Acta Oecologica xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Acta Oecologica

journal homepage: www.elsevier.com/locate/actoec

Transcriptome analysis of genes involved in the response of a pollinator fig wasp to volatile organic compounds from its host figs

Jiqing Zeng[a,b], Hui Yu[a,c,*], Finn Kjellberg[d]

[a] Guangdong Provincial Key Laboratory of Digital Botanical Garden, South China Botanical Garden, The Chinese Academy of Sciences, Guangzhou 510650, PR China
[b] Key Laboratory of South China Agricultural Plant Molecular Analysis and Genetic Improvement, South China Botanical Garden, Chinese Academy of Sciences, Guangzhou 510650, PR China
[c] Key Laboratory of Plant Resource Conservation and Sustainable Utilization, South China Botanical Garden, The Chinese Academy of Sciences, Guangzhou 510650, PR China
[d] CEFE, UMR 5175, CNRS - Université de Montpellier - Université Paul-Valéry Montpellier – EPHE, 1919 Route de Mende, F-34293 Montpellier Cédex 5, France

ARTICLE INFO

Keywords:
Ficus hirta
Fig
Fig wasp
Fig-fig wasp recognition
Mutualism
Transcriptome

ABSTRACT

The mutualism of figs and their pollinating fig wasps is widely regarded as a model for coevolved mutualism. A high degree of host specificity is ensured by female wasps only being attracted by their specific fig tree species through the volatile organic compounds (VOCs) released by the figs when they are ready to be pollinated. However, very little is known about the molecular mechanisms underlying the production of VOCs and how pollinators respond to these VOCs. Here we present transcriptome sequencing data from VOC-treated fig wasps and control fig wasps. Using Illumina paired-end sequencing, approximately 6.47 Gbp and 6.48 Gbp high quality reads were generated for fig wasps that had been exposed or not to VOCs of their host fig. After read trimming, the de novo assembly of both types of reads produced 58,192 unigenes with an average length of 817 bp. Then functional annotation and GO enrichment analysis was performed by aligning all-unigenes with public protein databases including NR, SwissProt, and KEGG. Differentially expressed genes (DEGs) were investigated using the RPKM method. Overall, 16 up-regulated genes and 13 down-regulated genes were identified. We further performed GO enrichment and metabolic pathway enrichment analyses. One gene involved in the synoptic vesicle cycle and two genes coding for odorant binding proteins (OBP) are likely to have potential impacts on the response of fig wasps to the VOCs emitted by their host figs. This is the first transcriptome sequencing of a fig wasp in the presence of VOCs of its host figs using the next-generation sequencing technology. Our studies suggest that the expression of some genes in the olfactory neural system of the fig wasps is affected by the VOCs released from the figs. This suggests the presence of a dynamic molecular system of detection and hence response to host plant VOCs. As such our findings provide indications for further mechanistic studies on the fig-fig wasp interactions.

1. Introduction

Fig trees (*Ficus* spp. Moraceae), have a largely pan-tropical distribution, and form one of the largest genera of woody plants, with more than 750 species (Berg, 2003). The interactions between fig trees and fig wasps provide one of the classic examples of pollination mutualism and present a model for investigating co-evolution (Cruaud et al., 2010). Fig trees produce figs which are urn-shaped inflorescences, lined on the inside by hundreds or thousands of tiny female flowers, each of which can produce one fig wasp or one seed. Gall induction coincides with oviposition and pollination and takes place after the entry into a inflorescence of one or more foundress pollinator fig wasps.

