Molecular response of whitefly to the wild tomato *Solanum habrochaites*

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Abstract

The whitefly *Bemisia tabaci* (Gennadius) causes severe damage to cultivated tomato in many regions of the world through direct feeding and indirectly through transmission of plant viruses. Field observations show that *B. tabaci* is rarely infested the non-host plants such as the wild tomato *Solanum habrochaites*; however, the response mechanism of whitefly to *S. habrochaites* is still unclear. In this study, we assessed the effects of *S. habrochaites* on the survival, fecundity, and egg hatchability of the Mediterranean (MED) species of *B. tabaci*. We found that *S. habrochaites* can significantly increase the mortality of *B. tabaci* adults and reduce both of fecundity and egg hatchability. Moreover, expression and splicing of stress-response genes in whitefly exposed to *S. habrochaites* was analyzed using RNA-sequencing data and alternative splicing analysis. These results indicated that the *S. habrochaites* treatment

can induce the expression of environmental stress genes in *B. tabaci*. We detected odorant-binding protein1 (OBP1) associate with the interaction of *S. habrochaites* and *B. tabaci* by WGCNA analysis. The function and binding characterization of OBP1 in *B. tabaci* were tested *in vivo* and *in vitro* against R-curcumene emitted by the wild tomato. Results showed that OBP1 can specific binding R-curcumene act as repellents to *B. tabaci*. This study may help us to a better understanding of the molecular mechanisms involved in the whitefly response and recognition of wild tomato relative.

Keywords: whitefly, *Solanum habrochaites*, volatile, RNA-sequencing, R-curcumene

1. Introduction

The Whitefly *Bemisia tabaci* (Gennadius) is one of the most important pests that causes economic damage to crop plants [1]. *B. tabaci* has a high oviposition rate and rapid population growth [2]. The Middle East-Asia Minor1 (MEAM1) and Mediterranean (MED) subspecies are the most invasive *B. tabaci* whiteflies worldwide [3].

B. tabaci is one of the main tomato pests that reduces tomato growth through direct feeding and indirectly through transmission tomato yellow leaf curl virus (TYLCV) [4]. *B. tabaci* is the only vector of TYLCV, so whitefly control is one of the key measures used to manage TYLCV[5]. Chemical insecticides remain the main tools in the management of whitefly, but the rapid development of resistance to several chemical classes of insecticides has made it so difficult to control. Therefore, there are urgent demands for safe alternative strategies in modern pest management. One of

these novel approaches is based on the fact that chemical communication via the olfactory system drives essential behaviors of insects. Indeed, detecting appropriate chemical signals is essential for insects to find their mating partners, oviposition sites and food sources [6, 7]. Understanding of the mechanisms and signals that are involved in non-host selection behavior of *B. tabaci* may lead to strategies that exploit the odorous alert signals to enhance crop resistance.

B. tabaci is attracted to cultivated tomato, but it is repelled by the wild tomato relative *S. habrochaites*. The repellent behavior response of *B. tabaci* exposed to *S. habroachaites* as non-host plant was found to be associated with the emission of 7-epizingiberene and R-curcumene compounds[8]. This two terpene volatiles have shown strong repellency against *B. tabaci* and induced *S. habrochaites* to develop enhanced resistance to insect infestation [9, 10]. On the other hand, *B. tabaci* did not elicit behavioral responses to the two corresponding chiral isomers: α-zingiberene and S-curcumene extracted from ginger [11]. Transformation of cultivated tomato with the gene encoding *S. habrochaites* 7-epizingiberene synthase has become strongly repellent to *B. tabaci* that reduced whitefly fecundity up to 87% [12]. These studies showed that *S. habrochaites* can exert strong repellent and toxic stresses aganist *B. tabaci*, but it is unclear how it exerts its effects on the physiological and molecular levels.

The olfactory system of many insects is important to discriminate between host and non-host plants, dealing with a complex and overlapping signals within the odor landscape [13]. However, the molecular mechanism involved in the olfactory

response of *B. tabaci* to non-host plant odors such as *S. habrochaites* is still unclear.

The process of olfactory reception in insect begins with the adsorption of mostly hydrophobic odorant molecules on the surface of the antenna from where the molecules have to pass through pores and pore tubules of the olfactory hair cuticle to enter the sensillum [14]. Detailed analyses of sensillum lymph from many insect species have led to the discovery of high concentrations of soluble proteins that bind hydrophobic compounds, including pheromone as well as general plant odorant molecules. These small globular proteins have been classified as pheromone-binding proteins (PBPs) and general odorant-binding proteins (GOBPs) [15].

In *B. tabaci*, eight OBPs genes have been identified in the MEAM1 and MED cryptic whitefly species using the genome and head transcriptome data [16-18]. Among them, only the preliminary functions of some OBPs of the MED cryptic species have been reported [19]. But, to date, we do not have any knowledge of which OBPs can bind strongly and efficiently to *S. habrochaites* volatiles and can therefore be used to develop environmentally-friendly and sustainable control strategy against whitefly by regulating the behavior of *B.tabaci* or designing highly active compounds through key target gene.

In order to achieve the goal, it was necessary to study the biological changes and expression-related genes that may be affected in the whitefly *B. tabaci* after exposure to the wild tomato relative *S. habrochaites*. Furthermore, it was interesting to compare the functional characterization of some key functional genes in *B. tabaci* interaction with *S. habrochaites*.

2. Materials and methods

2.1 Insects rearing and plant materials

The MED cryptic species of whitefly was reared on tomato plants (Xianke 8) under greenhouse conditions of 25 ± 2 °C, 65 ± 5% relative humidity (RH), and a 16:8 h (L:D) photoperiod. The tomato plant was placed into cage measuring (60 cm × 60 cm × 60 cm). The source of MED was collected from a greenhouse located in Beijing, China and identified as MED by mitochondrial cytochrome oxidase I (mtCOI) gene (Brown et al., 2005). One pair of newly emerged adults was used for colony initiation, and the MED source after six generations was used for subsequent experiments.

2.2 Biological studies

Whiteflies were anesthetized with carbon monoxide for 10s, and the male and female were distinguished by an abdomen distribution and body type differences. For the mortality and fecundity analysis: five pairs of newly emerged adults were placed in 10 cm × 12 cm nylon bags. The bag contained a fresh leaf of *S. habrochaites*. The experiment had 30 independent biological replicates. The male and female mortality were recorded after 6, 24 h and 7 d whereas the fecundity was recorded after 7 d. Then, the adults and nymphs were removed, and only the eggs were retained. The hatching rate of fertilized eggs was determined after 10 d. The experimental conditions were the same as above set at 25 ± 2°C, 65 ± 5% RH, and a 16:8 h (L:D) photoperiod. The biological differences of whiteflies after feeding on common tomatoes (Xianke 8) and *S. habrochaites* (LA2175 and LA2329) were evaluated. Analysis of variance and post hoc Fisher's LSD test were used to detected significant

statistical differences in mean mortality, fecundity and hatching rate.

