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RESEARCH ARTICLE

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

ZHANG Yong, FAN Jia, SUN Jing-rui, CHEN Ju-lian

State Key Laboratory for Biology of Plant Diseases and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing 100193, P.R.China

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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ZHANG Yong, E-mail: zhangyongnky@163.com;
Correspondence CHEN Ju-lian, Tel: +86-10-62813685, E-mail: jlchen@ippcaas.cn

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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

```
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.

contains an open reading frame (ORF) of 573 bp encoding a protein with 190 amino acids. The molecular mass of predicted protein is 21.5 kDa and the amino acid sequence contains a signal peptide with a cleavage site at N terminal side of Ala[24] predicted by SignaIP. BLAST results showed that this gene had no homology with any species other than Aphididae. Multiple sequence alignment and homology analysis revealed that *SgC002* had the highest sequence identity (69%) to the one in *Toxoptera citricida* (Fig. 2).

2.2. Expression of *SgC002* in different tissues of *S. graminum*

Expression of *SgC002* in the head, thorax, abdomen, salivary gland, gut, and salivary gland-removed head of *S. graminum* was studied using real-time PCR. As shown in Fig. 3, in contrast to the low expression of *SgC002* detected in the head, thorax, abdomen, gut, and salivary gland-removed head of *S. graminum*, it was highly expressed in the salivary gland (P<0.01), indicating that *SgC002* is specifically expressed in salivary gland of *S. graminum*.