The specificity of the relationship between a *Ficus* species and its pollinating wasps is maintained by a series of biological filters: volatile organic compounds (VOCs), ostiole (entry into the fig) structure and life history matching (Weiblen, 2002; Cook and Segar, 2010). To locate receptive figs, fig wasps mainly rely on identifying the volatile compounds the figs release, and are able to locate these figs despite the presence of other odors in the environment (Hossaert-Mckey et al., 1994; Grison-Pigé et al., 2002; Hossaert-Mckey et al., 2010). In the fig-fig wasp interaction system, the molecular interaction between floral smell and the fig wasps antennae is essential for the location and selection of hosts (Grison-Pigé et al., 2002; Raguso, 2008; Soler et al.,

* Corresponding author. Guangdong Provincial Key Laboratory of Digital Botanical Garden, South China Botanical Garden, The Chinese Academy of Sciences, Guangzhou 510650, PR China.
E-mail address: yuhui@scib.ac.cn (H. Yu).

https://doi.org/10.1016/j.actao.2018.01.003
Received 22 February 2017; Received in revised form 18 December 2017; Accepted 24 January 2018

2011). The odor emitted by figs varies according to inflorescence development stage and species. To locate appropriate breeding sites, the fig wasps must accurately identify the VOCs produced by receptive figs of their host fig tree species, differentiating these VOCs from those produced by co-occurring non-host-fig species (Grison-Pigé et al., 2002). Once a wasp has landed on the surface of a receptive fig, it typically brushes the surface of the fig with its antennae suggesting that tactile, gustative and olfactory cues may be involved in making the final decision to enter the fig (Gibernau et al., 1998). Moreover, inside the dark and moist inflorescences, an efficient physical and chemical sensing system is required for the fig wasps to successfully oviposit and for their offspring to mate (Weiblen, 2002). Therefore, in addition to morphological adaptations of fig wasps (van Noort and Compton, 1996), they must develop a perception mechanism of VOCs produced by the receptive inflorescences (Snyder et al., 1988; Hossaert-Mckey et al., 2010). .

Chemoreception is essential for the survival and reproduction of insects through processes such as the detection of food, predators, hosts, oviposition sites and mates (Sanchez-Gracia et al., 2009). Olfaction and taste have been reported as two major chemosensory mechanisms in insects (Vosshall and Stocker, 2007). In insects, volatile molecules are generally detected by olfactory sensory neurons (OSNs) that are located in antennal sensilla. The surface of sensilla present multiple pores, and the dendrites of OSNs are infiltrated by the sensillum lymph, which contains small, water soluble, odorant binding proteins (OBPs) and chemosensory proteins (CSPs) (Sanchez-Gracia et al., 2009). The cell membrane of OSNs also contains various receptor proteins that bind odor ligands (de Bruyne and Baker, 2008). Odor receptor genes expressed on insect OSNs are classified into three families (Benton et al., 2009; Touhara and Vosshall, 2009; Kaupp, 2010), which include odorant receptors (ORs), ionotropic receptors (IRs), and gustatory receptors (GRs) (Kwon et al., 2007). Most of the VOCs released by plants are lipid soluble small molecule compounds, which bind to the odorant binding proteins in the lymph and are transported to the olfactory receptor, causing nerve impulses that allow the insect to detect external stimuli. It has been shown that OBPs participate in the first step in odour detection (Pelosi et al., 2005). However, OBP family members are highly divergent among species of insects, and the minimum overall sequence identity between species can be as low as 17% (Vieira and Rozas, 2011a,b). The numbers and functions of these OBP genes are variable and diverse.

Although previous studies have shown that in insects chemical signals are detected by members of multigene families that encode odorant-binding proteins (OBPs), chemosensory proteins (CSPs), olfactory receptors (ORs), gustatory receptors (GRs) and ionotropic receptors (IRs) (Pelosi and Maida, 1990), the molecular mechanism of how VOCs affect the behavior of fig wasps is unclear and little genomic information is available for this non-model group of insects.

High-throughput RNA sequencing (RNA-Seq) using next-generation sequencing (NGS) has become a powerful technology to profile transcriptomes due to its accuracy and reproducibility (Wang and Gerstein, 2009). For fig wasps, the whole genome of a single species, *Ceratosolen solmsi marchali*, has been sequenced. Five GRs and forty-six OR genes were found and may be associated with the process of host specialization (Xiao et al., 2013). *Ceratosolen solmsi marchali*, the expression of two OBP genes (CsolOBP4 and CsolOBP5) was further confirmed using real-time quantitative PCR (Wang et al., 2014). In order to better understand the attraction mechanism of fig wasp to the VOCs, we need genomic or transcriptome studies on more fig wasps species and to identify more genes related to their chemoreception.

