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The identification and expression analysis of candidate chemosensory genes in the bird cherry-oat aphid *Rhopalosiphum padi* (L.)

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Abstract

The bird cherry-oat aphid *Rhopalosiphum padi* (L.) is one of the most important wheat pests with polyphagia and autumn migrants. And, chemosensory genes were thought to play a key role in insect searching their hosts, food and mate. However, a systematic identification of the chemosensory genes in this pest has not been reported. Thus, in this study, we identified 14 odorant-binding proteins, nine chemosensory proteins, one sensory neuron membrane protein, 15 odorant receptors, 19 gustatory receptors and 16 ionotropic receptors from *R. padi* transcriptomes with a significantly similarity (E-value $< 10^{-5}$) to known chemosensory genes in *Acyrthosiphon pisum* and *Aphis gossypii*. In addition, real-time quantitative polymerase chain reaction (RT-qPCR) was employed to determine the expression profiles of obtained genes. Among these obtained genes, we selected 23 chemosensory genes to analyze their expression patterns in different tissues, wing morphs and host plants. We found that except *RpOBP1*, *RpOBP3*, *RpOBP4* and *RpOBP5*, the rest of the selected genes were highly expressed in the head with antennae compared with body without head and antennae. Besides that, the stimulation and depression of chemosensory genes by plant switch indicated that chemosensory genes might be involved in the plant suitability assessment. These results not only provide insights for the potential roles of chemosensory genes in plant search and perception of *R. padi* but also provide initial background information for the further research on the molecular mechanism of the polyphagia and autumn migrants of it. Furthermore, these chemosensory genes are also the candidate targets for pest management control in future.

Keywords: *Rhopalosiphum padi*, transcriptome, chemosensory gene, phylogenetic analysis, expression pattern

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Introduction

As shown in many cases of insect chemosensory systems, olfaction play critical roles in the interactions of insects with their environment, such as foraging, oviposition behaviors,

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mating choice and the communication of social insects (Hallem *et al.*, 2006; Carey *et al.*, 2010; Zheng *et al.*, 2013). Various chemosensory genes are involved in the capture of volatiles from environment and signal transduction, including odorant-binding proteins (OBPs), chemosensory proteins (CSPs), sensory neuron membrane proteins (SNMPs), odorant receptors (ORs), gustatory receptors (GRs) and ionotropic receptors (IRs) (Hallem *et al.*, 2006; Kang *et al.*, 2017*a*). In general, odors in environment were recognized and bound by two classes of small, water-soluble, extracellular-binding proteins, OBPs and CSPs, which transferred odorants through the sensilla lymph to insect chemoreceptors at the membrane surface of olfactory sensory neurons (OSNs), that were thought to be the first biochemical step in odors reception (Zheng *et al.*, 2013; Chang *et al.*, 2015; Northey *et al.*, 2016).

The functions of insect OBPs have been proposed: (1) transporting hydrophobic odorants across the sensilla lymph to the chemoreceptors; (2) solubilizing hydrophobic odors, (3) concentrating odors in the sensilla lymph fluid; (4) combine with odors degrading enzymes to remove or deactivate odorants after simulating the chemoreceptors (Gu *et al.*, 2015; Zhu *et al.*, 2016). In aphid, the systemic identification of OBPs only reported in the pea aphid *Acyrthosiphon pisum*, cotton aphid *Aphis gossypii* and the English grain aphid, *Sitobion avenae* (Fabricius) (He *et al.*, 2011; Zhou *et al.*, 2010; Zhou *et al.*, 2015; Xue *et al.*, 2016). In *Megoura viciae* and *Nasonovia ribisnigri*, only the aphid pheromone (*E*-beta-farnesene) binding protein, OBP3, was cloned and functionally analyzed which was similar with the OBP3 in *A. pisum* (Qiao *et al.*, 2009; Northey *et al.*, 2016). Consist with the results of OBP3 in *A. pisum*, *OBP7* in the wheat aphid *S. avenae* showed high binding affinity with (*E*)-beta-farnesene (Zhong *et al.*, 2012). Similarly to OBPs, CSPs also mainly functioned in olfaction by solubilizing and transporting odors (Wang *et al.*, 2014; Cui *et al.*, 2017). However, in the vetch aphid *Megoura viciae*, two CSPs *MvOS-D1* and *MvOS-D2* showed no binding affinity for any of 28 compounds (Jacobs *et al.*, 2005). To consistent with this result, in *Camponotus japonicas* and *Chrysoperla sinica*, some CSPs are highly expressed in the non-chemosensory organs in the carpenter ant (Hojo *et al.*, 2015; Li *et al.*, 2015*c*). Thus, all these results suggested that CSPs may have varied functions in other biological function process. Additionally, SNMPs has been proposed to play an essential role in insect olfaction, especially in the process of pheromone reception in insects (Liu *et al.*, 2014; Jiang *et al.*, 2016).

Chemoreceptors, including three large and divergent families: ORs, GRs and IRs, are responsible for receiving, transporting and triggering responses to semiochemicals (Hallem *et al.*, 2006; Li *et al.*, 2015*b*; Liu *et al.*, 2015). In insect, chemoreceptors have been identified in various insect species (Ahmed *et al.*, 2016; Niu *et al.*, 2016; Sheng *et al.*, 2017). In aphid, chemoreceptors have been thoroughly identified in *A. pisum*, *D. noxia* and *A. gossypii* (Cao *et al.*, 2014; Nicholson *et al.*, 2015). Based on the real-time quantitative polymerase chain reaction (RT-qPCR) results of *A. gossypii*, almost all of the putative ORs were mainly expressed in the head (Cao *et al.*, 2014). Based on the RNAi experiment, *SaveOrco* was considered to play a critical role in the aphid's response to pheromones and other volatiles in *S. avenae* (Fan *et al.*, 2015). In the Chinese white pine beetle *Dendroctonus armandi*, the silencing of *Orco* led to significantly declining in EAG response to 11 major volatiles of its host (Zhang *et al.*, 2016*a*). As ORs serve as the molecular interface between the insect and its odor environment, GRs were considered to play a central role in co-ordinating insect feeding behaviors (Agnihotri *et al.*, 2016; Xu *et al.*, 2016). *In vitro* studies with GRs from the silkworm *Bombyx mori*, *BmGR8* and *BmGR9* showed strong responses to *myo*-inositol and D-fructose (Sato *et al.*, 2011). In the cotton bollworm *Helicoverpa armigera*, *HaGr1* and *HaGr3* were indispensable and sufficient for CO_2 sensing in labial palps (Ning *et al.*, 2016). As the third type of chemoreceptors, IR has facilitated dissection of the mechanism by which these olfactory receptors localize to OSN sensory cilia, recognize odors and produce neuronal depolarization (Wang *et al.*, 2015*b*; Hussain *et al.*, 2016). For example, the co-expression of *Ir8a* was necessary for the successful expression of *Ir84a* in *Or22a* neurons or in *Xenopus oocytes* (Abuin *et al.*, 2011). And co-expression of *Ir8a* with *Ir75a* conferred responses to a different ligand, propionic acid (Ai *et al.*, 2010). By contrast, in *Locusta migratoria*, the IR-based pathway was not responsible for the attractive behavior of gregarious nymphs (Wang *et al.*, 2015*b*). All of these work suggested that chemosensory genes play a critical role in insect-environment interactions.

