Identification and tissue expression profiling of odorant binding protein genes in the red palm weevil, *Rhynchophorus ferrugineus*

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Abstract

**Background:** The red palm weevil, *Rhynchophorus ferrugineus*, is a lethal pest of the palms. The identification of odorant binding protein (OBP) genes will be helpful for clarifying the mechanism of odorant detection of this pest. By sequencing the full length cDNA library of its antennae, 11 OBP genes (*RferOBP1*-11) were identified.

**Findings:** The result showed *RferOBP1*-7 and *RferOBP8*-11 belonged to the minus-C and classic family, respectively qPCR revealed that *RferOBP1*-10 highly transcribed in the antennae, of which *RferOBP1*, *RferOBP4*, *RferOBP8* and *RferOBP10* were obviously male-biased expression. *RferOBP7* and *RferOBP11* exhibited highly expression in female head and male thorax. *RferOBP2*, *RferOBP5* and *RferOBP6* were highly expressed in the female thorax, leg and abdomen respectively.

**Conclusions:** The results paved the way towards a future understanding of the olfaction in this species.

Keywords: Expression pattern, Odorant binding protein, Olfactory, *Rhynchophorus ferrugineus*

Background

The red palm weevil, *Rhynchophorus ferrugineus*, belongs to Coleoptera: Dryophthoridae family and is native to tropical Asian regions which have become a most threatening pest of palm trees worldwide (Yan et al. 2015). *R. ferrugineus* is a concealed tissue borer that resides inside the trunk of the palms with a highly aggregated population distribution pattern and tree injuries are often severe when it is discovered (Abraham et al. 1998). The widespread nature of this pest is due to their ability of adoption to a broader host range, to a wider variety of climates, and practice of shipping the palm trees between different territories (Murphy and Briscoe 1999). In the newly invaded areas, this pest generally causes serious damage to the trees and significant economic losses are associated by minimizing the production of palms, and increases cost management in eradication of the pest (El-Sabea et al. 2009). Due to its concealed nature and exceptional colonization capability, integrated pest management strategies play an important role in controlling the red palm weevil (Vacas et al. 2014). With respect to this, large quantities...
of insecticides are still applied to prevent and control *R. ferrugineus* infestations (Llácer et al. 2013). However, trapping systems which include aggregation pheromone, date fruits, bait traps have been effectively and environmentally used to detect and control this pest (Abuagla and Al-Deeb 2012). New management strategies from the aspect of chemical ecology should be developed by understanding the *R. ferrugineus* olfactory system, which will be efficient in reducing the weevil populations and the usage of pesticides. However, most of the olfactory genes have been deciphered by transcriptome sequencing are fragmental and their expression patterns are not well known (Yan et al. 2015; Antony et al. 2016). Thus, the information regarding the molecular mechanisms underlying the olfaction in this species is scarce.

Odorant-binding proteins (OBPs) are hydrophilic soluble proteins which are composed of approximately 130 amino acids and typically contain six conserved cysteine residues (Leal et al. 1999) which are secreted by the accessory cells around the olfactory neurons and are accumulated in the sensillar lymph (Klein 1987). OBPs are essential for insect olfaction which acts by transporting hydrophobic compounds through aqueous sensillar lymph to the receptors embedded on dendritic membranes of olfactory receptor neurons from the external environment and is thought to be the first step in the recognition of chemical signals (Krieger and Breer 1999; Leal and Leal 2015). Hence, it is necessary to understand the mechanism of olfaction of *R. ferrugineus* OBPs which has an important role. Hence, in our study we reported the identification of 11 OBP genes from the antennae of this pest and analyzed their tissue expression patterns.

**Methods**

**Insects and sample collection**

*Rhynchophorus ferrugineus* was collected from cocoons but was originally collected in naturally infested palms in the suburbs of Hainan province. The lab colony was maintained as described in the study by Yan et al. (2014). The antennae, heads (without antenna), thoraxes, abdomens, and legs of male and female were collected separately and frozen immediately in liquid nitrogen.