2.3 Identification of wild tomato volatiles

S. habrochaites were placed into the cages measuring (60 cm × 60 cm × 60 cm) and grown under greenhouse conditions of 25 ± 2°C, 65 ± 5% RH, and a 16:8 h (L:D) photoperiod. Six-week-old plants were used for volatile analysis. We placed leaves of a healthy tomato plant into a glass bottle (45 mL, cleman) and an SPME fiber 50/30-μm divinylbenzene/carboxen/polydimethylsiloxane (Supelco; Sigma-Aldrich, http://www.sigmaaldrich.com) was used for analyte extraction. GC-MS analysis was performed on an Agilent 7890 (Agilent Technologies, Tustin, CA, USA) gas chromatography and Agilent 5975 mass selective detector. Samples were separated using both HP-5 and DB-WAX (both 30 m × 0.25 mm i.d., 0.25 mm film thickness, Agilent Technologies, Tustin, CA, USA). Helium was used as carrier gas at a flow rate of 1.7 mL/min, and the GC inlet was set in the split-less mode. The injector temperature was 220 °C. The optimization of method parameters of SPME and GC-MS were performed following to the method previously described (Li et al., 2018). R-curcumene has been identified using GC-MS techniques by comparing its retention time and mass spectrum with the same parameters from a reference compound. As the 7-epizingiberene is thermally unstable (Bleeker et al., 2011)[11], therefore, the R-curcumene was used for conducting the behavioral and olfactory studies. The synthesis of R-curcumene and S-curcumene was performed according to the method previously reportedS (Song et al., 2012). The synthesized compounds were confirmed by using various spectral technique like mass spectrometry and NMR

detection. The α-zingiberene was purchased from Sigma-Aldrich (St. Louis, MO, USA).

2.4 RNA sequencing and bioinformatics analysis

To evaluate the effect of *S. habrochaites* on whiteflies at the molecular level, RNA-sequencing was performed using MED females and males, which have been fed on tomato Xianke 8 and the wild tomato *S. habrochaites* LA2175 for 6 and 24 h, respectively. Sequencing libraries were constructed using strand-specific libraries. FastQC [20] was used to assess the quality of the sequencing data while Fastp [21] was employed to remove adapters, contamination, and duplicate sequences. The sequenced clean data were mapped to the MED whitefly genome v1.0 using HISAT2 [22]. FeatureCounts [23] was used to calculate the expression of each gene, edgeR [24] was used to analyze the difference in gene expression (we used Log2 Fold Change>1, Adjusted P-value=0.05 as the threshold), rMATS [25] was used to analyze the alternative splicing, and Sashimi plot was used to draw the alternative splicing of genes. Finally, WGCNA [26] was used to calculate the correlation between the gene expression level (TPM values) and the mortality phenotypes of whitefly.

2.5 Identification and expression pattern of OBP -related genes in *B. tabaci*

Initially, we identified the OBPs that recognize R-curcumene using two methods: First, based on the results of WGCNA analysis, we identified the OBP genes related to the whitefly mortality phenotype. Second, we put 50 male and female whiteflies in a ratio of 1: 1 in a clean and well-sealed glass bottle (45 mL, cleman, France) containing the wild tomato *S. habrochaites* LA2175 leaves for 20 min. Glass bottles

with tomato leaves (Xianke 8) were used as controls. After the 20-min exposure, whiteflies were removed from the bottles, quick frozen in liquid nitrogen for RNA extraction. qRT-PT30a (+) expression vector and CR was used to examine the gene expression changes of eight OBPs in the whiteflies exposed to the leaves of the two different tomato species. Real-time RT–PCR (qRT–PCR) was performed with specific primers (listed in Supplementary Table 2).

2.6 Competitive fluorescence binding assay

The OBP1 and OBP4 of the MED whitefly were subcloned to the pEtransformed into the BL21 (DE3) strain. The recombinant protein expression was induced by IPTG. The recombinant protein was purified by a HisTrap chromatography column, the target protein was eluted using 50 mM Tris-HCl (pH 7.4). and then dialyzed overnight at 4°C. Using 1-NPN (N-phenyl-1-naphthylamine) or 1-ANS as the fluorescent probe, the excitation wavelength in the fluorescence spectrometer was set to 337 nm, and the scanning wavelength was 350–500 nm.

We determined the binding constants of 1-NPN or 1-ANS by the Scatchard formula and tested the binding affinity of OBPs to R-curcumene. The IC_{50} value (concentration when 50% of 1-NPN or 1-ANS was replaced by competing ligands) was used to calculate the binding constant of R-curcumene to each of the OBPs, and the target OBPs were identified. The dissociation constant K_i was calculated by the formula: $K_i = [IC_{50}]/(1 + [L]/K_L)$, where $[IC_{50}]$ was the concentration of the ligand at which the fluorescence intensity was reduced by half, [L] was the concentration of the free fluorescent probe, and K_L was the binding dissociation constant of the protein

and the fluorescent probe.

2.7 Homology modeling and molecular docking studies

We conducted OBP1 homology modeling using MODELLER [27], molecular docking analysis of OBP1 protein and R-curcumene using CDOCKER. We also identify the key amino acid residues of OBP1 binding to R-curcumene using virtual mutation. All analyses were performed by using Discovery Studio (Accelrys, San Diego, CA, USA) version 4.0. For mutagenesis, the site-directed mutagenesis kit (Clontech, Palo Alto, CA, USA) was used. Different mutant cDNAs were ligated to the pET30a(+) vector for expression and purification. Each mutant exhibits a distinct product profile that used to determine which amino acid residues are key to the function of OBP1 binding.

2.8 *In vivo* function characterization of OBP1

We selected the target fragments for RNAi according to the coding DNA sequences of whitefly OBP1 and confirmed the specificity of the fragment by blastn. We constructed the design of the hairpin dsRNA into the pbsk vector and then ligated it into the pbi21 expression vector. The constructed expression vector were transformed into *A. thaliana* Colombia wild-type using the floral dip method [28]. Seeds were sown and seedlings were sprayed with phosphinothricin (BASTA) to select for transformants. The genetically transformed positive plants was confirmed by PCR and sequencing. Whiteflies were fed on leaves of transgenic *A. thaliana* in 10 cm × 12 cm nylon bags, and qRT-PCR was used to detect changes in the expression of the OBP1 gene to assess the effect of interference, (primers were listed in Supplementary Table

2). The following behavioral studies were performed on the MED whiteflies that successfully interfered with OBP1.

2.9 Behavioral assays

A binary-choice bioassay tube was used to measure repulsion to volatile chemicals. Here, we determine the behavioral response of whitefly to R-curcumene compared with the mineral oil (control). The bioassays were conducted 9:00 a.m. to 5:00 pm in in a temperature-controlled room at 27 °C and 40% RH. The bioassay arena was constructed of clear glass (45 cm in length by 3 cm in diameter) with three stoppers (left, right, and upper). The lateral stoppers were covered with a 2.5-cm-diameter filter paper liner circle with coarse fabric netting outside to prevent the whiteflies from direct contact with the chemicals. Ten microliters of R-curcumene (dissolved in mineral oil) was added to a 2.5-cm-diameter filter paper on one end, and a control (mineral oil) was added to the other end of the test tube (Du et al., 2016). A total of 100 adult whiteflies of unknown sex and age were introduced into the tube from the middle hole. After the incubation period of 60 min, the number of whiteflies in the two test zones (7.5 cm away from both ends) was counted. The experiment was repeated three times. Two concentrations of R-curcumene (1 and 10 ng/μl) were used. In addtion, we use 100ng α-zingiberene as negative control to test the stability of the behavioral system. A chi-square test was used to calculate the difference in the number of whiteflies in the two zones. SPSS Statistics version 19 [29] (Chicago, IL, USA) was used for all statistical analyses.

