



Odorant-Binding Protein 2 is Involved in the Preference of *Sogatella furcifera* (Hemiptera: Delphacidae) for Rice Plants Infected with the Southern Rice Black-Streaked Dwarf Virus

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Odorant-binding protein 2 is involved in the preference of *Sogatella furcifera* (Hemiptera: Delphacidae) for rice plants infected with the *Southern rice black-streaked dwarf virus*

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Abstract

Southern rice black-streaked dwarf virus, transmitted by the white-backed planthopper (*Sogatella furcifera* [Horváth]) (Hemiptera: Delphacidae) was first found in Guangdong Province, China. A previous study has demonstrated that the host plant preferences of *S. furcifera* are altered by infection with the virus, with virus-free *S. furcifera* preferring virus-infected rice plants over healthy rice plants, whereas viruliferous *S. furcifera* prefer uninfected plants. To test the hypothesis that odorant-binding proteins (OBPs) are involved in the preference of *S. furcifera* for virus-infected rice plants, we first compared the expression levels of *SfurOBP2* and *SfurOBP11* in virus-free and viruliferous *S. furcifera*. The results show that mRNA transcript of these 2 genes were significantly reduced in viruliferous *S. furcifera*. We then used RNAi-mediated gene silencing to confirm the function of these 2 odorant-binding proteins in host selection of *S. furcifera*. The results showed that silencing of the *SfurOBP2* gene caused virus-free *S. furcifera* to no longer prefer virus-infected rice plants over uninfected rice plants, but the ability to locate host plants was maintained. These results indicate that *SfurOBP2* appears to play a crucial role in the preference of *S. furcifera* for virus-infected rice plants.

Key Words: white-backed planthopper; host plant choice; gene silencing; olfactory protein

Resumen

El virus del enanismo de raya negra del arroz del sur, transmitido por el delfácido de espalda blanca (*Sogatella furcifera* [Horváth]) (Hemiptera: Delphacidae) se encontró por primera vez en la provincia de Guangdong, China. Un estudio anterior ha demostrado que la preferencia de las plantas hospederas de *S. furcifera* se ven alteradas por la infección del virus, con los *S. furcifera* libres de virus prefiriendo las plantas de arroz infectadas con el virus sobre las plantas de arroz sanas, mientras que los *S. furcifera* virulíferos prefiriere las plantas no infectadas. Para probar la hipótesis de que las proteínas de unión a odorantes (PUO) están implicadas con la preferencia de *S. furcifera* por las plantas de arroz infectadas por virus, primero comparamos los niveles de expresión de *SfurOBP2* y *SfurOBP11* en *S. furcifera* libre de virus y con virus. Los resultados muestran que el transcrito de ARNm de estos 2 genes se redujo significativamente en *S. furcifera* virulífero. Luego utilizamos el silenciamiento de genes mediado por RNAi para confirmar la función de estas 2 proteínas de unión a odorantes en la selección del hospedero de *S. furcifera*. Los resultados mostraron que el silenciamiento del gen *SfurOBP2* causó que los *S. furcifera* sin virus, dejara de preferir las plantas de arroz infectadas por el virus en lugar de las plantas de arroz no infectadas, pero se mantuvo la capacidad de localizar las plantas hospederas. Estos resultados indican que *SfurOBP2* parece jugar un papel crucial en la preferencia de *S. furcifera* por las plantas de arroz infectadas con virus.

Palabras Clave: delfácido de espalda blanca; selección de la planta hospedera; silenciamiento de genes; proteína olfativa

Ecosystems containing plants, plant pathogens, and insect vectors are characterized by complex interactions (Stout et al. 2006; Lu et al. 2016). For example, some plant viruses have pathogenic effects on the development and fecundity of insect vectors (Czosnek & Ghanim 2012), and also can modify the host selection behavior of their vectors directly or indirectly. Previously documented examples showed enhanced attractiveness of insect vectors to virus-infected plant hosts compared to healthy plant hosts (Alvarez et al. 2007; Mauck et al. 2010a, b; McMenemy et al. 2012; Liu et al. 2013). The first evidence that acquisition of

a plant virus directly alters host selection behavior by its insect vector showed that virus-free *Rhopalosiphum padi* (L.) (Hemiptera: Aphididae) aphids preferred *Barley yellow dwarf virus*-infected wheat plants compared to healthy plants, whereas the aphids that had acquired virus during feeding preferred noninfected plants (Ingwell et al. 2012). More recently, the rice pest white-backed planthopper (*Sogatella furcifera* [Horváth]) (Hemiptera: Delphacidae) (Hoang et al. 2011; Matsukura et al. 2013; Zhou et al. 2013), was shown to transmit *Southern rice black-streaked dwarf virus* in a persistent propagative manner (Zhou