2.3. Expression of *SgC002* at different developmental stages *of S. graminum*

The expression levels of *SgC002* at different developmental stages (the 1st, 2nd, 3rd and 4th instars and adult) of *S. graminum* were examined using real-time PCR. The results showed that its expression level varied among different stages (Fig. 4). The expression level was the lowest at the 1st instar, rapidly enhanced to the highest at the 2nd instar (P<0.01), gradually decreased at the 3rd and 4th instars, and slowly increased again at the adult stage.

2.4. Relative expression of *SgC002* after feeding on siRNA

The silencing efficiency of the two siRNAs on *SgC002* gene was examined using real-time PCR. The results showed that the expression of *SgC002* gene was significantly decreased after feeding on the diet containing 476-siRNA. It was only (30.78±2.75), (13.98±1.78) and (8.48±0.67)% of the original level at day 3, 5 and 7, respectively, and signifi-

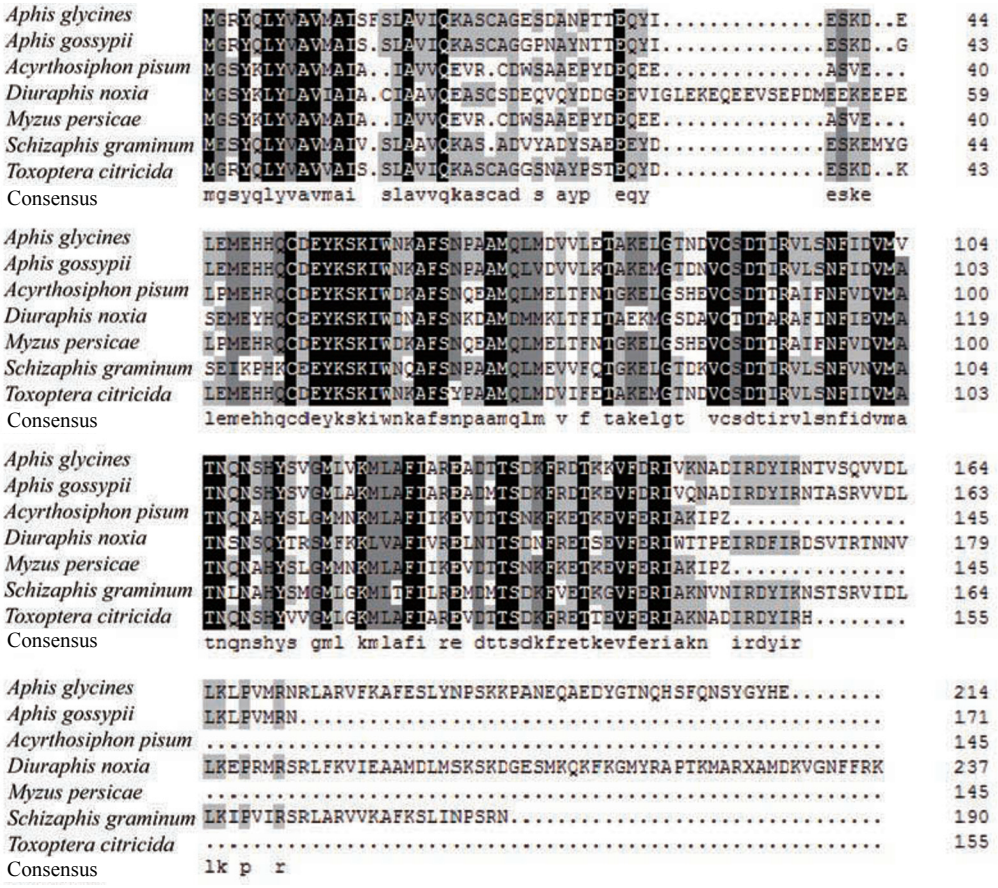

Fig. 2 Amino acid sequence alignment of C002 in different aphid species. *Aphis glycines*, soybean aphid; *Aphis gossypii*, cotton aphid; *Acyrthosiphon pisum*, pea aphid; *Diuraphis noxia*, Russian wheat aphid; *Myzus persicae*, green peach aphid; *Schizaphis graminum*, greenbug; *Toxoptera citricida*, brown citrus aphid.

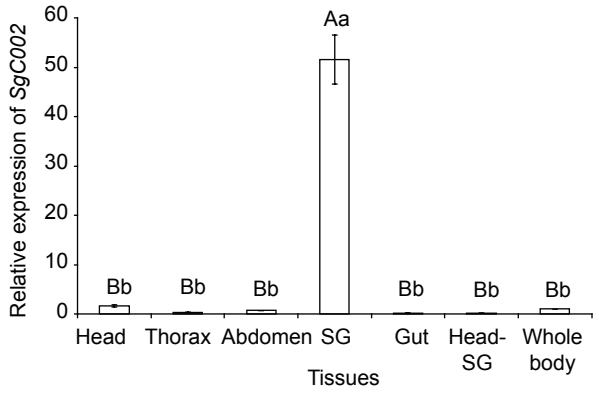

Fig. 3 The expression level of *SgC002* in different tissues of *S. graminum*. SG, aphid salivary gland; Head-SG, salivary gland-removed head. Lower case (P<0.05) and upper case letters (P<0.01) indicate statistical significance at different levels (one-way ANOVA). The same as below.

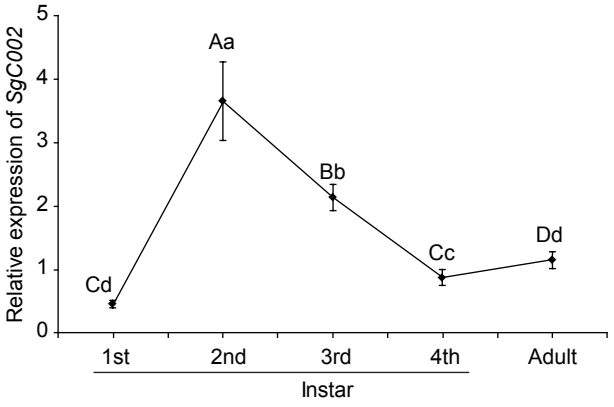

Fig. 4 The expression level of *SgC002* at different developmental stages of *S. graminum*.