3. Results

3.1 The biological effects of *S. habrochaites* on the survival of whitefly

We evaluated the biological effects of *S. habrochaites* LA2175 and LA2329 on the survival of the whitefly *B. tabaci*. After 7 d feeding, the mortality rates of male and female whitefly adults on LA2175 were 98.82% and 88.63%, respectively, and the mortality rates of male and female whitefly adults on LA2329 were 92.22% and 53.61%, respectively (Fig. 1). These data were significantly different from the mortality rates on common tomato (Xianke 8) (Fig. 1). Also, at the earlier time point of 24h feeding, the average mortality of male and female adults on LA2175 was higher (8.22% and 18.52%, respectively) than the average mortality of male and female adults on common tomato (1.61% and 2.22%, respectively) (Fig. S1).

After 7 d, the total eggs laid per female on LA2175 and LA2329 were 1.09 and 2.6, respectively, while the mean number of eggs per female on common tomato was 24.13 (Fig. 1). These differences were statistically significant $p<0.05$. The average egg hatching rate on LA2175 and LA2329, at 10 d, was 7.7% and 22.7%, respectively while the egg hatching rate on common tomato was 79% (Fig. 1).

3.2 7-epizingiberene and R-curcumene are the main volatile components of *S. habrochaites*

We synthesized a high-purity R-curcumene and confirmed its identity by mass spectrometry and NMR (Supplementary Data 1). We found that 7-epizingiberene and R-curcumene are the main volatile compounds of *S. habrochaites* using SPME and GC-MS detection. GC-MS analysis revealed that these two volatile compounds were not detected in the volatile blends of common tomato plants (Fig. 2). It was also found

that the peak areas ratio of 7-epizingiberene and R-curcumene in LA2175 was 2.37- and 4.27-fold that of LA2329, respectively (Fig. 2). The data showed that LA2175 leaves had a relatively higher content of terpene volatiles than LA2329. This is consistent with the biological analysis showing that LA2175 was more resistance to whiteflies. Therefore, we chose and employed LA2175 for subsequent whitefly high-throughput sequencing studies.

3.3 Expression of whitefly-related genes after stress exposure of *S. habrochaites*

A total of 24 samples of male and female whitefly adults (including two time points and four treatments) were used for transcriptome sequencing (Fig. 3). About 21–24 million reads with an average length of 151 bp were aligned to the MED whitefly reference genome by HISAT2 (Table 1). About 78% of the reads mapped to unique loci (Table 1). After gene expression value analysis by featurecount and edgeR, we found four significantly upregulated genes in wild tomato 6-h-treated female adults (WF6) and wild tomato 24-h-treated female adults (WF24) (Fig. 3; Supplementary Data 2). They were BTA016109 (Multicopper oxidase), BTA027707 (Sulfotransferase), BTA001651 (OHCU decarboxylase), and BTA011604 (Heat shock protein). Wild tomato 6-h-treated male adults (WM6) and wild tomato 24-h-treated female adults (WM24) also included four genes that are both significantly upregulated (Fig. 3; Supplementary Data 2). They were BTA003886 (Heat shock protein), BTA016667 (Unknown), BTA005900 (serine threonine-protein kinase SBK1-like), and BTA011604 (Heat shock protein). One gene (BTA011604, Heat shock protein) was significantly upregulated together in these four treatments. There were two genes

that were significantly downregulated in WF6 and WF24, BTA009028 (Unknown) and BTA013802 (Farnesyl-diphosphate farnesyltransferase). Eighteen genes were significantly downregulated in WM6 and WM24 (Fig. 3; Supplementary Data 2). Among these, BTA022129 is solute carrier family 3, member 1, and the other genes had unknown functions. These results indicated that the *S. habrochaites* treatment can induce the expression of environmental stress genes such as heat shock protein in whiteflies.

Alternative splicing is often related to the response of the organism to a stressful environment [30-32]. We determined if whiteflies were stressed by exposure to *S. habrochaites* and whether genes were able to respond to the stress through alternative splicing. We performed alternative splicing analysis on differently treated samples (comparison of male and female samples treated with *S. habrochaites* and then treated with common tomato) through rmats. There were five types of alternative splicing, including skipped exon (SE), mutually exclusive exon (MXE), alternative to 5′splice site (A5SS), alternative to 3′splice site (A3SS), and retained intron (RI). Among these, only SE and MXE had significant differences between the two treatments (FDR <0.05), and the SE type accounted for the main proportion (Table 2; Supplementary Data 3). GO enrichment analysis showed that genes with alternative splicing of the SE type were significantly enriched in three GO items including O-acetyltransferase activity, glycerol ether metabolic process , and ether metabolic process (Supplementary Data 4). The MXE-type alternative splicing genes were not significantly enriched.

3.4 Analysis of gene expression associated with whitefly mortality

To detect genes associated with whitefly mortality, we conducted the system biology analysis method WGCNA to study the expression network of whitefly genes. This method performs hierarchical clustering analysis by weighted correlation, and divides the clustering results to obtain different gene modules. Through a co-expression network analysis of gene expression levels in 24 samples, we found that these genes can be mainly divided into six distinct modules (labeled by different colors) (Fig. 4A). After correlating the gene with whitefly mortality, we found that only the the brown block had a high statistical significant correlation with this phenotype ($r = 0.87$, $p = 3e^{-08}$). GO gene enrichment analysis of brown block genes showed that these genes were enriched in 77 items, of which the first 10 items were behavior, iron ion binding, catalytic activity, locomotory behavior, circadian rhythm, rhythmic process, plasma membrane, regulation of biological quality, secondary active transmembrane transporter activity, and ammonium transmembrane transporter activity (Supplementary Data 4).

3.5 Identification and expression pattern of OBP -related genes in *B. tabaci*

To study the olfactory mechanism used by whiteflies to recognize volatiles from *S. habrochaites*, we first identified OBPs that whiteflies use to recognize R-curcumene. We searched the brown block obtained in the above WGCNA analysis results and found that OBP1 belonged to this block (Supplementary Data 5). In addition, we used qRT-PCR to study the changes of all eight OBPs gene expression in whiteflies after exposure to *S. habrochaites*. The expression levels of OBP1 and OBP4 were

significantly increased after *S. habrochaites* treatment ($p < 0.05$) (Fig. 5). Based on these data, we chose OBP1 and OBP4 as candidate genes for functional studies.

Protein prokaryotic expression and purification and fluorescence binding competition analysis of OBP1 and OBP4 were performed. OBP1 can bind to R-curcumene which reduced the fluorescence value of fluorescent probe 1-NPN to less than 50%. The K_d value was 1.89, but OBP1 cannot bind S-curcumene and a-zingiberene (Fig. 6). OBP4 cannot be combined with any of R-curcumene, S-curcumene, and a-zingiberene (Fig. 6). We performed homology modeling analysis on OBP1 and analyzed the docking of OBP1 and R-curcumene using CDOCKER. The calculated binding energy of OBP1 and R-curcumene was -0.534364, and the interaction energy was -22.1801.