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et al. 2008; Pu et al. 2012). Further, it was shown that virus-free *S. furcifera* significantly prefer virus-infected rice plants to healthy plants. In contrast, viruliferous *S. furcifera* prefer healthy plants over infected plants (Wang et al. 2014). Previous works on the vector-virus-plant interaction focused on the feeding behavior, development, and fecundity of *S. furcifera* (Tu et al. 2013; Xu et al. 2014; Lei et al. 2016), and the changes in the nutrients composition and the volatiles emission of rice plants after the infection of the virus (He et al. 2014; Wang et al. 2017). However, limited research analyzed the mechanism through which the virus affects the *S. furcifera* host plant preferences.

Olfaction plays a crucial role in guiding insects to find food sources, mating partners, and oviposition sites. Insects detect and locate food sources for reproduction based on the volatile chemical signals emitted from the host plants (Anfora et al. 2009; Allmann et al. 2013). Generally, odorant-binding proteins (OBPs) are the first proteins that interact with odors in the sensillum lymph when odors enter olfactory sensilla (Acín et al. 2009). Convincing evidence has documented that odorant-binding proteins participate in the olfactory perception in the insect. For instance, RNA interference (RNAi) knockdown of *LmigOBP1* in *Locusta migratoria* (L.) (Orthoptera: Acrididae) abolished electrophysiological responses by locusts to maize leaf volatiles (Li et al. 2016). Analogous results have been obtained in *Bactrocera dorsalis* (Hendel) (Diptera: Tephritidae), wherein blocking of *BdorOBP83a-2* decreased the behavioral responses to attractant semiochemicals (Wu et al. 2016). Researchers have also proposed that odorant-binding proteins play a role in host plant choice, and it has been demonstrated that *OBP57d* and *OBP57e* are implicated in the host plant preference of *Drosophila sechellia* Tsacas and Baechli (Diptera: Drosophilidae) (Matsuo et al. 2007).

Ten odorant-binding proteins have been identified recently in the brown planthopper (*Nilaparvata lugens* Stål) (Hemiptera: Delphacidae) (He et al. 2011; Zhou et al. 2014), and the results of RNAi experiments show that *NlugOBP3* is involved in nymph olfaction on rice plants (He et al. 2011). In *S. furcifera*, 12 *SfurOBP* genes have been identified, and the relative expression of 2 of them, *SfurOBP2* and *SfurOBP11*, was found to be significantly higher than those of other *SfurOBPs* in the antennae (He & He 2014). Little is known, however, about the role of *SfurOBPs* in *S. furcifera* host selection, especially the role of *SfurOBPs* in the preference of virus-free *S. furcifera* for virus-infected rice plants vs. healthy rice plants.

Generally, the antennae are the most important olfactory organ for the insect (Vogt & Riddiford 1981). To test our hypothesis that *SfurOBPs* are involved in the preference of virus-free *S. furcifera* for virus-in-

fecting rice plants, we measured the expression levels of *SfurOBP2* and *SfurOBP11* in antennae-enriched genes in virus-free and viruliferous *S. furcifera*. We then used RNAi technology to knockdown *SfurOBP2* and *SfurOBP11* with dsRNA (dsOBP2 and dsOBP11). At 24 h after the dsRNA injection, we tested the behavioral responses of *S. furcifera* for air (empty pot with soil), healthy plants, and virus-infected rice plants by using a glass Y-tube olfactometer.