**cDNA library construction and sequencing**

Total RNA was extracted from approximately 100 antennae of both sexes after 5 days of emergence by Trizol regent. The quantity and quality of the total RNA was validated using spectrophotometer. 5 μg of total RNA was subjected to construct full length cDNA library using Creator SMART cDNA Library Construction Kit (Clontech, Mountain View, CA, USA) according to the manufacturer’s instructions. During the library construction, 400–1200 bp fragments were selected. About 1000 clones were randomly sequenced from 5′-end of the gene. After BlastX annotation, the genes were assigned to the OBP family and were completely sequenced to obtain their full length cDNA. The signal peptide of the protein sequences were predicted using SignalP 4.0 server version (Petersen et al. 2011). Sequence alignments were performed using the program ClustalX (Thompson et al. 1997). Phylogenetic trees were constructed by MEGA version 5 based on the neighbor-joining algorithm method at bootstrap 1000 (Tamura et al. 2011).
qPCR analysis
Total RNA from different tissues were extracted as mentioned above. After DNase treatment, the cDNA was synthesized from 1 μg total RNA using a GoScript Reverse Transcription System (Promega, USA). qPCR was performed using gene-specific primers (Table 1) by SYBR Premix EX Taq™ (TaKaRa, Dalian, Liaoning, China) in three biological and technical replicates with different samples. PCR reactions were performed in a 3-step amplification process at 94 °C for 30 s, 55 °C for 30 s, and 72 °C for 30 s. Expression levels of the genes were calculated relative to the control gene 18S RNA expression using the $2^{-\Delta\Delta C_T}$ method (Livak and Schmittgen 2001). The total expression of one gene in all the tissues was set as 100 % and the percentage of one gene in each tissue was used to measure the expression level.

Results and discussion
Characteristics of OBP sequences
In total, 11 full length OBP genes from 36 clones named as RferOBP1-11 were identified from the antennal cDNA library sequencing, which have been deposited in the NCBI under the accession numbers KR780571 to KR780581 (Table 2). Compared to the 49 OBPs identified in the genome of Tribolium castaneum, more OBP genes might be expected to be identified in R. ferrugineus by massive sequencing strategies and by using more different tissues that may also express OBPs (Tribolium Genome Sequencing Consortium 2008; Zhu et al. 2013). The cDNA length ranged from 497 bp to 579 bp. The open reading frame (ORF) ranged from 396 to 441 bp, which encoded approximately

| Table 1 | qPCR primers |
|---|---|
| Primer name | Sequence (5’ → 3’) |
| RferOBP1 Reverse | TCTCGCCCAACATTTAC |
| Forward | TTTGCCGCCCTGTCCTCA |
| RferOBP2 Reverse | TAGTCAAGGCAGTTCACA |
| Forward | GTTACCACGCTTCTC |
| RferOBP3 Reverse | TCCGACGCTTCC |
| Forward | GACATTTATAGCAGG |
| RferOBP4 Reverse | GAGAAGCCTCAGCTCA |
| Forward | CGAACAATATTCCTT |
| RferOBP5 Reverse | CTGACAGGGCCAGGG |
| Forward | TATCCCAAACATACCA |
| RferOBP6 Reverse | CTGACTGGCGGAGG |
| Forward | TATCCCAAACATACCA |
| RferOBP7 Reverse | TGGTGTGGCCATC |
| Forward | CTGCGCCCTGCTGTTT |
| RferOBP8 Reverse | TGATGGTATGGGACCT |
| Forward | ATGTTGAGGCTGAGT |
| RferOBP9 Reverse | AGCGACTGAGGAGTAG |
| Forward | TGTCCTCTTGAGGAT |
| RferOBP10 Reverse | GTGACTCTGCTGGT |
| Forward | ATCCTAGATCCCCTT |
| RferOBP11 Reverse | AACAGGAGCAAGAAAGAT |
| Forward | ATAGCTGGCGGTAGGTT |
Table 2 Characteristics of *Rhynchophorus ferrugineus* OBP genes

| Gene name     | Nucleotide length (bp) | ORF length (bp) | Complete protein (aa) | Signal peptide (aa) | Molecular weight (kDa) | Isoelectric point | Accession number       |
|---------------|------------------------|-----------------|-----------------------|---------------------|-----------------------|-------------------|-----------------------|
| RferOBP1      | 556                    | 405             | 135                   | 19                  | 15.27                 | 8.43              | KR780571              |
| RferOBP2      | 569                    | 396             | 132                   | 18                  | 15.18                 | 4.42              | KR780572              |
| RferOBP3      | 568                    | 402             | 134                   | 20                  | 15.03                 | 5.25              | KR780573              |
| RferOBP4      | 497                    | 396             | 132                   | 18                  | 14.86                 | 5.02              | KR780574              |
| RferOBP5      | 509                    | 399             | 133                   | 17                  | 15.16                 | 4.43              | KR780575              |
| RferOBP6      | 533                    | 399             | 133                   | 17                  | 15.16                 | 4.43              | KR780576              |
| RferOBP7      | 580                    | 441             | 147                   | 19                  | 16.68                 | 6.59              | KR780577              |
| RferOBP8      | 556                    | 432             | 144                   | 18                  | 16.16                 | 7.01              | KR780578              |
| RferOBP9      | 579                    | 432             | 144                   | 21                  | 15.56                 | 7.78              | KR780579              |
| RferOBP10     | 570                    | 429             | 143                   | 19                  | 15.82                 | 4.6               | KR780580              |
| RferOBP11     | 571                    | 402             | 134                   | 18                  | 14.96                 | 4.29              | KR780581              |