To determine the key amino acids involved in the binding of OBP1 to R-curcumene, we selected the amino acid residues 3 Å from R-curcumene in the pocket region of OBP1 and R-curcumene as the candidate amino acids. We performed virtual mutations, and 19 single mutations affected the binding energy of OBP1 to R-curcumene. We selected two mutations for subsequent verification, including TRP67> SER and ASN108> SER (Supplementary Table 1). The mutation energy of these two mutations was 0.97 and 0.64 kcal/mol, respectively. We performed site-directed mutagenesis to build two single mutations and one double mutation for a total of 3 OBP1 mutants, and then performed prokaryotic expression, protein purification, and fluorescence competition experiments on these three mutants. The OBP1 W67 mutation did not affect the binding of OBP1 to R-curcumene. The

calculated Kd value of OBP1 for R-curcumene was 1.67, which was slightly stronger than that of wild-type OBP1 and R-curcumene (the K_d value was 1.89), but the mutation did not affect the combination of OBP1 W67 with S-curcumene and a-zingiberene. OBP1 W67 still could not bind these two compounds (Fig. 7). Surprisingly, OBP1 N108 and OBP1 W67N108 prevented OBP1 from binding to R-curcumene (Fig. 7). These results indicated that a single amino acid mutation (ASN108> SER) of OBP1 can affect the binding of OBP1 to R-curcumene.

3.6 *In vivo* function characterization of OBP1

To clarify the role of OBP1 in whiteflies, we conducted RNA interference studies, based on the fluorescence competition and binding results, to determine the function of OBP1 in whiteflies. Because of the small size of whiteflies, it is difficult to carry out RNAi by injection. We therefore expressed the reverse dsRNA of OBP1 in *Arabidopsis thaliana* to obtain host plant materials that stably express dsRNA of OBP1. After feeding whiteflies on this material for 7 d, qRT-PCR detection revealed that the expression level of OBP1 in the treated whiteflies was only 23% that of whiteflies fed on wild-type *A. thaliana* for 5 d (Fig. S2). Behavioral studies revealed that R-curcumene could significantly repel whiteflies at both 10 and 100 ng doses ($p < 0.01$). But for 100ng of α-zingiberene, whitefly's behavior selection before and after OBP1 were silenced were not significantly affected ($p > 0.05$). This indicates that R-curcumene has a strong repellent effect on whitefly. Whiteflies subjected to OBP1 interference showed no significant repellency to R-curcumene at either of the two doses studied ($p > 0.05$) (Fig. 8).

4. Discussion

Previous studies have shown that *S. habrochaites* strongly repels whiteflies and can reduce whitefly fecundity [8, 11, 12]. We found that *S. habrochaites* can significantly reduce the number of whitefly eggs. This finding is consistent with previous reports [11, 12]. In addition, *S. habrochaites* can significantly reduce whitefly survival. *S. habrochaites* volatiles 7-epizingiberene and R-curcumene, under confined conditions, are toxic to whiteflies [11]. The mortality of whiteflies in this study may be related to the effects of R-curcumene. Our GC-MS results confirmed that 7-epizingiberene is the main sesquiterpene in *S. habrochaites*, and the plant volatile compounds are the same as those identified in previous studies [11]. *S. habrochaites* can significantly reduce the hatching of whitefly eggs. These results show that *S. habrochaites* is toxic and strongly resistant to whiteflies. This plant species may have application value in the breeding of commercial tomatoes resistant to whiteflies. In addition to the volatiles identified, *S. habrochaites* also produces acyl sugar, which can stick to whiteflies and cause their death [33, 34]. The resistance of *S. habrochaites* to whiteflies appears to result from the combination of volatile repellents and acyl sugars.

We explored molecular and biochemical mechanisms of whitefly response to *S. habrochaites* stress using high-throughput sequencing and bioinformatics analysis. Differential gene analysis showed that whiteflies responded to the stress by upregulating expression of HSP and related metabolic enzyme genes. Several genes that affected the survival of whiteflies were found by WGCNA analysis. The specific

functions of these genes require additional study for verification. In addition, alternative splicing of genes can play a role in insect stress [35-37]. We found that *S. habrochaites* stress can induce alternative splicing in whiteflies, and the types of alternative splicing are SE and MXE. The type of alternative splicing in insects is mainly SE [38, 39], and the type of alternative splicing in plants is RI [31, 40]. The difference in the type of alternative splicing between plants and insects may result from differences in their Intron processing mechanism [30, 32]. How the alternative splicing of these genes affects whiteflies will require additional study.

Herbivorous insects typically rely on their sensitive olfactory system to identify plant volatile compounds and find suitable host plants [41] or to avoid non-host plants [42]. Insects gain this recognition ability in two ways: the first is acquired through experience and learning [43],while the second is genetically programmed. Bleeker et al found that whiteflies, which have never been exposed to R-curcumene, can show strong repellency behavior and believed that the whitefly response to R-curcumene is innate [11]. Therefore, the olfactory system should be involved in this recognition process. We previously found that OBP1 can be specifically overexpressed in the head of females, which may be because *S. habrochaites* volatiles are danger signals to whiteflies [17]. OBP1 has the highest expression level in the whole body among all 8 OBPs reported in whitefly, and this may be related to the characteristics of *R*-curcumene. Whiteflies can recognize 7-epizingiberene and R-curcumene at a distance [11]. Low concentrations of 7-epizingiberene and R-curcumene can repel whiteflies, while high concentrations of 7-epizingiberene and R-curcumene are lethal

[11]. A previous study showed that R-curcumene elicited electroantennogram (EAG) responses at very low concentration of 0.0001 dilution [11].Moreover, R-curcumene can be volatilized in the air at a low concentration and can repel whiteflies over long distances. The whitefly olfactory gene OBP1 that identify R-curcumene volatiles are highly expressed. This helps whiteflies to identify the potential danger that may affect survival and reproduction. Previous studies have shown that the repellency of this volatile is innate that was hypothesized by those authors [11], however not proven, which is consistent with the high expression of the OBP1 gene.

We used R-curcumene to test the binding of whitefly OBP1 and to conduct behavioral studies because of the instability of 7-epizingiberene. We also tested the binding ability of OBP1 to α-zingiberene, and the results showed that OBP1 could not bind to α-zingiberene. A previous study showed that α-zingiberene extracted from ginger was not active against whiteflies [11]. This finding was confirmed at the molecular level and indicated that whiteflies can distinguish between different chiral structures and have specific recognition ability for chirality. These data provide a basis for the development and practical application of these compounds. In addition, the weakened behavior of whiteflies after RNAi treatment may be due to interference with the transportation process. OBP is the first step in insect olfactory recognition. In insect olfactory recognition, odor molecules first enter pores in the sensilla on the antennae or maxillae and combine with OBPs to form a complex. This passes through the sensory lymphatic fluid to bind to an olfactory receptor, which then triggers an insect response[7].