In this study, we investigate the response of a female wasp's transcriptome to exposure to the VOCs produced by their host tree. We surmised that, on recognizing this odor, a whole set of genes would be upregulated or downregulated in preparation for the next set of physiological and behavioural challenges facing the wasp. For instance, mobilizing carbohydrates stored in fat bodies is central to fueling flight (Beenakkers et al., 1984), and on reaching a receptive fig flight comes to an end. Narrowing in towards a receptive fig, saturation of receptors by increasing concentrations of VOCs may become a problem and could potentially stimulate higher turnover of odor-binding molecules. The wasp also needs to switch on the fig entering behavior and the set of behaviors associated with the act of oviposition. To provide preliminary elements to answer these questions, we produce a database of unigenes from VOC treated and non-treated fig wasps (*Valisia javana*) through Illumina transcriptome sequencing and de novo sequence assembly. This allowed us to analyze the functional composition of the transcriptome. Here we particularly focus on identifying relevant candidate genes responsible for odour detection in fig wasps, and specifically examine their expression in VOC-treated fig wasps and control wasps. We hypothesize that the match between the olfactory neural system of figs wasps and VOCs released by their host's inflorescences greatly contributes to fig-fig wasp interactions. Our studies show that the transcription of some genes in the olfactory neural system is modulated by exposure again to fig VOCs suggesting a role in the attraction mechanism. Our findings shed further light on fig-fig wasp interactions and provide a better understanding of the coevolved mutualism.

2. Materials and methods

Valisia javana sensu lato (super-family *Chalcidoidea*, family *Agaonidae*; Cruaud et al., 2010) is the pollinator of the dioecious shrub or small tree, *Ficus hirta* (family *Moraceae*, subgenus *Ficus*, section *Eriosycea*, sub-section *Eriosycea*; Berg, 2003), distributed from tropical South-East Asia (Indonesia) to tropical and subtropical northeast India and south China (Zhou and Gilbert, 2003).

In *F. hirta*, as in other functionally dioecious figs, female trees produce inflorescences containing only female flowers, which produce seeds and no wasp offspring. Male trees, in contrast, serve as hosts for fig wasps, producing 'male' inflorescences containing pollen-bearing male flowers and female flowers that are each capable of supporting a single developing fig wasp but no seeds.

The development of male figs can be divided into five phases (A–E; Yu and Compton, 2012). The pre-receptive A-phase comes first, with the fig containing young male and female flowers and not emitting pollinator attracting VOCs. The receptive B-phase is second, with the inflorescence emitting VOCs that attract pollinating wasps. In the three subsequent developmental phases (C-E), female flowers can contain developing pollinators. In the D-phase figs, female pollinators become adult, and mate with the males before escaping in search of B-phase figs.

2.1. Insect material, RNA extraction and sequencing

Eight male figs close to D-phase were collected in the South China Botanical Garden and divided equally into two tubes in the laboratory. The next morning, the fig wasps flew out of the figs and more than 50 adults were collected two tubes. Then, eight B-phase male inflorescences and eight B-phase female inflorescences were put into one tube of fig wasps (the SM sample), and the other tube of fig wasps was used as a control (the CK sample). After 35 min, both tubes of fig wasps were collected and immediately frozen in liquid nitrogen, and stored at −80 °C until further processing. Total RNA was extracted from the adult wasps using Trizol reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. The quantity of RNA in the samples was assessed using 1.1% agarose gel electrophoresis and a NanoDrop 2000 spectrophotometer (NanoDrop, Wilmington, DE, USA).

For each sample, a messenger RNA-Seq library was constructed using an Illumina TruSeq™ RNA Sample Preparation Kit (Illumina, San Diego, USA) following the manufacturer's recommendations. The isolation of messenger RNA (mRNA), fragment interruption, complementary DNA (cDNA) synthesis, adaptor ligation, PCR amplification, and RNA-Seq were performed by Novogene Bioinformatics Technology