  N  V  N  I  R  D  Y  I  K  N  S  T  S  R  V  I  D  L
541    CTCAAGATACCAGTGATTAGAAGTCGACTAGCTAGAGTAGTGAAAGCCTTCGAGAGTCTG
        L  K  I  P  V  I  R  S  R  L  A  R  V  V  K  A  F  E  S  L
601    ATTAACCCATCTAGAAATTAACAAAGGGTCAAGCAGAAAATTAATGGACTACCTAGTAAA
        I  N  P  S  R  N
661    ATTTCTAATAAACATGTTCATATAACAGACTAGTGATATTTATGTATAATTATATATATA
721    TATTCATTAAATATACATTTACTTAAAAAAAAAAAAAAAAAAAAAA
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Fig. 1 The nucleotide sequence and deduced amino acid sequence of *SgC002* gene. Arrow indicates the cleavage site of signal peptide. The initial 24 amino acid residues consist of a signal peptide.

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