The bird cherry-oat aphid, *Rhopalosiphum padi* (L.), is well known as one of the most important pests of wheat. Due to direct feeding and transmitting the *Barley yellow dwarf virus* (BYDV), this aphid caused a dramatic reduction of the quality and yield of wheat crops globally (Li *et al.*, 2015*b*; Wang *et al.*, 2015*a*). However, *R. padi* is a polyphagous pest of barley, oats, wheat, common choke-cherry, bird cherry *Prunus padus L.* and other species with alternation of hosts: its winter hosts are Rosaceae, and its summer hosts are Gramineae (Dixon, 1971; Li *et al.*, 2015*b*). It has been found that the volatile components emitted from its host plant cause the switch between winter and summer hosts (Pettersson *et al.*, 1994). Methyl salicylate produced by *P. padus* has been identified as a take-off stimulus of spring migrants and significantly reduced the initial aphid settling in filed spraying experiment (Park *et al.*, 2000; Pettersson *et al.*, 1994). Besides, the nonvolatile components of its host plants play a critical role in the host acceptance (Dewhirst & Pickett, 2009; Nam & Hardie, 2014). And the feeding behaviors of *R. padi* in different hosts were significantly different (Nam & Hardie, 2014; Li *et al.*, 2015*b*). All of these results revealed that *R. padi* has evolved comprehensive chemosensory systems to search for the suitable host plants during their migration and alter their feeding strategy based on the host plants' nutrition and defense condition. In this study, we identified and annotated the putative chemosensory genes in *R. padi* using next-generation sequencing. To have an initial prediction of these genes function, the expression patterns of these genes among the different tissues, hosts and wing morphs were conducted by qPCR.

Materials and methods

Identification of the chemosensory genes in R. padi

The transcriptome data were downloaded from the National Center for Biotechnology Information (NCBI) Sequences Read Archive (SRA) database (Accession number: ERR983159, ERR983160, ERR983161, ERR983162, ERR983163 and ERR983164), which were conducted by Illumina HiSeq 2000 paired-end sequencing. The raw reads were cleaned by removing adapter sequences, low-quality sequences (reads with ambiguous bases 'N'), and reads with >50% Q <20 bases. Cleaned reads shorter than 60 bases were removed because short reads might represent sequencing artifacts. Then, transcriptome *de novo* assembly was carried out using a short-

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reads assembling program – Trinity, which combines three independent software modules: Inchworm, Chrysalis and Butterfly, to overcome the quality and polymorphism issues. The previously described OBPs, CSPs, SNMPs, ORs, GRs and IRs sequences from *A. pisum*, *A. gossypii* and *D. melanogaster* were used as queries searching and verifying against the *R. padi* database using TBLASTX program with e-value $<10^{-5}$.

Phylogeny analysis of the chemosensory genes

Amino acid sequences of candidate OBPs, CSPs, SNMPs, ORs, GRs or IRs were aligned by MAFFT through FFT-NS-I iterative refinement method with JTT200 scoring matrix, unalignlevel 0.3, 'leave gappy regions' set and other default parameters. Bioedit Sequence Alignment Editor 7.1.3.0 (Ibis Pharmaceuticals, Inc., Carlsbad, CA, USA) was used for the further manual editing. Phylogenetic trees were subsequently constructed by the Maximum likelihood (ML) method using PhyML3.1 based on the best-fit model LG+G estimated by ProtTest2.4. SH-like approximate likelihood ratios (aLRT-SH) supports were used to evaluate the reliability of internal branches. The trees were further edited using the ITOL tool. And all amino acid sequences used in this work were presented in table S2.

The rear condition and treatment of R. padi

In this study, the bird-cherry oat, *R. padi* was kindly provided by Dr Kang Wang (Northwest A&F University, Yangling, Shaanxi, China), and maintained on seedlings of wheat cultivar 'Xinong 979' at 23 ± 1°C, a photoperiod of L16:D8, and relative humidity of 60 ± 5%. For the host switch treatment, the third nymph of *R. padi* was transferred from wheat to maize (*Zea mays* L., var. 'Zhengdan 985') and chili pepper (*Capsicum annuum* L., var. 'Lingxiudajiao F1') upon to adult. The whole bodies of five *R. padi* from different treatment were collected as a biological replicate and each treatment with three biological treatments. Then, the collected samples were frozen in liquid nitrogen and immediately stored at −80°C for further processes.

Expression profiles of chemosensory genes via qPCR

Total RNA was extracted by TRIzol reagent (Takara Bio, Tokyo, Japan) per manufacturer's instructions. The RNA integrity was verified by 1% agarose gel electrophoresis and the quantity was assessed with a Nanodrop ND-2000 spectrophotometer.

qPCR was performed to validate the expression of candidate chemosensory genes in *R. padi*. Then, 1 µg of RNA was used to synthesize the first strand complementary DNA (cDNA) using a PrimeScript® RT reagent Kit with gDNA Eraser (perfect Real Time) (Takara, Tokyo, Japan) following the manufacturer's protocol. The synthesized cDNA was stored at −20°C. Gene-specific primers were designed by Primer Premier 5 (PREMIER Biosoft International, Palo Alto, CA, USA) and were shown in table S1. qPCR was conducted in 20 µl reactions containing 50x SYBR Premix Ex Taq 10 µl, primer (10 mM) 0.8 µl, sample cDNA 0.8 µl, and sterilized ultra-pure grade H_2O 7.6 µl. Cycling conditions were: 95°C for 30 s, 40 cycles of 95°C for 5 s and 55°C for 30 s. Each sample was done in triplicate technical replicates and three biological replicates. Relative quantification was performed by using the Comparative $2^{-\Delta\Delta CT}$ method. Transcription levels of these genes were normalized by Actin (Zuo *et al*., 2016; Kang *et al*., 2017*b*).

Results

Identification of putative OBPs

Fourteen transcripts encoding candidate OBPs in *R. padi* were identified based on the PSI-blast. The number of putative OBPs in this work is more than that of *A. gossypii* and *A. pisum*. All of these putative OBPs had full-length ORFs, and only *RpOBP10* without signal peptide. The detail information on the putative OBPs was shown in table 1. And a phylogenetic tree was constructed using the identified OBPs from *A. pisum*, *A. gosyypii* and *R. padi* (fig. 1). In the phylogenetic tree, *RpOBP2*, *RpOBP4*, *RpOBP5*, *RpOBP6*, *RpOBP8*, *RpOBP7* and *RpOBP9* were clustered in a specific subgroup.

Identification of putative CSPs

Nine transcripts encoding candidate CSPs in *R. padi* were identified and the detail information on the putative CSPs was shown in table 1. The number of putative CSPs in *R. padi* is similar with *A. gossypii* and *A. pisum* and more than that of *S. avenae*. Of the nine putative CSPs, all of them had full-length ORFs and only *RpCSP8* without signal peptide. To analyze the relationship between the CSPs and those of other species, a phylogenetic tree was presented in fig. S1, which includes the identified OBPs from *R. padi*, *A. gossypii* and *N. lugens*. In the phylogenetic tree, all of these identified CSPs were clustered in a specific subgroup.