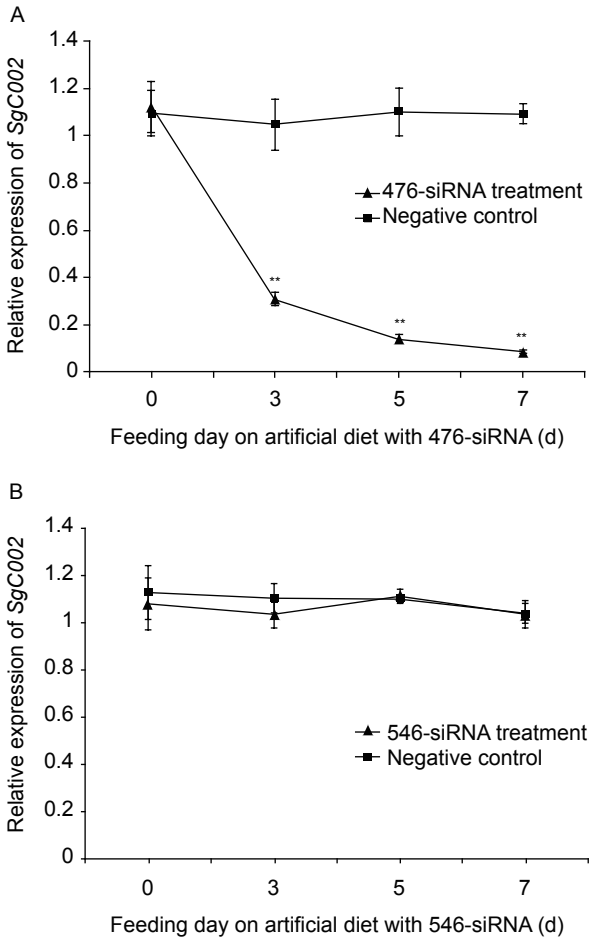

Fig. 5 The relative expression level of *SgC002* of *S. graminum* at different time points after aphid fed on two different siRNAs and their controls. A, the relative expression level of *SgC002* after aphid fed on 476-siRNA and negative control at day 0, 3, 5, 7. B, the relative expression level of *SgC002* after aphid fed on 546-siRNA and negative control at day 0, 3, 5, 7. ** indicates highly statistical significance (P<0.01, t-test). The same as below.

cantly lower than that of the negative control group (P<0.01) (Fig. 5-A). In contrast, the expression of *SgC002* gene was not suppressed by feeding the diet containing 546-siRNA (Fig. 5-B). The results suggested that feeding 476-siRNA but not 546-siRNA could effectively silence *SgC002* gene.

2.5. Effects of *SgC002* silencing on survival rate of *S. graminum*

The effects of *SgC002* silencing on the survival rate of *S. graminum* were detected. Firstly, aphids were fed on artificial diet with 476-siRNA for 3 days to silence the target gene and then transferred onto pure artificial diet (without any siRNA) and aphid-susceptible wheat, respectively. Fig. 6-A shows that the survival rate of *S. graminum* fed on pure artificial diet for 7 days after being pretreated with 476-siRNA for 3 days was not significantly different from that of *S. graminum* in the negative control group. However, as shown in Fig. 6-B, the survival rate of *S. graminum* fed on the wheat rapidly decreased to (84.45±3.85)% at day 2, significantly lower than that of the control group (P<0.05), and further decreased to (48.89±3.85)% at day 4, much lower than that of the control group (P<0.01). At day 7, the survival rate dropped to (37.77±3.85)%, significantly lower than that of the control group (P<0.01).