A practical challenge for the production and application of these allelochemicals is how to convert 7-epizingiberene and R-curcumene into stable, safe, and effective products to protect susceptible crops against whiteflies. The 7-epizingiberene and R-curcumene have excellent repellent properties, and they have great potential for future field applications. However, in consideration of the stability, and cost of 7-epizingiberene and R-curcumene, it may be necessary to modify the structure of 7-epizingiberene and R-curcumene to develop a whitefly repellent product that is stable, safe, effective, and economical. Research on the key structural regions of OBP1 can guide research and development on the structural modification of these two compounds. This type of research has been successfully performed on *Anopheles gambiae*, *Bactrocera dorsalis*, and aphids, and has promoted N, N-diethyl-m-toluamide (DEET), and aphid alarm pheromone (*E*)-β-farnesene (EBF) analogs and other insect behavior modifying chemicals [44, 45]. In this study, experiments on molecular docking of OBP1 with R-curcumene, site-directed mutations, and other experiments helped us to identify the key amino acid residues in the binding process of OBP1 and R-curcumene. The key structural regions combined with R-curcumene are conducive to future work such as the modification of these compounds. The binding energies obtained in this study are not completely consistent with the actual site-directed mutation results. This may be due to the bias caused by the OBP1 structural model we obtained using the homology modeling method. Subsequent crystal structure analysis techniques can be used to obtain the precise structure of OBP1 to effectively promote the research and application of repellent

compounds.

5. Conclusions

This study provides the first gene expression profile of whitefly *Bemisia tabaci* corresponding to the stress of wild tomato *Solanum habrochaites*. These gene expression profile explain the molecular mechanism of the survival, fecundity, and egg hatchability effects of *S.habrochaites* on *B.tabaci*. Combining WGCNA and qRT-PCR analysis, we identified the odor-binding protein OBP1 as one of the key functional genes in *S.habrochaites* and *B.tabaci* interaction. Then, the function and binding characterization of OBP1 in *B. tabaci* were tested *in vivo* and *in vitro* against R-curcumene emitted by the wild tomato. This study provide valuable resources for future studies investigating functions associated with response of whitefly to wild tomato.

Data availability conflict of interest

RNA-seq data have been deposited and are available in NCBI (BioProject ID is PRJNA622388).

Conflict of interest: The authors declare no conflict of interest.

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Table legends

Table 1. Summary of the RNA-Seq Data. CKF6_1-3, whitefly females grown on common tomato for 6 h replicates 1–3. CKM6_1-3, whitefly males grown on common tomato for 6 h replicates 1–3. CKF24_1-3, whitefly females grown on common tomato for 24 h replicates 1–3. CKM24_1-3, whitefly males grown on common tomato for 24 h replicates 1–3. WF6_1-3, whitefly females grown on wild tomato LA2175 for 6 h replicates 1–3. WM6_1-3, whitefly females grown on wild tomato LA2175 for 6 h replicates 1–3. WF24_1-3, whitefly females grown on wild tomato LA2175 for 24 h replicates 1–3. WM24_1-3, whitefly males grown on wild tomato LA2175 for 24 h replicates 1–3.

Table 2. Genome-wide effects of *Solanum habrochaites* stress on alternative splicing of whitefly. WF6: wild tomato (*Solanum habrochaites*) 6-h-treated female adults; WM6: wild tomato 6-h-treated male adults; WF24: wild tomato 24-h-treated female adults; WM24: wild tomato 24-h-treated male adults. CKF6: common tomato 6-h-treated female adults; CKM6: common tomato 6-h-treated male adults; CKF24: common tomato 24-h-treated female adults; CKM24: common tomato 24-h-treated male adults.

Table 1

	No. of paired-end reads	No. of mapped paired-end reads	Proportion of mapped paired-end reads
CKF6_1	22729871	19170373	84.34%
CKF6_2	24263436	20446798	84.27%
CKF6_3	24587112	20852330	84.81%
CKM6_1	22886414	18199276	79.52%
CKM6_2	22949620	18146265	79.07%
CKM6_3	22716792	18100740	79.68%
CKF24_1	22159172	18759955	84.66%
CKF24_2	22190660	18620183	83.91%
CKF24_3	23055250	19278800	83.62%
CKM24_1	22912092	18384663	80.24%
CKM24_2	22576192	17744887	78.60%
CKM24_3	22106168	17342289	78.45%
WF6_1	21695473	17994225	82.94%
WF6_2	25184704	21089671	83.74%
WF6_3	24590350	20636222	83.92%
WM6_1	23754024	19112488	80.46%
WM6_2	24277496	19358875	79.74%
WM6_3	22011538	17624639	80.07%
WF24_1	22934225	19340432	84.33%
WF24_2	22669251	18994565	83.79%
WF24_3	24285715	20487429	84.36%
WM24_1	23292401	19060172	81.83%
WM24_2	21426439	17460405	81.49%
WM24_3	24205186	19790160	81.76%

Table2

AS Events Summary	WF6 vs CKF6	WM6 vs CKM6	WF24 vs CKF24	WM24 vs CKM24
SE	7	13	11	14
MXE	0	1	2	5
RI	0	0	0	0
A5SS	0	0	0	0
A3SS	0	0	0	0

Figure legends

Fig.1: The effect of *Solanum habrochaites* on mortality, fecundity and egg hatchability of whitefly. A. Mortality of whitefly adults. LA2175_M: Mortality of whitefly male adults on LA2175. LA2175_F: Mortality of whitefly female adults on LA2175. LA2329_M: Mortality of whitefly male adults on LA2329. LA2329_F: Mortality of whitefly female adults on LA2329. Xianke8_M: Mortality of whitefly male adults on Xianke8. Xianke8_F: Mortality of whitefly female adults on Xianke8. B. Eggs per female of whitefly adults on wild tomatos LA2175, LA2329 and common tomatos Xianke 8. C.Hatchability of whitefly eggs on wild tomatos LA2175, LA2329 and common tomatos Xianke 8.

Fig.2: GC–MS analysis of the volatile profiles of wild tomato *Solanum habrochaites* LA2175, LA2329, and common tomato Xianke 8. 1. R-curcumene, 2. 7-epizingiberene, 3 . *p*-cymene, 4. β-phellandrene, 5. elemene, 6. α-copaene, 7. unknow, 8.β-caryophyllene, 9. α-humulene.

Fig.3: Analysis of the RNA-Seq data of whiteflies treated by common tomato and wild tomato. (A) Principal component analysis (PCA) plot showing clustering of RNA-Seq of whiteflies. (B) Bar graph showing the number of upregulated (upper bars) and downregulated (lower bars) genes in whitefly male and female adults. (C) Venn diagram showing the number of induced differently expressed genes unique to or common within the four conditions. (D) Venn diagram showing the number of suppressed differently expressed genes unique to or common within the four conditions. WF6: wild tomato (*Solanum habrochaites*) 6 hours treated female adults; WM6: wild tomato 6 hours treated male adults; WF24: wild tomato 24 hours treated female adults; WM24: wild tomato 24 hours treated male adults.

Fig.4: Coexpression network of whitefly identified by WGCNA.(A) Hierarchical clustering tree (dendrogram) of genes based on coexpression network analysis. Hierarchical cluster tree showing coexpression modules identified by WGCNA. Each leaf in the tree corresponds to individual gene. The color rows below the dendrograms indicate module membership. (B) Cluster dendrogram showing global relationship between biological replicates and among different whitefly samples. (C) Eigengene adjacency heatmap that show the correlations of different modules. (D) Modules and

whitefly mortality association by correlation analysis. Each row corresponds to a module. The numbers in each module are correlation coefficients, and the numbers in parentheses are p-values of correlation analysis.

Fig.5: Expression analysis of OBPs of whiteflies on common tomato (Xianke 8) leaves compared with on wild tomato (LA2175) leaves. Data are presented as means±SE (n=3). Statistical significance between common tomato and wild tomato treated whiteflies are indicatedby asterisks (*, $P<0.05$; **, $P<0.01$; Student's t-test).