130 amino acid residues, with a predicted molecular weight of about 15 kDa. Except for RferOBP1, 8 and 9, the predicted pH of others were acidic. The amino acid sequences identity of RferOBP5 and RferOBP6 was 99 %, while their DNA sequence homology was only 62 % (Fig. 1). RferOBP5 and RferOBP6 might be the result of gene duplication events. Similar to the characteristics of OBP with a rather low level of sequence similarity (Forêt and Maleszka 2006; Zhu et al. 2012), RferOBP1-4 and RferOBP7-11 shared 8–65 % amino acid residual identities with each other. In addition, they contained the characteristic sequence features for these proteins, such as conserved cysteine residues and signal peptide. All identified OBPs were used to construct a phylogenetic tree clustering with OBPs from *T. castaneum* (Fig. 2), which was the only species with

**Fig. 1** Alignment of the amino acid sequences of *Rhynchophorus ferrugineus* OBPs
| Gene       | Female |           | Thorax | Abdomen | Leg  | Male     |           | Thorax | Abdomen | Leg  |
|------------|--------|-----------|--------|---------|------|----------|-----------|--------|---------|------|
|            |        | Anntena   | Head   |         |      | Anntena  | Head      |         |         |      |
|            |        | 33.21 ± 1.01 | 2.66 ± 0.22 | 0.45 ± 0.03 | 0.24 ± 0.04 | 0.16 ± 0.01 | 61.54 ± 1.23 | 0.07 ± 0.01 | 1.12 ± 0.02 | 0.39 ± 0.04 | 0.17 ± 0.01 |
| RfrOBP1    |        | 18.06 ± 1.54 | 98.8 ± 0.26 | 23.67 ± 0.01 | 0.93 ± 0.00 | 17.17 ± 0.01 | 11.04 ± 1.68 | 0.71 ± 0.03 | 4.24 ± 0.12 | 1.01 ± 0.01 | 13.28 ± 0.02 |
| RfrOBP2    |        | 29.79 ± 0.07 | 583 ± 0.44  | 0.20 ± 1.11  | 1.05 ± 0.16 | 496 ± 0.79  | 29.53 ± 0.75  | 1.74 ± 0.04 | 0.02 ± 0.22 | 0.33 ± 0.12 | 26.55 ± 0.40 |
| RfrOBP3    |        | 24.91 ± 1.15 | 511 ± 0.82  | 4.07 ± 0.01  | 0.50 ± 0.13 | 1045 ± 0.22 | 39.00 ± 2.14  | 1.11 ± 0.09 | 0.90 ± 0.00 | 0.92 ± 0.03 | 13.04 ± 0.58 |
| RfrOBP4    |        | 15.05 ± 0.54 | 107 ± 0.21  | 5.65 ± 0.29  | 15.94 ± 0.05 | 35.88 ± 0.47 | 12.86 ± 1.28  | 0.40 ± 0.17 | 1.06 ± 0.02 | 0.47 ± 0.13 | 11.62 ± 1.28 |
| RfrOBP5    |        | 15.30 ± 1.94 | 147 ± 0.08  | 1.03 ± 0.29  | 27.38 ± 1.87 | 15.27 ± 2.67 | 22.20 ± 1.10  | 0.04 ± 0.04 | 2.99 ± 0.04 | 2.48 ± 0.04 | 11.84 ± 1.09 |
| RfrOBP6    |        | 12.30 ± 1.33 | 2806 ± 0.08 | 2.38 ± 0.07  | 0.91 ± 4.53  | 1.78 ± 0.32  | 9.05 ± 0.98  | 1.31 ± 0.01 | 28.85 ± 0.35 | 8.87 ± 0.16 | 6.50 ± 1.79 |
| RfrOBP7    |        | 30.21 ± 0.71 | 218 ± 1.18  | 0.39 ± 0.16  | 0.16 ± 0.12  | 0.09 ± 0.16  | 65.51 ± 1.23  | 0.05 ± 0.18 | 0.83 ± 2.40 | 0.45 ± 0.50 | 0.13 ± 0.93 |
| RfrOBP8    |        | 30.92 ± 1.37 | 1768 ± 0.13 | 0.78 ± 0.04  | 3.20 ± 0.02  | 4.73 ± 0.01  | 23.60 ± 1.35  | 0.83 ± 0.01 | 5.44 ± 0.04 | 8.83 ± 0.04 | 4.00 ± 0.00 |
| RfrOBP9    |        | 32.84 ± 0.24 | 1023 ± 1.17 | 0.03 ± 0.04  | 0.58 ± 0.20  | 3.64 ± 0.66  | 43.84 ± 1.11  | 1.51 ± 0.18 | 0.22 ± 0.86 | 4.08 ± 0.85 | 3.03 ± 0.33 |
| RfrOBP10   |        | 1.17 ± 0.09  | 4884 ± 2.22 | 3.60 ± 0.03  | 2.92 ± 0.18  | 0.52 ± 0.02  | 3.48 ± 0.11  | 1.99 ± 0.18 | 36.33 ± 2.14 | 0.30 ± 0.03 | 0.85 ± 0.13 |
| RfrOBP11   |        | 30.92 ± 1.37 | 1768 ± 0.13 | 0.78 ± 0.04  | 3.20 ± 0.02  | 4.73 ± 0.01  | 23.60 ± 1.35  | 0.83 ± 0.01 | 5.44 ± 0.04 | 8.83 ± 0.04 | 4.00 ± 0.00 |
known genomic sequence from Coleoptera and were distributed into all three groups. The phylogenetic tree had relatively large minus-C and classic OBPs, and one plus-C OBP branches. Minus-C OBPs lack the second and fifth cysteine residues, and plus-C OBPs contained three additional conserved cysteines and one conserved proline (Xu et al. 2009). Four (RferOBP8-11) of the candidate sequences represented classic OBPs, each containing the six conserved cysteine residues. RferOBP1-7 showed minus-C OBPs, and there was no plus-C OBP. Of the 12 OBPs, RferOBP2-6 were clustered with each other together, rather than those from other insect species suggesting that they may be the products of recent gene duplications (Li et al. 2015a, b). These OBPs from different clades may have different functions, which are interesting to be determined.