3. Discussion

Aphids are sap-sucking insects, they can secrete a variety of salivary proteins to assist the feeding process. Salivary protein C002 has been confirmed to play an important role in their feeding process. Researches on C002 can provide new insight into mechanisms of aphid-host plant interaction and viral transmission, and help to screen new target genes

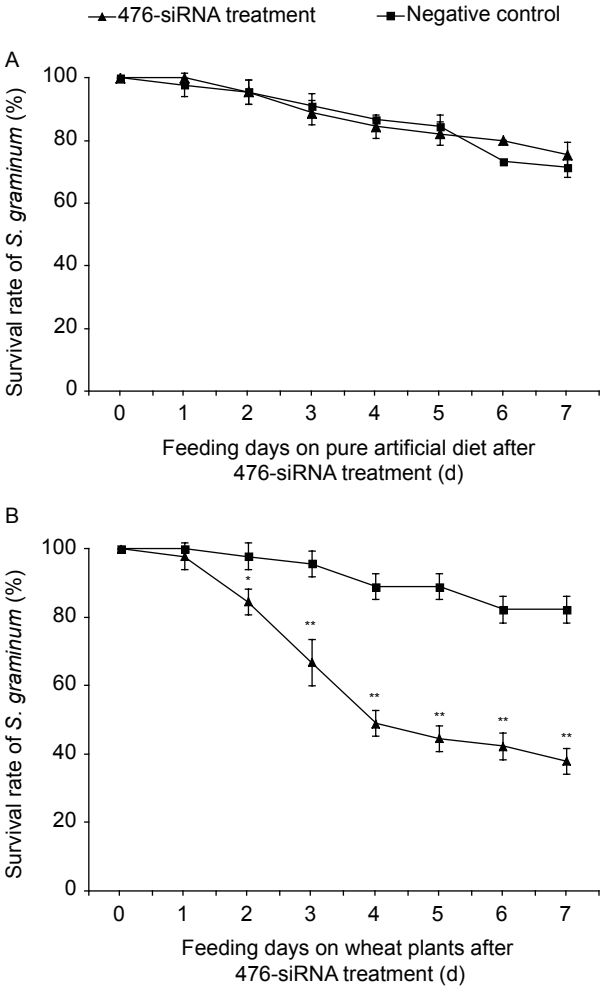

Fig. 6 Effects of *SgC002* silencing on the survival rate of *S. graminum.* A, the survival rate of *S. graminum* fed on pure artificial diet after fed on 476-siRNA and negative control for 3 days. B, the survival rate of *S. graminum* fed on aphid-susceptible wheat after fed on 476-siRNA and negative control for 3 days.

for pest control.

In this study, we cloned full length *C002* cDNA from *S. graminum* and named it *SgC002*. Amino acid sequence alignment among different species of aphids indicated that C002 had typical conservative regions as well as variable regions, suggesting this group of genes is undergoing rapid evolution. Pitino and Hogenhout (2013) found that C002 protein from different aphids showed inter-species-specificity in terms of promoting colonization. In other words, reproduction of *M. persicae* increased significantly when fed on transgenic *A. thaliana* which over-expressed *M. persicae* salivary protein C002, whereas *M. persicae* reproduction did not increase when fed on transgenic *A. thaliana* which over-expressed *A. pisum* salivary protein C002. It is speculated that this may be related to different selection pressures that aphids face, such as different host plant defense responses and nutritional conditions.

Tissue-specific expression analysis showed that *SgC002* is specifically expressed in the salivary glands of aphids, which is consistent with Pitino *et al.* (2011) but slightly different from Mutti *et al.* (2008), in which they found that *C002* also expressed at low level in the intestine of *A. pisum*. This discrepancy needs further verification. Expression level of *SgC002* is the highest at the 2nd instar, which is possibly related to different nutrients requirement at different stages because the growth rate of *S. graminum* is much higher at the 2nd and 3rd instar stages than other stages. Additionally, the elevated expression of *SgC002* may also be related to stronger plant defense responses accompanied by relatively high feeding rate. RNAi technology is a very useful reverse genetic tool and has been widely used in insect research, mainly in insect functional genomics study. In recent years, due to increased pest resistance to traditional pesticides and insect-resistant crops, screening new and efficient insect-resistant genes and creating new insect-resistant crops are of significant importance. Since RNAi has characteristics of efficiency, specificity and stable geneticity, it has become one of the most popular and promising techniques used in genetically engineering crops resistance to insect pests (He *et al.* 2009; Yang *et al.* 2009).