Identification of putative SNMPs

Only one transcript that encoded putative SNMP with ORFs of 1470 bp (table 1). The E-value for Blastp search was 0, indicating that they were homologous to known sequences in *A. pisum* and *Diuraphis noxia*. The phylogenetic tree was constructed (fig. S2).

Identification of putative ORs

We identified transcripts encoding 14 putative ORs. Of the 14 ORs, *RpOrco1*, *RpOr17* and *RpOr31* likely represented full-length genes, encoding proteins of longer than 300 amino acids. And the E-value for Blastp search of *RpOrco1* was 0 comparing with that of *A. pisum* and *A. gossypii*. The detail information on ORs was shown in table 1, and phylogenetic tree was constructed and presented in fig. 2. *RpOrco1*, *AgOrco1* and *ApOr1* were clustered in a specific subgroup called odorant co-receptor (Orco).

Identification of putative GRs

We identified 19 transcripts encoding putative GRs (table 1). However, none of them were close to full-length genes with bigger than 1000 bp ORFs. A phylogenetic tree was constructed with sequences from *R. padi*, *A. pisum* and *D. melanogater*. In the phylogenetic tree, *RpGr14* was clustered as carbon dioxide receptor (CO_2 receptor), and *RpGr1*, *RpGr2*, *RpGr3*, *RpGr4* and *RpGr6* were found in a clade with sugar receptors, which included GRs identified from *D. melanogater* (fig. 3).

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Table 1. The identified chemosensory genes in *R. padi* transcriptome

Gene type	Gene name	Length (bp)	ORF (aa)	Status	Blast query sequences	E-value	Identify (%)
OBPs	*RpOBP1*	798	165	Complete	*ApOBP1*	1e-025	40
	RpOBP2	1400	243	Complete	*ApOBP2*	1e-128	90
	RpOBP3	933	141	Complete	*ApOBP3*	2e-086	87
	RpOBP4	1608	199	Complete	*ApOBP4*	2e-120	89
	RpOBP5	1202	221	Complete	*ApOBP5*	1e-146	88
	RpOBP6	907	215	Complete	*ApOBP6*	2e-101	87
	RpOBP7	819	148	Complete	*ApOBP7*	3e-077	81
	RpOBP8	798	165	Complete	*ApOBP8*	3e-093	91
	RpOBP9	815	166	Complete	*ApOBP9*	2e-096	86
	RpOBP10	770	176	Complete	*ApOBP10*	3e-073	72
	RpOBP11	933	141	Complete	*ApOBP11*	1e-060	74
	RpOBP13	1380	329	Complete	*ApOBP13*	4e-070	94
CSs	*RpCSP1*	1583	165	Complete	*AgCSP1*	4e-110	93
	RpCSP2	849	131	Complete	*AgCSP2*	7e-079	91
	RpCSP4	2167	145	Complete	*AgCSP4*	3e-083	96
	RpCSP5	591	139	Complete	*AgCSP5*	2e-081	93
	RpCSP6	782	131	Complete	*AgCSP6*	1e-082	88
	RpCSP7	719	152	Complete	*AgCSP7*	3e-103	93
	RpCSP8	856	110	Complete	*AgCSP8*	3e-095	89
	RpCSP9	744	186	Complete	*AgCSP9*	8e-070	69
	RpCSP10	622	151	Complete	*AgCSP10*	4e-081	82
SNMP	*RpSNMP1*	2151	460	Complete	*ApSNMP1*	0	85
ORs	*RpOrco1*	1851	463	Complete	*ApOr1*	0	96
	RpOr3	427	116	3′5′lost	*ApOr3*	2e-071	92
	RpOr4	215	57	3′5′lost	*ApOr4*	3e-016	65
	RpOr16	453	148	3′5′lost	*ApOr16*	3e-078	76
	RpOr17	485	132	3′5′lost	*ApOr17*	5e-055	68
	RpOr24	583	195	3′5′lost	*ApOr24*	2e-030	58
	RpOr26	270	67	3′5′lost	*ApOr26*	3e-015	43
	RpOr27	232	77	3′5′lost	*ApOr27*	2e-019	47
	RpOr31	1888	419	Complete	*ApOr31*	2e-156	67
	RpOr43	331	109	3′5′lost	*ApOr43*	8e-056	83
	RpOr46	226	73	3′5′lost	*ApOr46*	1e-011	68
	RpOr45	218	72	3′5′lost	*ApOr45*	2e-024	76
	RpOr60	294	74	3′5′lost	*ApOr60*	2e-008	41
	RpOr70	1416	378	Complete	*ApOr70*	2e-017	47
	RpOr73	267	90	3′5′lost	*ApOr73*	4e-007	52
GRs	*RpGr1*	507	157	3′5′lost	*ApGr1*	2e-104	99
	RpGr2	929	159	3′5′lost	*ApGr2*	5e-104	93
	RpGr3	507	157	3′5′lost	*ApGr3*	2e-089	84
	RpGr4	635	181	3′5′lost	*ApGr4*	5e-072	78
	RpGr5	237	79	3′5′lost	*ApGr5*	1e-048	100
	RpGr6	333	110	3′5′lost	*ApGr6*	7e-066	96
	RpGr7	276	71	3′5′lost	*ApGr7*	6e-02	76
	RpGr8	280	63	3′5′lost	*ApGr8*	7e-015	79
	RpGr9	229	33	3′5′lost	*ApGr9*	1e-005	70
	RpGr10	706	31	3′5′lost	*ApGr10*	2e-026	90
	RpGr13	782	170	3′5′lost	*ApGr13*	9e-068	76
	RpGr14	406	134	3′5′lost	*ApGr14*	6e-057	78
	RpGr15	352	117	3′5′lost	*ApGr15*	3e-044	98
	RpGr16	495	73	3′5′lost	*ApGr16*	1e-037	95
	RpGr17	361	98	3′5′lost	*ApGr17*	2e-056	97
	RpGr18	212	70	3′5′lost	*ApGr18*	3e-029	87
	RpGr35	215	71	3′5′lost	*ApGr35*	2e-032	92
	RpGr37	346	114	3′5′lost	*ApGr37*	3e-030	75
	RpGr41	392	92	3′5′lost	*ApGr41*	6e-027	80
	RpGr43	338	69	3′5′lost	*ApGr43*	6e-023	86
	RpGr45	229	74	3′5′lost	*ApGr45*	3e-028	81

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Table 1. (*Cont.*)