Fig.6: Ligand-binding test of OBP1 and OBP4 to R-curcumene and its analog. (A) competitive combining of ligands with 1-NPN and OBP1 protein; and (B) competitive binding of ligands with bis-ANS and OBP4 protein.

Fig.7: Ligand-binding test of OBP1 mutants to R-curcumene and its analog. (A) competitive combining of ligands with 1-NPN and OBP1 W67 protein; (B) competitive binding of ligands with 1-NPN and OBP1 N108 protein; and (C) competitive binding of ligands with 1-NPN and OBP1 W67N108 protein.

Fig.8: Behavioural responses of the whiteflies to R-curcumene. Numbers in bars represent the total numbers of whiteflies responding to stimuli. The bars show the percentage of whiteflies responding to the stimuli. Data were analysed using $\chi 2$ analysis to evaluate differences in each choice experiment (** $P < 0.01$).

Fig.S1: Mortality of whitefly adults on wild tomatos LA2175 and common tomatos Xianke 8. WF6: wild tomato 6 hours treated female adults; WM6: wild tomato 6 hours treated male adults; WF24: wild tomato 24 hours treated female adults; WM24: wild tomato 24 hours treated male adults. CKF6: common tomato 6 hours treated female adults; CKM6: common tomato 6 hours treated male adults; CKF24: common tomato 24 hours treated female adults; CKM24: common tomato 24 hours treated male adults.

Fig. S2: Effects of RNAi induced gene silencing on OBP1 expression in whitefly

Fig.1

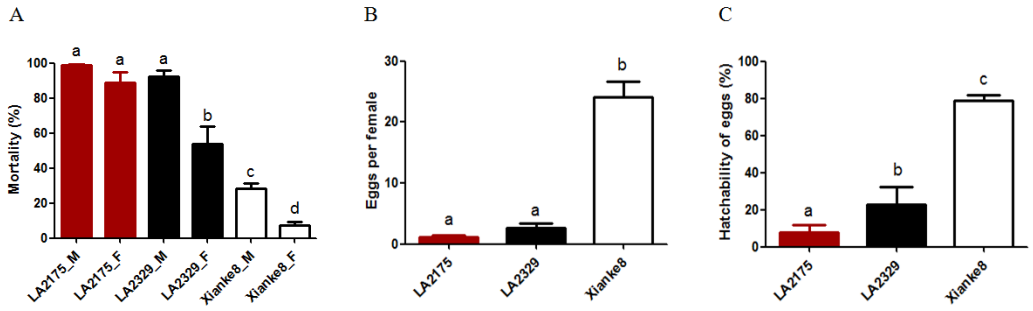

A
Mortality (%)
100
80
60
40
20
0
a
a
a
b
c
d
LA2175_M
LA2175_F
LA2329_M
LA2329_F
Xianke8_M
Xianke8_F
B
Eggs per female
30
20
10
0
a
a
b
LA2175
LA2329
Xianke8
C
Hatchability of eggs (%)
100
80
60
40
20
0
a
b
c
LA2175
LA2329
Xianke8