In this experiment, we introduced siRNA through diet and successfully silenced aphid salivary gland specific gene *SgC002*. The silencing efficiency is more than 90% at day 7 after feeding 476-siRNA. The results illustrated that feeding siRNA through normal dietary path to directly introduce RNAi is feasible. Pitino *et al.* (2011) found that partially silencing *MpC002* (40% reduction in expression) significantly reduced *M. persicae* reproduction rate, but not survival rate. In contrast, our results indicated that the complete knock-down of *SgC002* gene could cause the high mortality of aphids. Therefore, as an aphid-specific protein, *C002* has great potential in controlling aphid pest as the target gene.

Interestingly, siRNAs that target to different sites of the same gene can have different silencing efficiencies. In this paper, we designed two siRNAs: 476-siRNA and 546-siRNA targeting to the site of 476–498 bp and 546–568 bp, respectively. However, the experiment results showed that 476-siRNA but not 546-siRNA has the highest silencing efficiency and could effectively inhibit *SgC002* expression in a short time. Similar phenomena were also found in other studies using both mammals and insects as the model organisms (Holen *et al.* 2002; Ciladi *et al.* 2003; Song *et al.* 2003; Yoshinari *et al.* 2004; Yang *et al.* 2009). Far *et al.* (2003) found that the effectiveness of siRNA was closely related to the secondary structure of its target sites, such as local RNA folding, and the subsequent accessibility for siRNA. This study indicates that the loop segment is more

accessible than the stem segment of the mRNA. The secondary structure of *SgC002* mRNA was predicted using the Mfold Web Server (http://mfold.rna.albany.edu/?q=mfold). We found that target site of 476-siRNA mainly consisted of a loop segment with 15 nucleotides, but the target site of 546-siRNA was mainly composed of stem segments so different silencing efficiencies of 476-siRNA and 546-siRNA are very likely related to the difference of accessibility for siRNAs.

Another interesting finding is that feeding *S. graminum* on wheat plants but not pure artificial diet significantly affected the survival of *S. graminum* pretreated with 476-siRNA, indicating that silencing *C002* gene is conditionally lethal to aphids based on aphid-host plant interactions. However, its underlying mechanism is still unknown. In the next step, we will conduct comprehensive study using techniques such as eukaryotic expression, *in situ* hybridization and co-immunoprecipitation to verify C002 function and establish transgenic wheat capable to produce *SgC002* dsRNA to detect its effects of gene expression and aphid survival.

4. Conclusion

In this paper, we reported the full-length salivary gene *C002* from *S. graminum.* The gene is expressed specifically in the salivary glands of *S. graminum* and the highest expression level is at the 2nd instar. Artificial diet-mediated siRNA with specific target site can effectively silence *C002* gene and lead to the lethality of *S. graminum.*

5. Materials and methods

5.1. Materials

Clone of *S. graminum* was initially established from a single aphid collected from wheat field in Langfang, Hebei Province, China, and has been reared on wheat plants (Beijing 837 variety), which is susceptible to *S. graminum*, for 5 years (25–30 generations every year) in an indoor environment with temperature of (20±1)°C, relative humidity of (75–80)% and photoperiod of 14 h L:10 h D.

5.2. Cloning of full-length cDNA of *C002* from *S. graminum*

About 300 salivary glands were dissected from adult alate females of *S. graminum* in PBS buffer, pH 7.0, and homogenized in a 1.5-mL centrifuge tube. The total RNA was then extracted using Micro RNA Extraction Kit (PureLink RNA Mini Kit, Ambion, USA) according to the instructions provided by the manufacturer, during which genomic DNA was removed by DNase incubation (DNase I, Invitrogen, USA). The quality of RNA was examined by electrophoresis and the quantity was measured by NanoDrop 2000 (Thermo Scientific, USA). The synthesized cDNA from the above RNA template, *via* reverse transcription (Transcript One-Step gDNA Removal and cDNA Synthesis Supermix, TransGenBiotech, Beijing, China), was used for the following analysis.