Gene type	Gene name	Length (bp)	ORF (aa)	Status	Blast query sequences	E-value	Identify (%)
IRs	*RpIr7c*	1575	297	5′lost	*DmIr7c*	3e-005	40
	RpIr8a	1368	366	Complete	*DmIr8a*	7e-159	67
	RpIr21a	311	99	3′5′lost	*DmIr21a*	3e-016	28
	RpIr25a	1890	511	Complete	*DmIr25a*	0	72
	RpIr31a	2895	377	Complete	*DmIr31a*	8e-008	42
	RpIr40a	877	164	3′5′	*DmIr40a*	1e-029	35
	RpIr41a	1332	197	3′5′lost	*DmIr41a*	7e-006	43
	RpIr68a	1101	120	3′5′lost	*DmIr68a*	2e-018	38
	RpIr75b	3258	465	Complete	*DmIr75b*	1e-005	39
	RpIr75c	3424	324	Complete	*DmIr75c*	3e-007	42
	RpIr75d	314	100	3′5′lost	*DmIr75d*	2e-016	35
	RpIr76b	264	85	3′5′lost	*DmIr76b*	1e-030	66
	RpIr84a	3105	383	Complete	*DmIr84a*	5e-016	38
	RpIr93a	912	236	3′5′lost	*DmIr93a*	3e-048	41

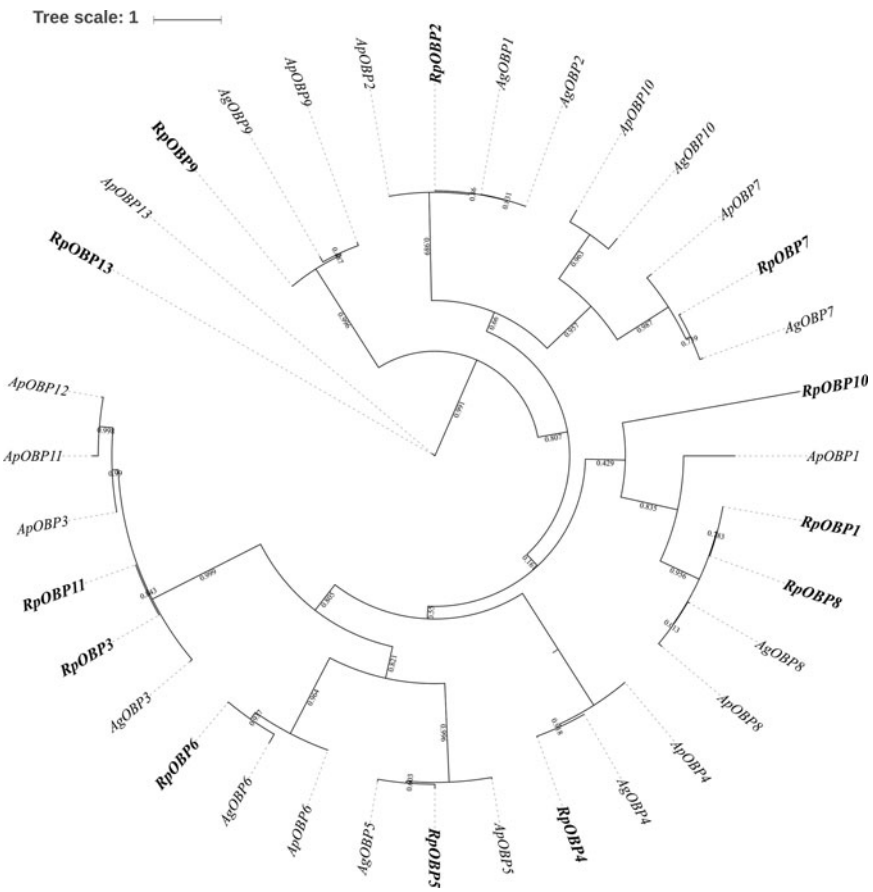

Fig. 1. Maximum likelihood phylogenetic tree of odorant-binding proteins (OBPs). OBPs from *R. padi* (Rp), *A. pisum* (Ap) and *A. gossypii* (Ag) are included.

Identification of putative IRs

We identified 16 transcripts encoding putative IRs (table 1). Among them, 12 IRs were close to the full-length gene. The E-value of *RpIr25a* was 0 comparing with the sequence of Ir25a in *Drosophila melanogaster*. In the phylogenetic tree, most of these IRs were clustered as a known group, like *Ir8a*, *Ir25a*, *Nmdar*, *Ir93a*, *Ir75b* and *Ir40a* (fig. 4).

The expression patterns of putative chemosensory genes

We verified the expression patterns of these chemosensory genes in different tissues, wing morphs and host plants. *RpOBP1*, *RpOBP3* and *RpOBP5* predominately expressed in body compared with head with antennae, while the rest selected genes, except *RpOBP4*, highly expressed in the head with antennae (fig. 5). When transferred to non-host plants

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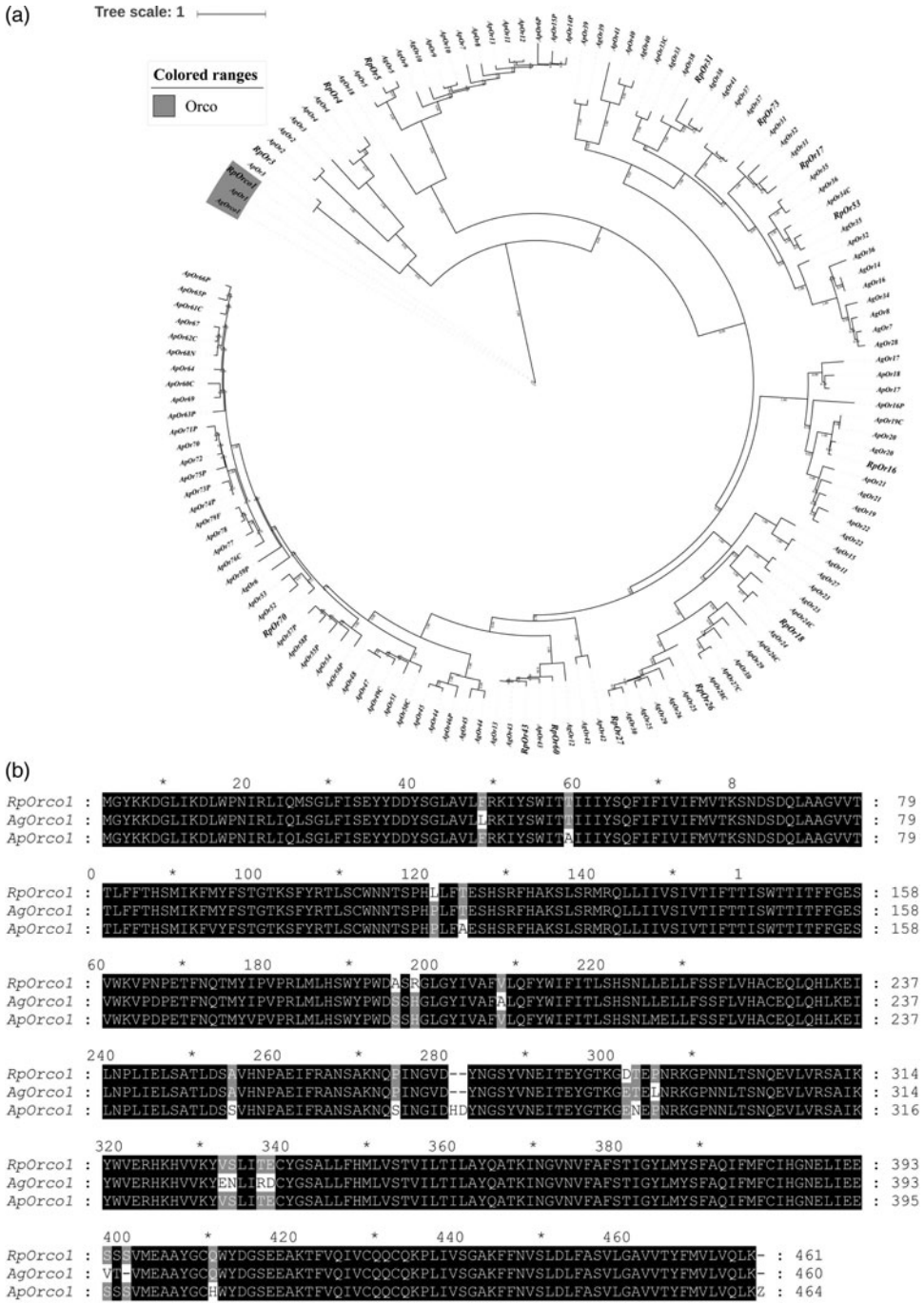