**Tissue expression pattern**
To uncover the tissue expression pattern, we used qPCR to assess the transcriptional abundance of the identified 11 OBP genes which showed varying degrees of expression in the antennae, head (without antenna), thorax, abdomen, and leg (Table 3). Except for RferOBP11 with abundant expression in female head and male thorax, RferOBP1-10 showed a relatively higher expression in the antennae suggesting that the OBP genes identified in current study may play an important role in the olfaction (Leal 2013). Of them, RferOBP1, RferOBP4, RferOBP8 and RferOBP10 were obviously male biased which may play the same role as pheromone binding proteins and could play a role in odorant perception of certain plant volatiles (Zhang et al. 2015). RferOBP3 was highly expressed in the legs with near identical expression with the antennae. With respect to the other genes, RferOBP2, RferOBP5 and RferOBP6 were highly expressed in the female thorax, leg and abdomen, respectively. As head, thorax and leg have taste sensilla and other olfactory sensilla, OBP expressed in these tissues may function in the perception of non-volatile host chemicals, gustatory reorganization and other olfactory function (Mitaka et al. 2011). These results were similar to the previous studies which showed that OBPs of some insect are expressed primarily or exclusively in non-antennae tissues or in larvae, which may have physiological functions independent of olfaction (Li et al. 2015a, b). This also reflects that the identification of these OBPs solely depend on the structural similarities and not function. Nonetheless, why OBPs are expressed in abdomen remains a mystery. RferOBP7 exhibited similar expression patterns with that of RferOBP11 and was expressed at a very high level in the female head and male thorax. The expression patterns make an important contribution to our understanding of OBPs in *R. ferrugineus* and might facilitate for their future functional characterization.

**Conclusion**
The work presented here brings an identification of 11 OBP genes from red palm weevil by sequencing the antennal full length cDNA library. In particular, the expression profile studies provided a clear map of these genes, which may further facilitate other functional studies on these genes. These data enables a basis to reveal the molecular mechanisms of olfactory functions in the red palm weevil.
Authors’ contributions
Conceived and designed the experiments: WY, LL and WQQ. Performed the experiments: YQL and NH. Analyzed the data: WY and LL. Contributed reagents/materials/analysis tools: WY, XZM and MI. Helped and advised preparations of the manuscript: YQL, XZM and NH. Wrote the paper: WY, LL and WQQ. All authors read and approved the final manuscript.

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Competing interests
The authors declare that they have no competing interests.

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