Fig.2

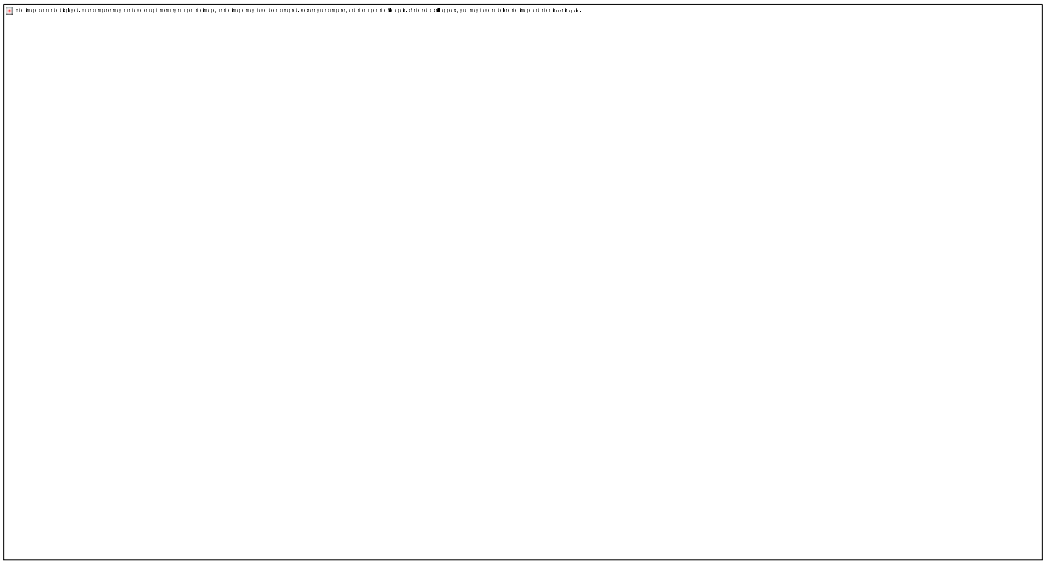


Fig.3

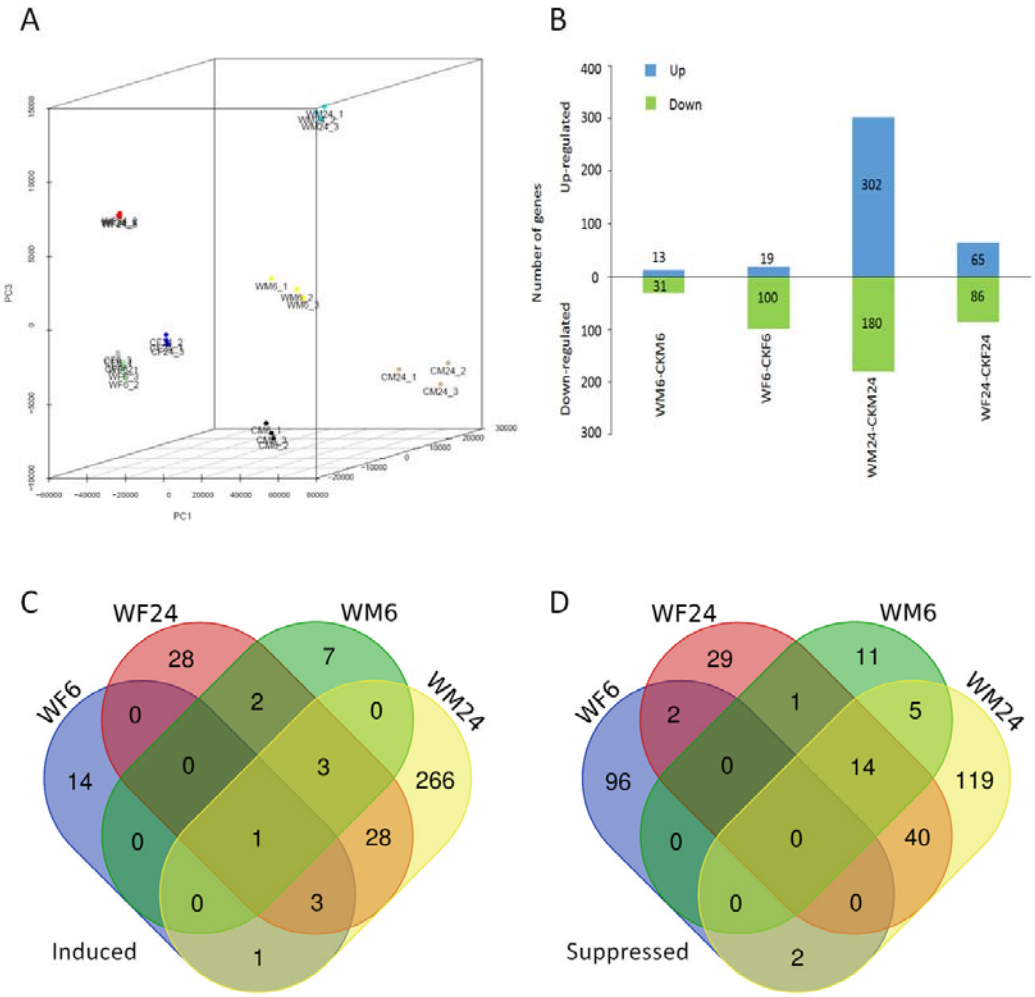

A
PC1
PC3
B
Up
Down
Number of genes
Up-regulated
Down-regulated
13
31
19
100
302
180
65
86
WM6-CKM6
WF6-CKF6
WM24-CKM24
WF24-CKF24
C
WF24
WM6
WF6
WM24
28
7
0
2
0
0
14
3
266
0
1
28
0
3
1
Induced
D
WF24
WM6
WF6
WM24
29
11
2
1
5
0
96
14
119
0
0
40
0
0
2
Suppressed

Fig.4

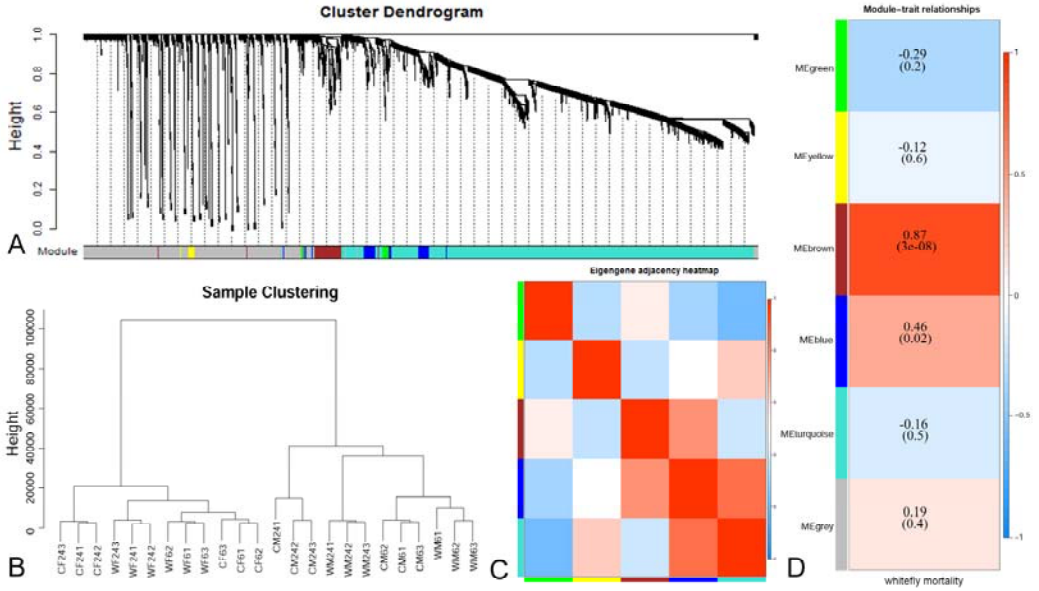

Cluster Dendrogram
Height
Module
A
Sample Clustering
Height
B
Eigengene adjacency heatmap
C
Module−trait relationships
MEgreen
MEyellow
MEbrown
MEblue
MEturquoise
MEgrey
-0.29
(0.2)
-0.12
(0.6)
0.87
(3e-08)
0.46
(0.02)
-0.16
(0.5)
0.19
(0.4)
whitefly mortality
D

Fig.5

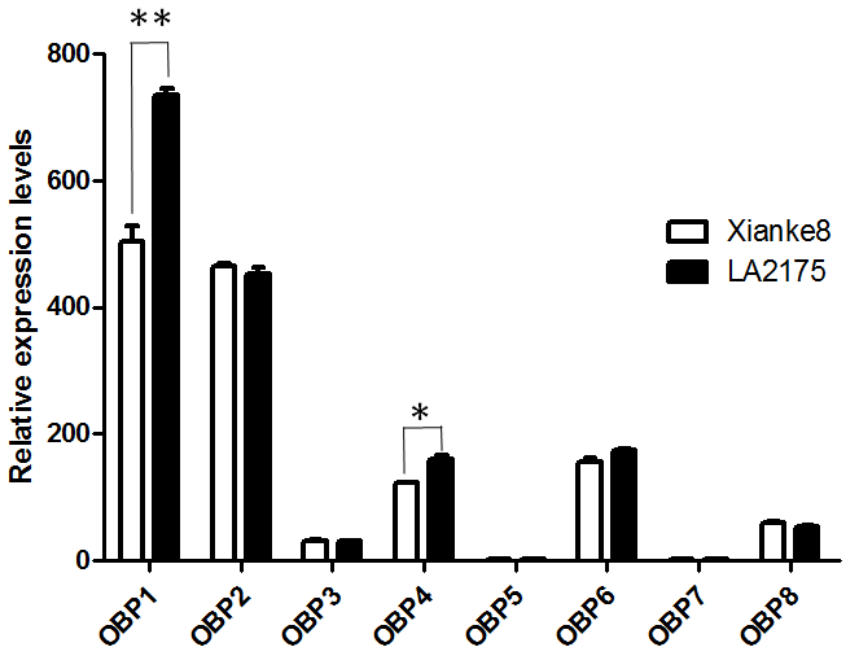

**
*
800
600
400
200
0
Relative expression levels
Xianke8
LA2175
OBP1
OBP2
OBP3
OBP4
OBP5
OBP6
OBP7
OBP8

Fig.6

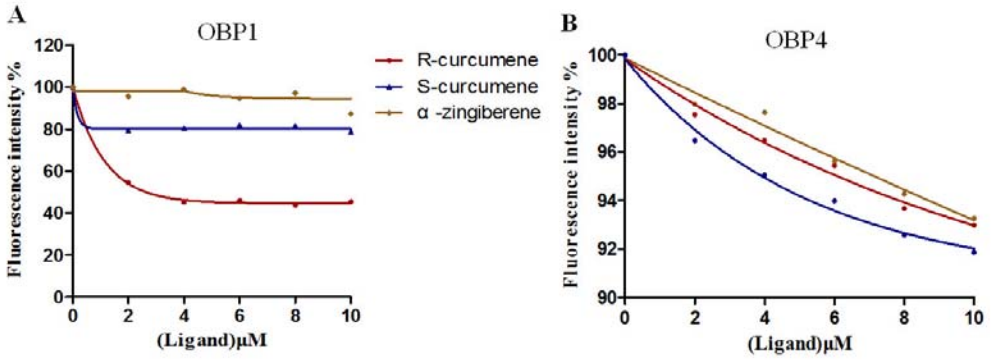

A
OBP1
Fluorescence intensity %
120
100
80
60
40
20
0
0
2
4
6
8
10
(Ligand)μM
R-curcumene
S-curcumene
α -zingiberene
B
OBP4
Fluorescence intensity %
100
98
96
94
92
90
0
2
4
6
8
10
(Ligand)μM