Fig. 2. Maximum likelihood phylogenetic tree of odorant receptors (ORs, A) and the amino acid sequences alignment of odorant co-receptors (Orco) of *R. padi* (B). ORs from *R. padi* (Rp), *A. pisum* (Ap) and *A. gossypii* (Ag) are included.

(chili pepper), the expression of *RpGr1*, *RpGr4*, *RpGr5*, *RpGr6*, *RpIr25a* and *RpNmdar2* in *R. padi* reared on maize were lower than that in *R. padi* reared on chili pepper (fig. 6). In the contrary, the expression of *RpOBP1*, *RpOBP2*, *RpOBP3*, *RpOBP4*, *RpOBP5*, *RpOBP6*, *RpOBP7*, *RpOBP9*, *RpOBP10*, *RpOBP11*, *RpORco*, *RpGr2*, *RpIr8a* and *RpIr76b* reared on maize were higher than that in *R. padi* reared on the chili pepper. *RpOBP2*, *RpOBP5*, *RpOBP6*, *RpOBP7*, *RpOBP8* and *RpOBP10* were abundantly expressed in winged aphid compared with wingless aphid whereas *RpIr8a*, *RpIr25a*, *RpIr41a*, *RpIr76b*, *RpNmdar1* and *RpNmdar2* predominately expressed in wingless aphid (fig. S3).

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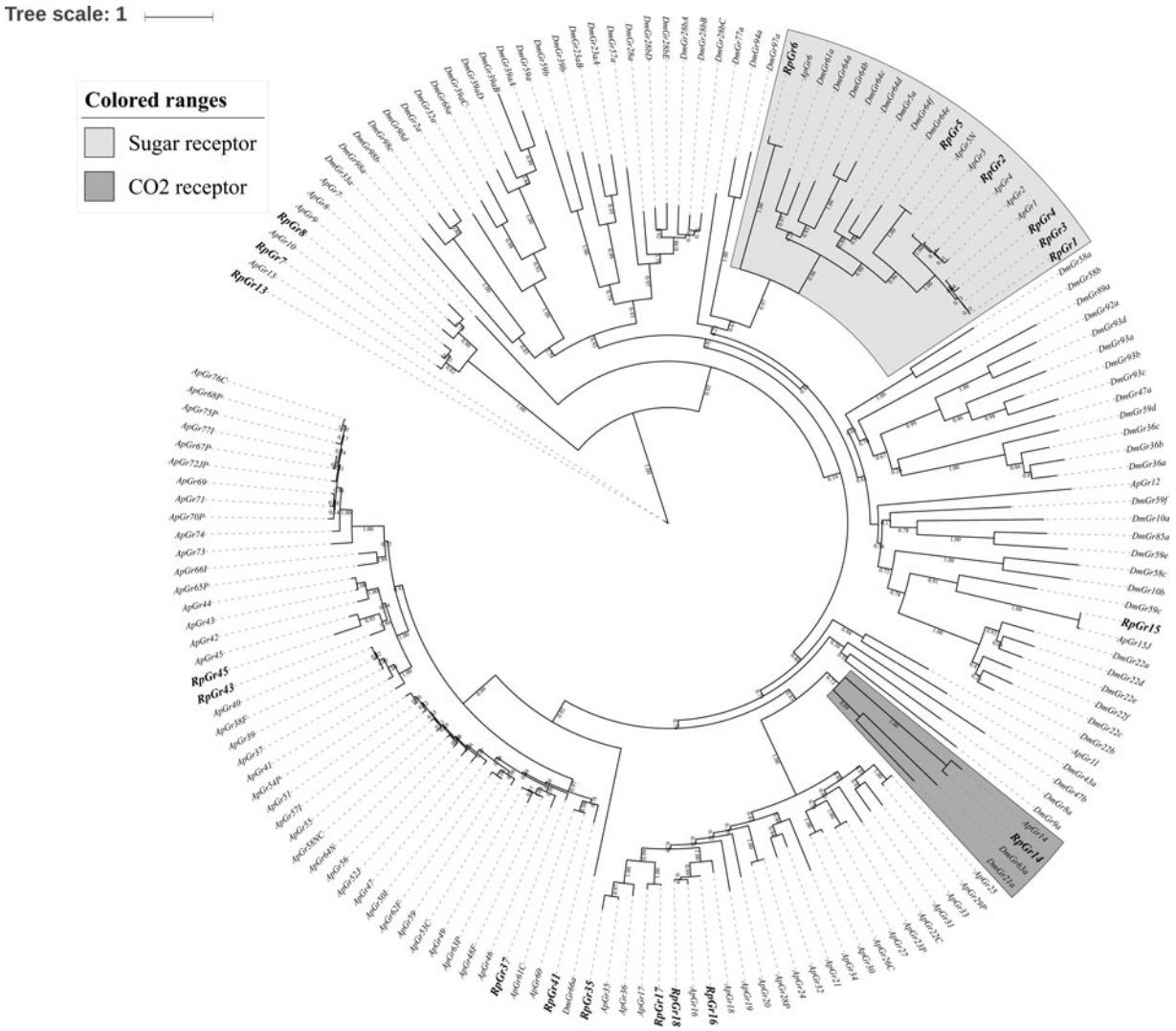

Fig. 3. Maximum likelihood phylogenetic tree of gustatory receptors (GRs). GRs from *R. padi* (Rp), *A. pisum* (Ap) and *D. melanogaster* (Dm) are included.

Discussion

Olfactory plays a critical role in insect survival and reproduction, such as foraging, aggregation, enemy avoidance, and mating (Hallem *et al.*, 2006). Because of their season-dependent alternation of hosts, *R. padi* has gained massive attention for studying the host resistance and chemical cues in this process (Dixon, 1971). No investigation has focused on the putative functions of olfactory systems in this process. Our discoveries provide the first extensive molecular insights into the olfactory system of *R. padi*.