Materials and Methods

INSECT REARING AND VIRUS-INFECTED PLANTS

The rice used in this study (cultivar 'Fengyuanyou 299') was purchased from Hunan Longping Seed Industry Co., Ltd., Changsha, Hunan Province, China. *Sogatella furcifera* were collected from rice fields in Changsha, Hunan Province, China, and reared in the laboratory with healthy rice plants for more than 3 generations at 26 ± 1 °C, 85% RH, under a 16:8 h (L:D) photoperiod. *Southern rice black-streaked dwarf virus*-infected rice plants were kindly provided by Guohui Zhou (South China Agricultural University, Guangzhou, China). The inoculated plants were subjected to reverse-transcription polymerase chain reaction (RT-PCR) as described previously (Wang et al. 2014).

COLLECTION OF ANTENNAE OF VIRUS-FREE AND VIRULIFEROUS PLANTHOPPERS

Newly hatched white-backed planthopper nymphs were reared on healthy or virus-infected rice plants. After the nymphs had developed into adults for 2 d, RT-PCR was used to confirm the presence of the virus in the viruliferous insects as described previously (Wang et al. 2014). The antennae of virus-free and viruliferous *S. furcifera* adults were collected and stored at -80 °C until further use. Approximately 200 antennae for each replicate were analyzed and 3 replicates for each treatment were done.

DSRNA SYNTHESIS

For dsRNA synthesis, the gene-specific primers conjugated with the T7 RNA polymerase promoter sequence (Table 1) were designed from the sequences (*SfurOBP2*, accession No. KF660218; *SfurOBP11*, accession No. KF732020), and were used to amplify target regions (*SfurOBP2*, 451 bp; *SfurOBP11*, 549 bp). The 441 bp segment of enhanced

Table 1. PCR primers used in this study.

Purpose	Primer name	Gene ID	Primer (5'→3')	E (%) ^a	R ²
qPCR	<i>OBP2</i> -F	KF660218	ATTCGAGCCAGCCATGACAA	110.0	0.999
	<i>OBP2</i> -R		TGAAGCAATCATCCACGGCT		
	<i>OBP11</i> -F	KF732020	CAGCGACAGTATATGGGCGA	105.2	0.996
	<i>OBP11</i> -R		GTCACCATTGGTCGCTTTGTT		
	<i>TUB</i> -F	KP735521	GAGGACACTACACCATCGGC	99.3	0.992
	<i>TUB</i> -R		TCAACAGCGAGGTGAATCCG		
	<i>EF1α</i> -F	KP735517	AAGATCGGTTACAACCCGGC	103.8	0.989
	<i>EF1α</i> -R		TCCTTGCGCTCAATGTCCA		
RNAi	<i>OBP2</i> -F		TAATACGACTCACTATAGGGTCTCACCCAACTCAAAG ^b	n.a.	n.a.
	<i>OBP2</i> -R		TAATACGACTCACTATAGGGGAAGTCACTTGGAGAAAGCTCTG		
	<i>OBP11</i> -F		TAATACGACTCACTATAGGGTTATACCGCAAGTGTGTGTTT	n.a.	n.a.
	<i>OBP11</i> -R		TAATACGACTCACTATAGGGGCTCAAGTGGGAATGTCTATCAC		
	<i>EGFP</i> -F	U55762	TAATACGACTCACTATAGGGAGGACGACGGCAACTACAAG	n.a.	n.a.
	<i>EGFP</i> -R		TAATACGACTCACTATAGGGGTCATGCCGAGAGTGATCC		

^aPCR efficiency; ^bT7 RNA polymerase promoter is underlined; n.a. = not applicable.

green fluorescent protein gene (*EGFP*, accession No. U55762) was amplified as a negative control. The dsRNA was synthesized using the T7 RiboMAX™ Express RNAi System, according to the manufacturer's instructions (Promega, Madison, Wisconsin, USA). The synthesized dsRNAs were individually isopropanol precipitated, resuspended in RNase-free water, and quantified by a spectrophotometer (NanoDrop™ 1000, Thermo Fisher Scientific, Wilmington, Delaware, USA) at 260 nm. The purity and integrity were determined by agarose gel electrophoresis. The dsRNA solution was stored at -80°C .

dsRNA DELIVERY BY INJECTION

Four treatments, namely RNase-free water (control), dsEGFP, dsOBP2 and dsOBP11, were established. We injected 50 nL dsRNA (2000 ng per μL) into the membrane between the meso- and meta-thoracic legs of the CO_2 anesthetized 1-d-old virus-free *S. furcifera* adults using a Nanoinjector (Drummond Scientific, Broomall, Pennsylvania, USA). Three replicates (40 adults for each replicate) were carried out for each treatment. The injected adults were placed on the healthy rice seedlings at $26 \pm 1^{\circ}\text{C}$, 85% RH, with a 16:8 h (L:D) photoperiod.