The degenerate primer pairs *C002*-hop-F and *C002*-hop-R (Table 1) used for *C002* gene cloning were designed using an online degenerate primer design software CODEHOP (http://blocks.fhcrc.org/codehop.html) based on the amino acid sequences of C002 from five species of aphids including *Aphis glycines* soybean aphid, *Aphis gossypii* cotton aphid, *A. pisum* pea aphid, *Diuraphis noxia* Russian wheat aphid, *M. persicae* green peach aphid and *Toxoptera citricida* brown citrus aphid. The PCR conditions were 3 min at 94°C, 35 cycles of 30 s at 94°C, 30 s at 50°C and 30 s at 72°C, and final 10 min at 72°C. The obtained cDNA fragment was sequenced and used as template to design the 3′ and 5′ RACE primers: *C002*-3′-GSP1, *C002*-3′-GSP2, *C002*-5′-GSP1 and *C002*-5′-GSP2 (Table 1). The 5′ and 3′ ends of the cDNA were amplified using RACE Kit (5′-Full RACE Kit, 3′-Full RACE Core Set, TaKaRa, Dalian, China) according to the instructions provided by the manufacturer. The obtained cDNA fragments were spliced using software DNAMAN to obtain the full-length cDNA sequence of salivary protein *C002* gene from *S. graminum*. The open read frame (ORF) of the gene was further verified using a specific primer pair *C002*-orf-F and *C002*-orf-R (Table 1) at the following conditions: 3 min at 94°C followed by 35 cycles of 30 s at 94°C, 30 s at 55°C and 30 s at 72°C and final 10 min at 72°C.

5.3. Sequence analysis

The ORF was predicted using ORF finder (http://www.ncbi.nlm.nih.gov/gorf/gorf.html). Amino acid sequence and protein physicochemical properties were deducted using software DNAMAN. Multiple sequence alignment was first performed using CLUSTAL W2 (http://www.ebi.ac.uk/Tools/msa/clustalw2/) and then refined using BoxShade (http://ch.embnet.org/software/BOX_form.html).

5.4. Expression of *SgC002* in different tissues and developmental stages of aphid

The expression of *SgC002* in different tissues and developmental stages of aphid was quantified by real-time PCR. Briefly, RNA were extracted from the head, thorax, abdomen, salivary glands, gut, salivary gland-removed head and whole body of adult alate *S. graminum* of different instars. The primers (*SgC002*-F1 and *SgC002*-R1, Table 1) specific to *SgC002* were designed as described above and *Actin* (*Actin*-F and *Actin*-R, Table 1) was used as reference gene

Table 1 PCR primers for *SgC002* cloning

Name of primer	Sequences of primers (5′→3′)	Amplicon length (bp)
C002-hop-F	GGAACACCACCAATGTGANGARTAYAA	176
C002-hop-R	TGGTGGCCATCACGTCNAYRAARTT	
C002-3′-GSP1	CGGCTATGCAGCTGATGGAAGTAG	375
C002-3′-GSP2	TTCAAACAGGTAAAGAATTGGGCAC	
C002-5′-GSP1	AGGACTCGAATTGTATCTGAGCACAC	350
C002-5′-GSP2	CGGTGCCCAATTCTTTACCTGTTTG	
C002-orf-F	ATTTATCGTCGTGTCGCA	633
C002-orf-R	ATTTTCTGCTTGACCCTT	
SgC002-F1	CGTCGGATAAGTTCGTAG	177
SgC002-R1	ATGGGTTAATCAGGCTCT	
Actin-F	CGGTTCAAAAACCCAAACCAG	260
Actin-R	TGGTGATGATTCCCGTGTTC	

(Pitino *et al.* 2011). A total of 1 μg RNA was reverse transcribed into cDNA, diluted by 10-fold and used as template to detect the relative expression of the target gene in a 20 μLreal-time PCR reaction system containing 2 μL of cDNA, 0.5 μL of 10 μmol L^{-1} forward primer and reverse primer each, 10 μL of 2× SYBR premix *Ex Taq*™ (Tli RNase H Plus) and 0.4 μL of 50× ROX reference dye II at the following conditions: 30 s at 95°C followed by 40 cycles of 30 s at 95°C, 30 s at 60°C. Each treatment was performed in triplicate and the differential expression was calculated using $2^{-\Delta\Delta CT}$ method (Livak and Schmittgen 2001).