We performed a bioinformatic searching for olfactory genes based on the *R. padi* transcriptome using the related sequences from other species (Zhou *et al.*, 2010; Cao *et al.*, 2014; Xue *et al.*, 2016). Finally, we identified a total of 71 olfactory genes including 11 OBPs, nine CSPs, one SNMP, 15 ORs, 19 GRs and 16 IRs. All these candidates have been confirmed by Blastp in NCBI. The number of identified chemosensory genes in *R. padi* were similar with that in other aphid species except ORs, which was significantly less than that in other aphid species (Zhou *et al.*, 2010; He *et al.*, 2011; Zhou *et al.*, 2015; Xue *et al.*, 2016). ORs identified in other aphid species were screened from the genome database. Thus, the less of identified ORs in *R. padi* might be the different sequencing strategy. And the sequencing tissues might be another reason for that (Kang *et al.*, 2017*a*). In this work, the transcriptome was conducted using the RNA extracted from the head with antennae.

As the first gate in the odorant recognition process, the functions of OBPs in various insect species have been well documented. For example, in the aphid, *OBP3* and *OBP7* in *A. pisum* and *S. avenae* were considered as the key delivery of aphid alarm pheromone whereas OBP1 only showed binding affinity with plant volatiles (Qiao *et al.*, 2009). Furthermore, OBP1 and OBP3 were found to be expressed in cornicle (De Biasio *et al.*, 2015). Consistent with this, in this work, we found RpOBP1 and RpOBP3 predominately expressed in

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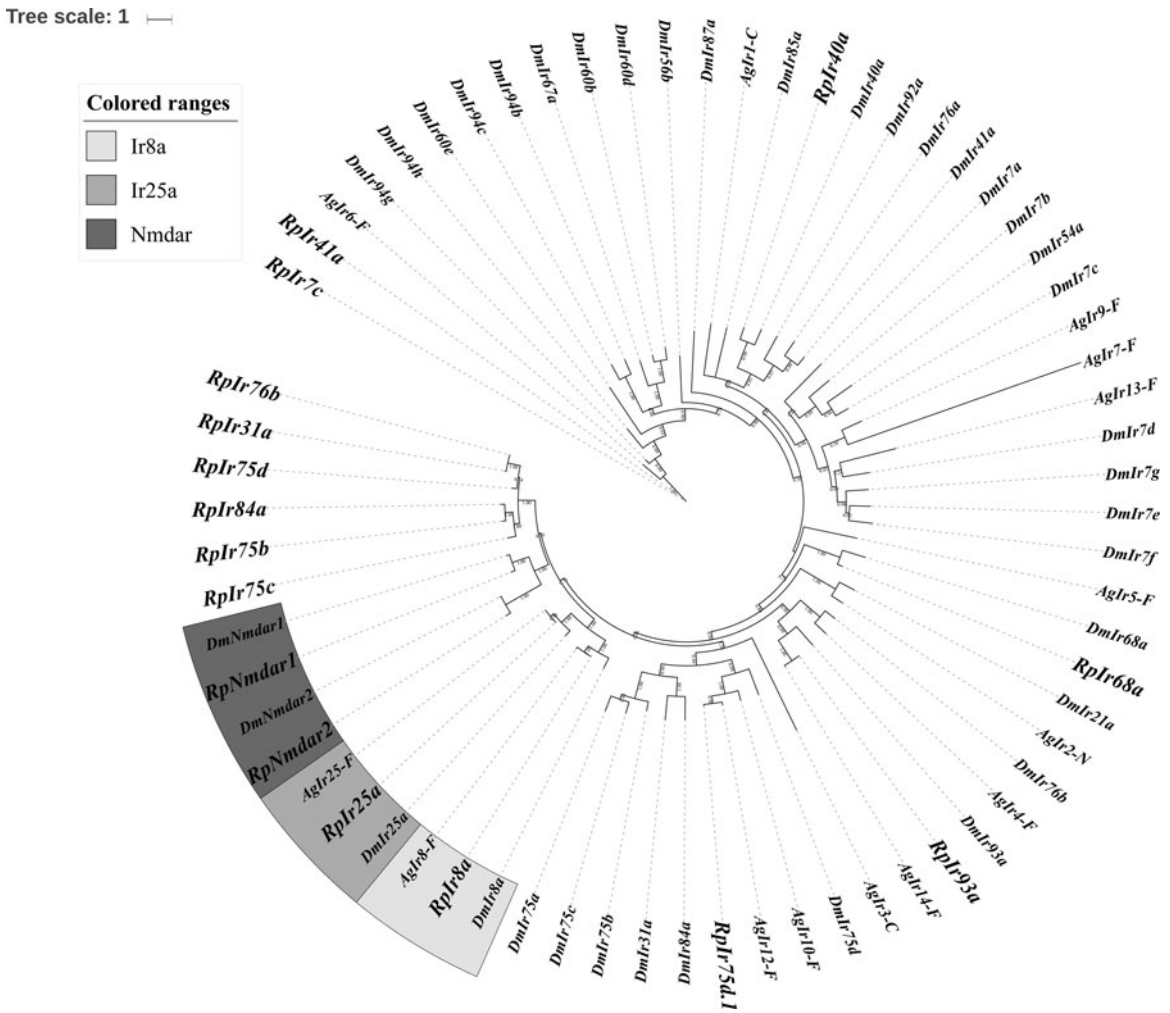

Fig. 4. Maximum likelihood phylogenetic tree of ionotropic receptors (IRs). IRs from *R. padi* (Rp), *A. pisum* (Ap), *D. melanogaster* (Dm) and *A. gossypii* (Ag) are included.

body compared with head with antennae. And, *RpOBP2*, *RpOBP5*, *RpOBP6*, *RpOBP7*, *RpOBP8* and *RpOBP10* were abundantly expressed in winged aphid compared with wingless aphid. Similarly, when reared on the crowd condition to induce wing type aphid, the expression of *OBP2*, *6*, *8* and *10* in *A. pisum* was significantly higher than that reared on the solitary condition (Vellichirammal *et al.*, 2016). Furthermore, when transferred to non-host plant, the expression of all the OBPs was significantly depressed except *RpOBP8*. As we all know, the plant quality, population density, alarm pheromones and interactions with predators, parasites will influence aphid to produce offspring with different wing morphs (winged or wingless) (Guo *et al.*, 2016; Vellichirammal *et al.*, 2016). All of these results suggested that *OBP* might be essential in the wing morph determination signal reception process of aphid.

ORco was thought to be responsible for the OR adopting the correct structure and worked as a selective ion channel during olfactory signal transduction. In *Dendroctonus armandi*, the silencing of *ORco* led to EAG declining to 11 major volatiles of its host (Zhang *et al.*, 2016*a*). RNAi and behavioral assay indicated that OR-based signaling pathway is mainly responsible for the attractive behavior of gregarious nymphs in the migratory locust (Wang *et al.*, 2015*b*). In *S. avenae*, (*E*)-beta-farnesene failed to induce production of winged aphids after the knockdown of *SaOrco* (Fan *et al.*, 2015). In our result, *RpOrco1* abundantly expressed in the head with antennae and reared on maize significantly up-regulated its expression. However, there was no significant difference between the expression of *RpOrco1* in the winged and wingless aphid. Besides the *ORco1*, there are 13 typical ORs in the *R. padi* transcriptome. And *ApOr5* was identified as an essential agent to EBF reception in *A. pisum* (Zhang *et al.*, 2016*b*). All of these results are consist with the previous work that ORs mediate the olfactory response to most food odors (Hallem *et al.*, 2006).