The host orientation preference of each *S. furcifera* was tested at 24 h after injection, and 10 individuals were randomly collected from each replicate to analyze the effectiveness of silencing the target genes.

HOST ORIENTATION PREFERENCE TEST

Three host orientation preference trials were performed; 1 experiment compared the behavioral response of *S. furcifera* for healthy or virus-infected rice plants; another experiment tested the behavioral response of *S. furcifera* for healthy rice plants or air (empty pot with soil); the last experiment tested the behavioral response of *S. furcifera* for virus-infected rice plants or air. The experiments were performed by a two-choice bioassay using the glass Y-tube olfactometer (one 13-cm stem and two 10-cm branched arms, 60° between 2 arms, 2 cm diam) as described by Wang et al. (2014). The Y-tube was placed horizontally in an airtight box ($70 \times 45 \times 30$ cm), and lighted from 25 cm above by a 25-W filament lamp. The rice plants (80 d after planting) or air were enclosed in the odor bottles (50 cm in height, 25 cm inner diam) that connected to the arms of the Y-tube. Air that was filtered through activated charcoal and humidified with doubly distilled water was pumped into both arms at a flow rate of 300 mL per min. A planthopper adult (starved for 1 h) was randomly chosen and placed in the stem of the Y-tube. Each insect was given 10 min to choose between the 2 arms of the Y-tube. The insect choice was noted if the planthopper reached one-half the length of an arm and stayed in the arm for more than 1 min. The behavioral trials were conducted in an environmentally controlled room ($25 \pm 1^{\circ}\text{C}$ and 50% RH). For each treatment, a chi-square test was used to detect the differences in insect orientation preferences between the 2 odor cues using SPSS19.0 software (SPSS Inc., Chicago, Illinois, USA).

QUANTITATIVE RT-PCR (qRT-PCR) ASSAY

Total RNA was isolated using the MiniBEST Universal RNA Extraction Kit (TaKaRa, Dalian, China), and first-strand cDNA was synthesized using the PrimeScript™ RT reagent Kit with gDNA Eraser (TaKaRa, Dalian, China) according to the manufacturer's instruction.

The expression levels of *SfurOBP2* and *SfurOBP11* were evaluated using qRT-PCR. All qPCR samples were run in triplicate using a CFX96 Touch™ Real-Time PCR Detection System (Bio-Rad Laboratories, Hercules, California, USA) and TB Green Premix Ex Taq™ II (TaKaRa, Dalian, China) according to the manufacturer's protocol. The *S. furcifera* α -1 tubulin (*TUB*, accession No. KP735521) and elongation factor 1- α

(*EF1- α* , accession No. KP735517) were used as the internal references as suggested by a previous study (An et al. 2016). The qPCR primers of *SfurOBP2*, *SfurOBP11*, *TUB* and *EF1- α* were designed by using the National Center for Biotechnology Information profile server (<http://www.ncbi.nlm.nih.gov/tools/primer-blast>). A 5-fold dilution series of cDNA was used to generate a standard curve to determine efficiency of each primer pair. The primers and amplification efficiency are shown in Table 1.

Gene expression data were analyzed using $2^{-\Delta\Delta\text{CT}}$ method (Livak & Schmittgen 2001; Pfaffl 2001), the geometric mean of the 2 internal control genes was used for normalization. Student's *t*-test was used to analyze the differences of *SfurOBPs* (*SfurOBP2* and *SfurOBP11*) expression levels in virus-free and viruliferous *S. furcifera*, and difference between treatment means were analyzed using 1-way ANOVA.