5.5. Preparation of aphid artificial diet and feeding apparatus

The aphid artificial diet and feeding apparatus were prepared according to Chen *et al.* (2000). 200-μL artificial diet was sandwiched between two lays of parafilm membrane and stretched to a glass tube with 21-mm diameter under sterile conditions. Artificial diet was sterilized by passing through a 0.2-μm filterand stored at –20°C before use.

5.6. siRNAs and their silencing efficiency

Two siRNA targeting against *SgC002* gene and corresponding siRNA controls were designed using BLOCK-iT™ RNAi Designer (http://rnaidesigner.invitrogen.com/rnaiexpress/) and named based on their target sites on ORF as 476-siRNA, 546-siRNA, 476-siRNA-control and 546-siRNA-control, respectively (Table 2).

The two siRNAs and their controls were diluted to 20 ng μL^{-1} in artificial diet. A total of 600 alate adult *S. graminum* were taken from fresh wheat. After starved for 3 h, 30 active *S. graminum* were transferred to each feeding device placed in an artificial climate chamber with temperature of (20±1)°C, humidity of 75% and photoperiod of 14 h L:10 h D. In total, there were 3 replicates for each treatment, e.g., 476-siRNA, 546-siRNA, 476-siRNA-control and 546-siRNA-control, and

Table 2 Sequences of siRNAs

Name of siRNAs	Sequences of siRNAs (5′→3′)
476-siRNA	CCCGAGUCAUCGACUUGCUCAAG
	AUCUUGAGCAAGUCGAUGACUCG
476-siRNA-control	CCCUGACCUAGUCGUUCACAAGG
	AUCCUUGUGAACGACUAGGUCAG
546-siRNA	GAGUCUGAUUAACCCAUCUAGAA
	AUUUCUAGAUGGGUUAAUCAGAC
546-siRNA-control	GAGGUUAAUCACCUAUCGAACUA
	AUUAGUUCGAUAGGUGAUUAACC

360 aphids were used. The aphids were kept on the diet for 7 days and the diet was changed every 3 days. One alive aphid was taken from each treatment at day 0, 3, 5, 7, and the total RNA was extracted and used to evaluate the silencing efficiency.

5.7. Detection of aphid survival after *SgC002* gene silencing

The effect of *SgC002* gene silencing on aphid survival in plant was investigated using 476-siRNA and 180 aphids were used in total. Briefly, after starved for 3 h, 90 active alate adult *S. graminum* were transferred to artificial diet containing either 20 ng μL^{-1} 476-siRNA or 20 ng μL^{-1} 476-siRNA-control. After feeding for 3 days aphids from each treatment were equally divided and transferred to the 3 replicates of new artificial diet (without any siRNA) and wheat plants (Beijing 837 variety). The number of survival aphids was recorded for 7 consecutive days and the percentage of the survival aphids was calculated.

5.8. Statistical analyses

The fold change of the expression of *SgC002* in different tissues and developmental stages to the whole adult alate aphid was calculated. The silencing efficiency of siRNA on different days was calculated with day 0 expression as

the baseline. All data were analyzed using SAS 9.0 software and the differences between or among groups were examined using *t*-test or one-way analysis of variance. *P* values less than 0.05 and 0.01 were considered as statistical significance and highly statistical significance respectively.

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