As the different plant has different nutritional and defensive substances, and all of these substances were the last process for host-plant acceptance as a food source (Dewhirst & Pickett, 2009; Nam & Hardie, 2014; Cao *et al.*, 2016; Liu *et al.*, 2016). These aphid feeding behaviors are significantly regulated by these compounds. For instance, the feeding behaviors can be affected by sucrose, glycosides and glucosinolates

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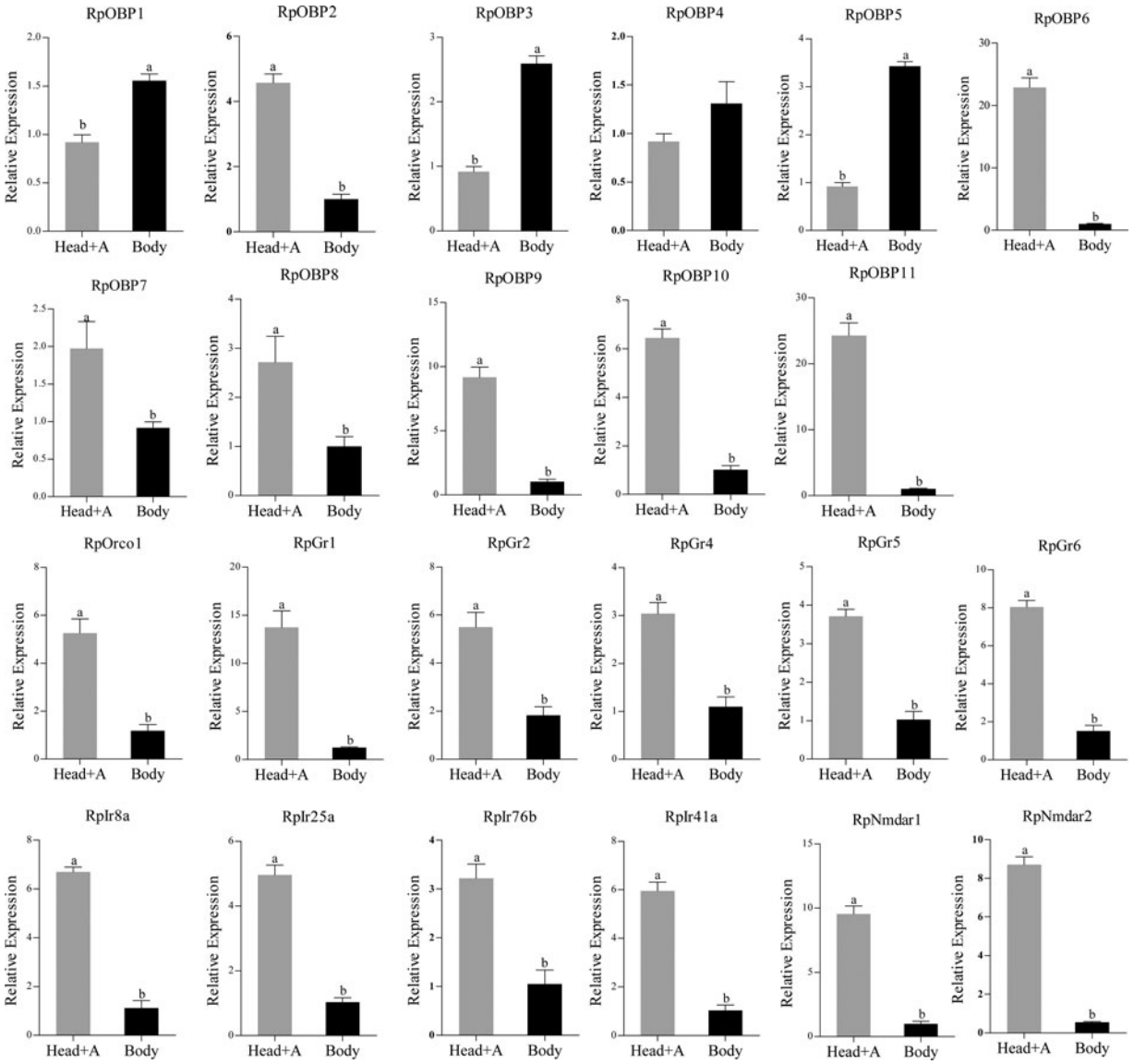

Fig. 5. The expression patterns of *R. padi* chemosensory genes in different tissues. Head+A: head with antennae; Body: only body without head and antennae. Data are presented as the mean of three replicates ($n = 3$) ± SE. The error bar represents standard error and the different small letters above each bar indicate significant differences in transcript abundances ($P < 0.05$).

(Takemura *et al*., 2006; Kim & Jander, 2007; Pescod *et al*., 2007). And Grs are considered as the detector of these semiochemicals to co-ordinating insect feeding behaviors based on the life condition (Xu *et al*., 2016). For example, *B. mori*, *BmorGr6* was thought as a chemical sensor to influence the feeding behavior of *B. mori* larvae based on the food ingestion (Mang *et al*., 2016). In *D. melanogaster*, two Grs, *Gr5a* and *Gr64a* underlay the sugar perception result from the no physiological and behavioral response to any tested sugar in Gr5a and Gr64a mutant. And *HaGr4* in *H. armigera* turned to D-fructose in antennal sensilla chaetica (Jiang *et al*., 2015). In this work, we found that the expression of *RpGr1*, *RpGr4*, *RpGr5* and *RpGr6* in *R. padi* reared on maize were lower than that of *R. padi* reared on the chili pepper. Contrary to this, *RpGr2* predominant expressed in *R. padi* reared on maize. Furthermore, all of the selected Grs in *R. padi* predominately expressed in the head with antennae, which also revealed that these selected Grs were involved in the aphid gustatory reception. All of these results suggested that Gr might be crucial for the detection of the plant nutrition quality or defense substances.