Results

EXPRESSION LEVELS OF *SFUROBP2* AND *SFUROBP11* IN THE ANTENNAE OF *SOGATELLA FURCIFERA*

In this research, qRT-PCR was used to investigate the expression levels of *SfurOBP2* and *SfurOBP11* post-viral infection in the antennae of *S. furcifera* adults. As shown in Figure 1, the expression level of *SfurOBP2* was reduced by 52.7% in the antennae of viruliferous *S. furcifera* ($t = 11.335$; $df = 2$; $P < 0.01$), and the expression level of *SfurOBP11* was reduced by 52.5% in the antennae of viruliferous *S. furcifera* ($t = 5.462$; $df = 2$; $P < 0.05$).

EFFECT OF RNAI TREATMENT ON *SFUROBP2* AND *SFUROBP11* TRANSCRIPT LEVELS

RNAi technology was used to reduce the expression of candidate genes potentially involved in host preferences of *S. furcifera*. At 24 h after injection of dsOBP2 and dsOBP11, the transcript levels of *SfurOBP2* and *SfurOBP11* in the treated virus-free *S. furcifera* adults were reduced by 68.6% ($F = 16.176$; $df = 2, 6$; $P < 0.01$) and 85.2% ($F = 26.527$; $df = 2, 6$; $P < 0.01$), respectively, relative to the expression in insect controls injected with water (Fig. 2A, B).

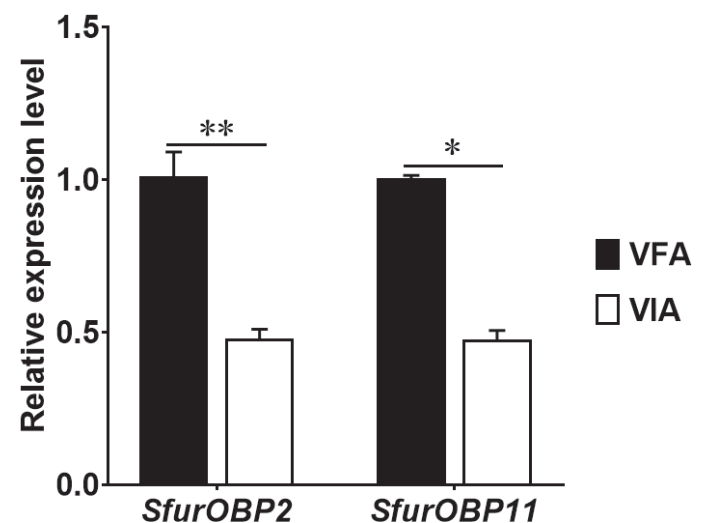


Fig. 1. Expression profiles of *SfurOBP2* and *SfurOBP11* in the antennae of virus-free and viruliferous *S. furcifera*. VFA: virus-free antennae; VIA: virus-infected antennae. The histogram bars represent mean \pm SE of 3 biological replicates. Asterisk above bars indicate significant differences (*t*-test: * $P < 0.05$, ** $P < 0.01$).

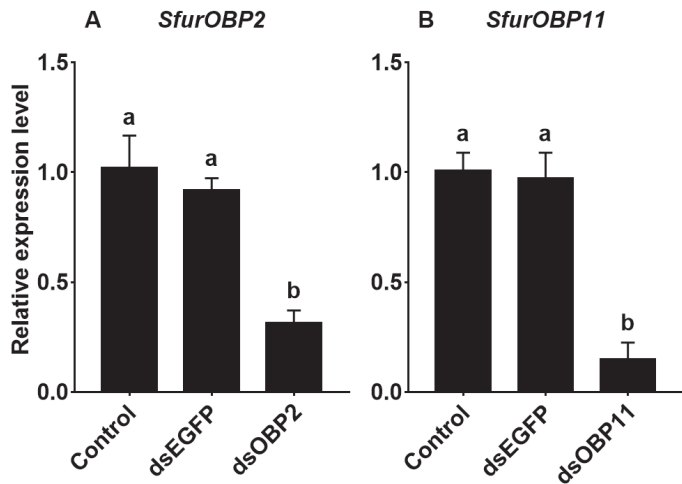


Fig. 2. Detection of the mRNA levels after RNA interference. The histogram bars represent mean \pm SE of 3 biological replicates. Different letters above bars indicate significant differences (1-way ANOVA: $P < 0.01$). (A) mRNA levels of *SfurOBP2* in *S. furcifera* injected with different dsRNA. RNase-free water injection (Control), *EGFP*-dsRNA (dsEGFP), *SfurOBP2*-dsRNA (dsOBP2). (B) mRNA levels of *SfurOBP11* in *S. furcifera* injected with different dsRNA. RNase-free water injection (control), *EGFP*-dsRNA (dsEGFP), *SfurOBP11*-dsRNA (dsOBP11).