Fig.7

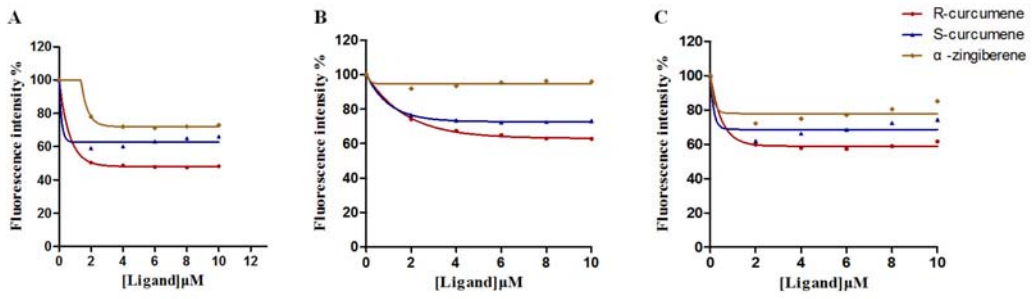

A
B
C
R-curcumene
S-curcumene
α -zingiberene
Fluorescence intensity %
Fluorescence Intensity %
[Ligand]μM

Fig.8

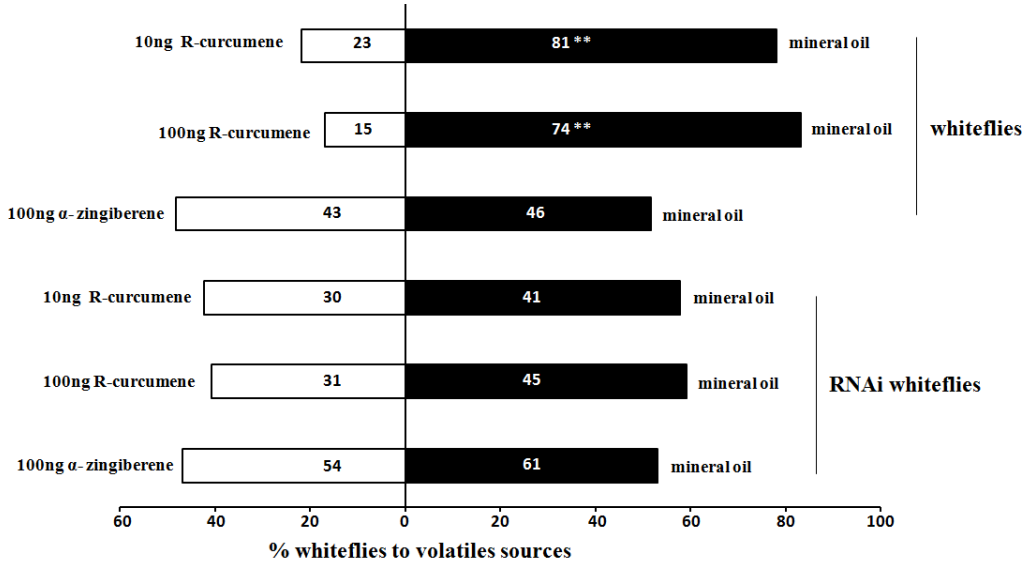

10ng R-curcumene
23
81 **
mineral oil
100ng R-curcumene
15
74 **
mineral oil
whiteflies
100ng α- zingiberene
43
46
mineral oil
10ng R-curcumene
30
41
mineral oil
100ng R-curcumene
31
45
mineral oil
RNAi whiteflies
100ng α- zingiberene
54
61
mineral oil
60
40
20
0
20
40
60
80
100
% whiteflies to volatiles sources

Fig. S1

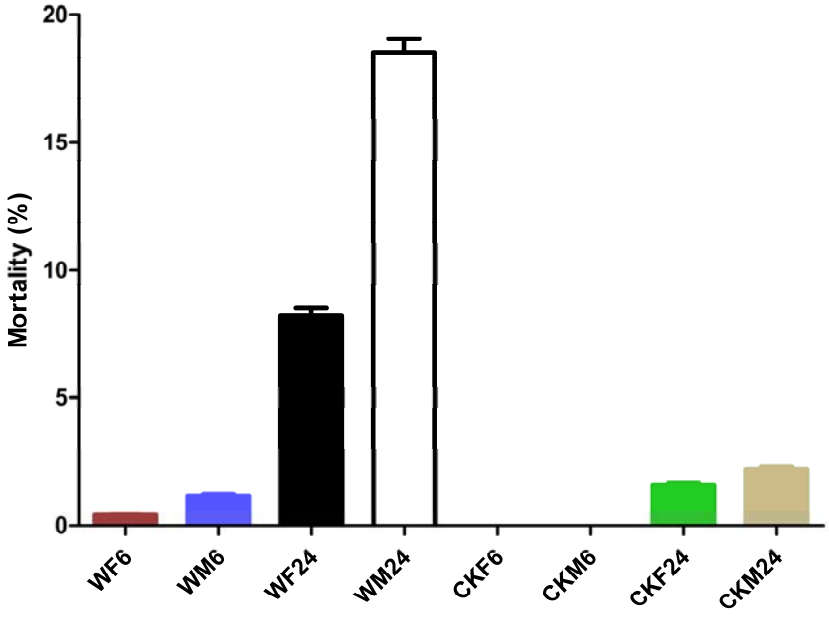
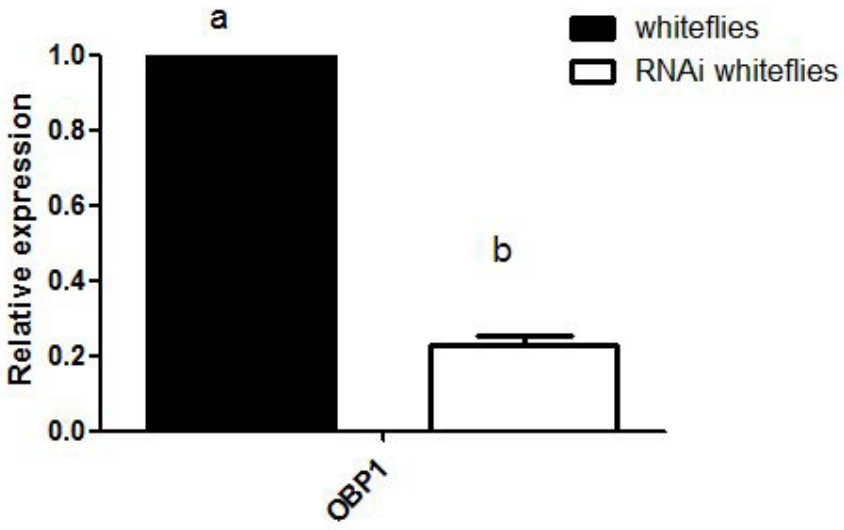


Fig. S2


a
b
whiteflies
RNAi whiteflies
Relative expression
1.0
0.8
0.6
0.4
0.2
0.0
OBP1