Biogenic amines are necessary components of living cells, protecting cellular macromolecules and biomembrane phospholipids against damage under stress conditions. The accumulation of amines concentration cause the programmed cell death in response to various abiotic and biotic stresses such as herbivorous feeding. In previous work, plant amines participate in plant defence responses to *R. padi* through disturbing its feeding behavior by higher concentration (Sempruch *et al*., 2016). And *R. padi* was able to reduce the accumulation of biogenic amines by depressed the activity of

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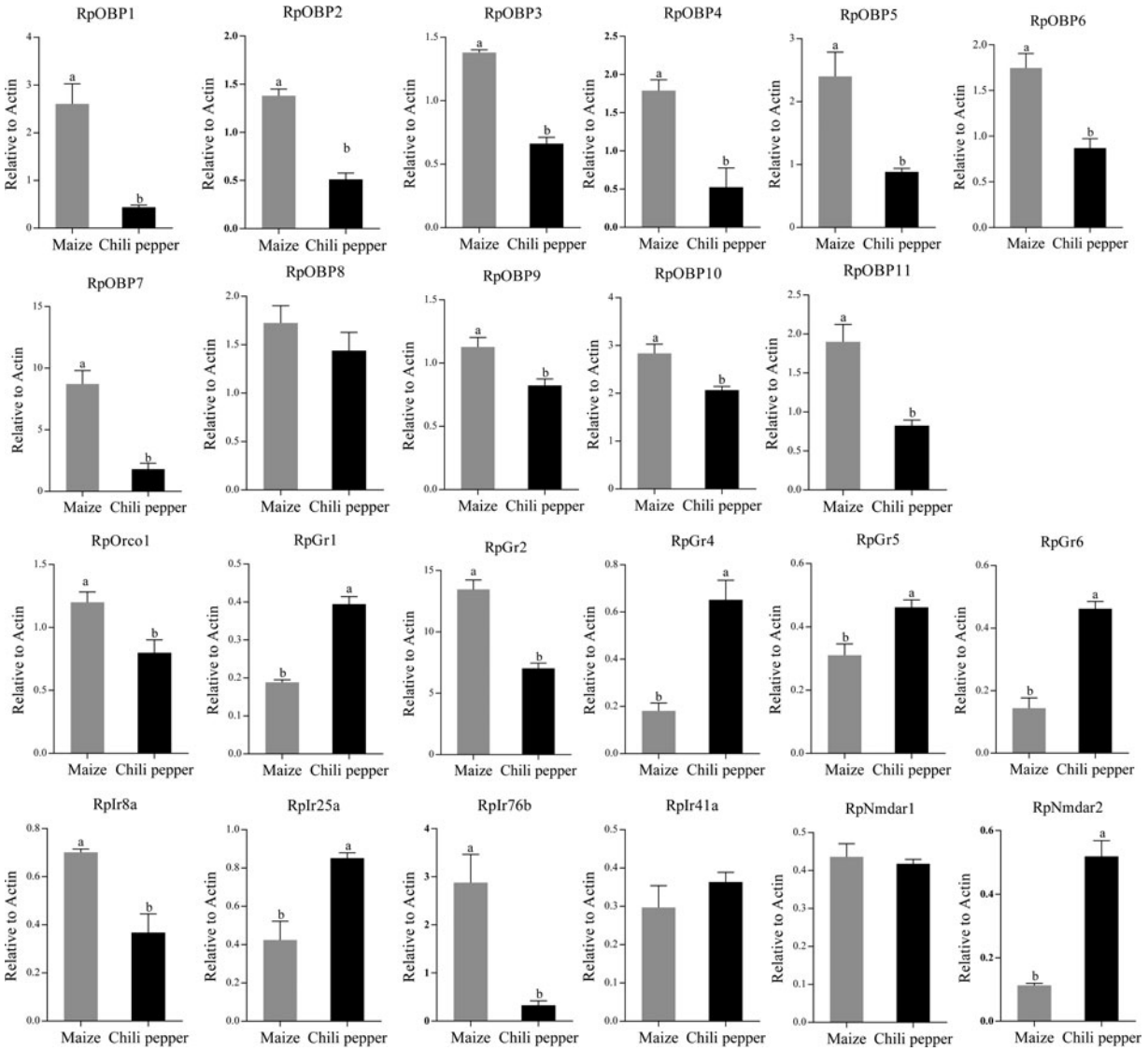

Fig. 6. The expression patterns of *R. padi* chemosensory genes in different host plants. Data are presented as the mean of three replicates ($n = 3$) ± SE. The error bar represents standard error and the different small letters above each bar indicate significant differences in transcript abundances ($P < 0.05$).

amino acid decarboxylation, which is a key step of biogenic amines biosynthesis (Sempruch *et al.*, 2013). In addition, a previous work posited a model of that pea aphid can regulate the biogenic amine levels depending on their reared condition revealed by olfactory perception (Vellichirammal *et al.*, 2016). In insect olfaction systems, IR was considered to mediate responsiveness of OSNs to organic acids, amines and alcohol (Hussain *et al.*, 2016). For example, *AgIr76b* mediated larval responses to butylamine (Liu *et al.*, 2010). In *D. melanogaster*, Ir20a mediates amino acid taste and blocks salt taste dependent on Ir76b (Ganguly *et al.*, 2017). Furthermore, *DmIr76b* and *DmGr66a* were used to assess the quality and valence of polyamine in the supplied diet (Hussain *et al.*, 2016). In this work, all of these selected IRs in *R. padi* were highly expressed in the head with antennae. Among them, the expression of *RpIr8a* and *RpIr76b* was lower in chili pepper reared aphid whereas the expression of *RpIr25a* and *RpNmdar2* were higher in chili pepper reared aphid. And in *Aphidius gifuensis*, IRs also exhibited a host-specific expression patterns (Kang *et al.*, 2017*a*). All of these results indicated that IRs system in *R. padi* may be the detector of plant defense and involved in the regulation of host suitability along with GRs.

As the key role of the olfactory system in insect survival, it has been thought to be a target for pest management. For example, olfaction of RNAi treated aphids was severely damaged and the activity of these aphids were significantly restrained. And the injection of *CqOr37/99*-dsRNA in *Culex quinquefasciatus* significantly reduced the egg-laying induction of 4-ethylphenol (Zhu *et al.*, 2013). Moreover, silenced *RpOrco* decreased blood-feeding volume, egg laying and molt rate in *Rhodnius prolixus* (Franco *et al.*, 2016). In *Apis cerana*, neonicotinoid insecticides disrupted olfactory cognitive behavior (Li

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et al., 2015*a*; Tan *et al.*, 2015). All of these results suggested that we could design the specific insecticides to disrupt the olfactory system in pest for controlling them. In addition, the key chemosensory receptor will be the target for designed or looking for the attractant, which might be used to kill pests combining with insecticides.

In this study, we not only identified the chemosensory genes in *R. padi* but also investigated the expression patterns of 23 selected genes among the different tissues, wing morphs and host plants. Combing with the demonstrated functions of related genes in other organisms, the tissue-, morph- and host-specific expression profile of these genes potentially revealed the candidate roles of these genes in the plant suitability assessment. Furthermore, the identification and expression patterns of chemosensory genes also provided initial background information for the further research on the molecular mechanism of the polyphagia and autumn migrants of it. Besides that, these chemosensory genes are also the candidate targets for pest management control in future.

Supplementary material

The supplementary material for this article can be found at https://doi.org/10.1017/S0007485317001171

Author contributions

These studies were conceived of and designed by Z.W Kang and T.X Liu; Whole experiments were performed by Z. W Kang, F.H Liu, R.P Pang, W.B Yu and Z.Q Zheng; Data analysis and paper writing were done by Z.W Kang, F.H Liu, X.L Tan, H.G Tian and T.X Liu.

Conflict of interest disclosure

The authors declare no conflict of interest.

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