HOST PLANT SELECTION BEHAVIOR OF *SOGATELLA FURCIFERA*

Twenty-four hours after injection of dsRNA, a glass Y-tube olfactometer was used to test the host plant selection behavior of dsRNA treated virus-free *S. furcifera* for air, healthy, and virus-infected rice plants. The results revealed that *S. furcifera* treated with RNase-free water and dsEGFP did not change their behavior, and preferred virus-infected rice plants when subjected simultaneously to healthy plants ($\chi^2 = 13.333$; $df = 1$; $P < 0.01$, and $\chi^2 = 9.227$; $df = 1$; $P < 0.01$, respectively) (Fig. 3A). In contrast, there was no significant difference ($\chi^2 = 0.180$; $df = 1$; $P = 0.671$ for dsOBP2, and $\chi^2 = 0.321$; $df = 1$; $P = 0.571$ for dsOBP11) in the preference of insects for infected plants when the *SfurOBP-2* or *-11* were silenced (Fig. 3A). When the dsRNA-treated *S. furcifera* were subjected to healthy or infected plants and air, the control, dsEGFP and dsOBP2 were significantly attracted to the rice plants whatever their infection status (infected $\chi^2 = 12.224$; $df = 1$; $P < 0.01$, or non-infected $\chi^2 = 14.500$; $df = 1$; $P < 0.01$ for dsOBP2) rather than air (Fig. 3B, C). Furthermore, the dsOBP11 treated *S. furcifera* did not significantly prefer healthy ($\chi^2 =$

0.723; $df = 1$; $P = 0.395$) or infected rice plants ($\chi^2 = 0.321$; $df = 1$; $P = 0.571$) when subjected simultaneously to air (Fig. 3B, C).

Discussion

Olfaction plays a critical role in numerous insect behaviors (Liu et al. 2012; Chang et al. 2017; Zhang et al. 2017), and odorant-binding proteins play a key role in host plant choice and oviposition in insects (Hallem et al. 2006; Matsuo et al. 2007; Pelosi et al. 2018). In this study, we found that the transcription level of *SfurOBP2* was significantly reduced in the antennae of viruliferous *S. furcifera*, and that after silencing the *SfurOBP2* gene, virus-free *S. furcifera* no longer displayed significant preference for virus-infected rice plants; however, the host-seeking ability of *S. furcifera* was not affected. Taken together, we propose that *SfurOBP2* is one of the key odorant-binding proteins responsible for the preference of *S. furcifera* for virus-infected rice plants. Actually, the *SfurOBP2* gene is expressed preferentially in antennae (He & He 2014), and experiments on the binding properties of odorant-binding proteins indicate that *SfurOBP2* has a relatively high affinity for the rice plant volatiles 2-tridecanone and β -ionone (He & He 2014), these results suggest that *SfurOBP2* plays a crucial role in the planthopper’s ability to discriminate between host plants.

The preference of the dsOBP2-treated individuals did not completely shift to healthy rice plants, which implied that some other *SfurOBPs* may play a role in determining the host preference of the *S. furcifera*, similarly as *SfurOBP2*. In fact, previous research has shown that the interactions of *CmedOBP2* and *CmedOBP3* have significant effects on the ability of *Cnaphalocrocis medinalis* (Guenée) (Lepidoptera: Crambidae) to detect host plant volatiles (Sun et al. 2016). However, further research is required to identify these *SfurOBPs*. Furthermore, *SfurOBP2* also is highly expressed in the abdomen, and there was lower homology (11.9%–25.7%) among *SfurOBP2* and other *SfurOBPs* at the amino acid level (He & He 2014), these characteristics of *SfurOBP2* may make a contribution to the special function.

Volatile chemical signals from host plants provide important cues for various insects to detect and locate appropriate host plants for reproduction (Anfora et al. 2009; Tasin et al. 2010; Allmann et al. 2013). Differences in the relative contents and composition of volatiles produced by healthy and virus-infected rice plants have been detected (He et al. 2014; Wang et al. 2017). However, the specific compounds playing a role in the behavior of *S. furcifera* for healthy and virus-infected rice plants have not yet been obtained. Furthermore, the composition of volatiles seems to differ greatly between different rice species (Wang

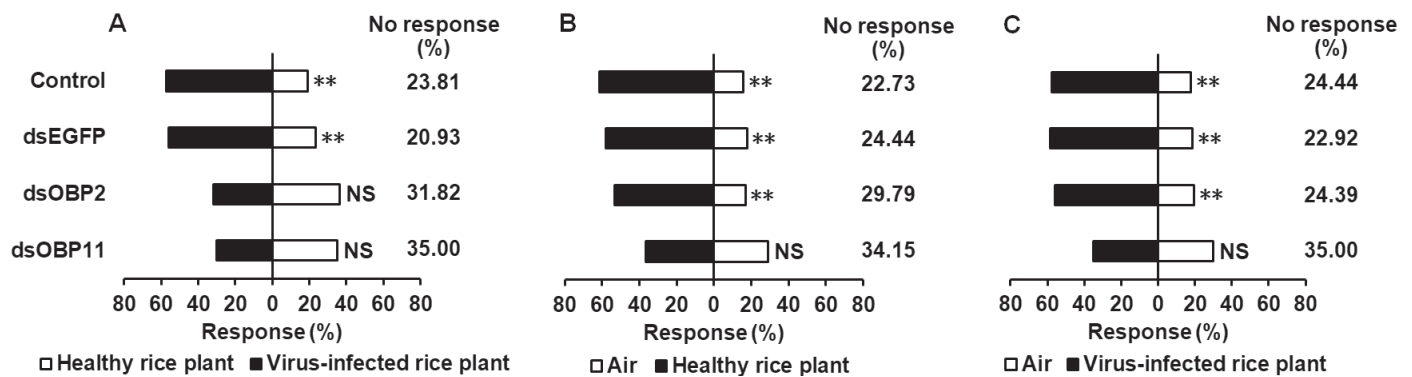


Fig. 3. Effects of RNAi of *SfurOBP-2* or *-11* on the preferences of *S. furcifera* for healthy, virus-infected rice plants or air (empty pot with soil). The data of non-responding insects were given on the right of the bar in the figure. Double asterisks indicate statistically significant difference (chi-square test: $**P < 0.01$), NS indicates no significant difference. (A) *S. furcifera* choice tests between healthy or infected rice plants after injection of dsRNA or water (Control); (B) *S. furcifera* choice tests between healthy rice plants or air after injection of dsRNA or water (Control); (C) *S. furcifera* choice tests between virus-infected rice plants or air after injection of dsRNA or water (control).

et al. 2017). The behavioral responses of *S. furcifera* to healthy and virus-infected rice plants does not vary due to the different cultivars of the rice plant (preliminary tests), implying that it is more the relative content than the composition of volatiles that are involved. Discovery of the special function of *SfurOBP2* means that binding assays of this protein to the volatiles showed a difference between healthy and virus-infected rice plants, and the behavior of *S. furcifera* for these changed volatiles after knockdown of *SfurOBP2* can be used to determine the volatiles that influence host plant choice of *S. furcifera*.

In this study, the *S. furcifera* did not significantly prefer virus-infected rice after *SfurOBP11* gene silencing by RNAi. In addition, when faced with the choice between healthy or infected rice plants and air, there was no significant difference between rice plants and air. These results led us to speculate that *SfurOBP11* is involved in the host plant location but not in the recognition of healthy and virus-infected rice plants. In fact, previous research has demonstrated already that silencing of *SfurOBP11* significantly reduced the number of nymphs attracted to rice plants (Jiang et al. 2016). Our results reinforce the role of *SfurOBP11* in the recognition of rice plants in *S. furcifera*.

In addition, our results provide additional evidence that plant viruses can influence the behavior of insect virus vectors (Ingwell et al. 2012; Moreno-Delafuente et al. 2013). Research on the influence of plant viruses on the olfactory system of vectors provides a new perspective for understanding the mechanisms through which these viruses modify vector feeding behavior.

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