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Identification and expression profile analysis of putative odorant-binding proteins in *Sitodiplosis mosellana* (Gehin) (Diptera: Cecidomyiidae)



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ABSTRACT

Odorant binding proteins (OBPs) contribute to the remarkable sensitivity of the insect's olfactory system and play important roles in the olfactory recognition. The orange blossom midge, *Sitodiplosis mosellana* is a cereal specialist, and utilizes pheromone and host odorant as a cue for its mating and oviposition. However, OBP genes have not been largely identified in *S. mosellana*. Based on the sequenced transcriptome database, twenty-six OBP genes were identified in *S. mosellana* for the first time. Phylogenetic analysis revealed that *S. mosellana* OBP genes are more closely related to *Mayetiola destructor* OBP genes than to *Aedes aegypti* OBP genes. Most OBP genes seemed to be antenna-specific, but differentially expressed in male and female antennae. Three OBP genes (OBP9, OBP19 and OBP23) are leg-specific. And also, most OBP genes have higher expression levels in adults. Only one OBP gene (OBP10) has higher expression levels in larval stages. These findings serve as an important basis for understanding the molecular mechanisms of chemosensory perception.

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1. Introduction

The orange wheat blossom midge (OWBM), Sitodiplosis mosellana (Gehin) (Diptera: Cecidomyiidae), is a periodic pest of wheat crops in the Northern Hemisphere and occasionally inflicts severe damage, particularly where a sequence of seasons favoring the midges triggers an outbreak [1]. S. mosellana is a cereal specialist, for which wheat is the most attractive crop for oviposition and in the absence of a wheat crop at a suitable growth stage, midges will fly to crops of rye, triticale, or barley, or may complete their life cycle in weed grasses [2]. The percentage of infested spike was above 50% in Roegneria ciliaris [3]. Female S. mosellana are attracted by volatile compounds from preanthesis wheat spikes [2]. Stimulatory volatiles may be the cues that females use to find potential hosts and initiate oviposition. But sometimes volatile compounds released by wheat spikes contribute to the reduced oviposition on some wheat genotypes and growth stages [4]. On the other hand, a monitoring system for male S. mosellana for use in pheromone traps in Canada and UK has been made [5]. But in China, it was not effective (data not shown). Therefore it is very important to study the recognition mechanism of S. mosellana.

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A better knowledge of the molecular mechanism of *S. mosellana* olfaction will contribute to the development of new tools for the control of this species since inhibition/artificial activation of the proteins implicated in the olfactory process would lead to the disruption of its chemical communication [6]. Secreted proteins including odorant-binding proteins (OBPs) play important roles in the olfactory recognition. Experimental evidence has demonstrated that OBPs could selectively bind odorants, pheromones or oviposition deterrents [7–15].

Despite the economical importance of *S. mosellana*, no data described the olfactory proteins. Here, we took advantage of next generation sequencing technologies (NGS) to enrich the study of the olfactory in this pest. We identified genes encoding OBPs. The tissue and the development stages specificity of the transcripts of these genes were analysed. These finding would serve as an important basis for identification of the *S. mosellana* OBPs that is required perception of the host compound or pheromone.

2. Materials and methods

2.1. Insects

The cocoons of *S. mosellana* were originally collected in Wuzhi, Henan province, China, during March 2011. The field studies did

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not involve endangered or protected species. No specific permits were required for the described field collections, and the location is not protected in any way. *S. mosellana* is common agricultural pest and is not included in the "List of Protected Animals in China". The third instar larvae, pupae and male and female adults were collected, respectively. Meanwhile, the emerged adults were collected within 24 h and temporarily stored at -80 °C. Each part of *S. mosellana* (male and female and female legs) was dissected on ice and collected in an Eppendorf tube. Finally, these samples were frozen at -80 °C until use.

2.2. Identification of odorant binding protein genes from S. mosellana transcriptome

The available odorant binding protein gene sequences from other insect species including (*Anopheles gambiae*, *Aedes aegypti* and *Culex quinquefasciatus*) were used as references to screen the *S. mosellana* transcriptomic database [16]. OBP genes were identified by searching the sequences in the transcriptome database for keywords (OBP, odorant binding protein) or by using the basic local alignment search tool (BLAST) algorithm to search for other

SmosOBP5	1 MKYFVAVLLLAAVVFADAEWKIQTNENLNAYRPECANSLSIPEDKVNEYKK 1 MQLIATAIFLAFVGFCWSVETNADIFSEHLKWQETLGTARREQTLHVSEDAVREHGL 1 MKSLILVACVVIGFCEP-WSKE
SmosOBP1	1MQLIATAIFLAFVGFCWSVETNADIFSEHLKWQETLGTARRECQTTLHVSEDAVREHGL
SmosOBP2	1MKSLIILLVACVVIGFCEP-MSKEBRIANFMK I ASE <mark>C</mark> AVTEGATDDEMAEIFQ
SmosOBP13	1LlavvCatigfSeQaitkeQaiemykklaeeCaakegasaadiQeaya
SmosOBP8	1EKMAAMKIVMDQAAKENASQADLEELFA
SmosOBP14 SmosOBP10	1MKAFYSIVIFS-VLFAVCMAKLTPEQRKELEAKLLSECKGPSGATDDDVGOLKE
SmosOBP15	
SmosOBP12	
SmosOBP11	1HDV@VGRTGVSEDVIKRFSD
SmosOBP21a	1KKIVLMVLGVVGLSLAALEIPEHLKAAVKIMRKACLVESGVDEKYVNQSRD
SmosOBP21b	1 MKVLLIVIGVVGLSLAALDIPDH
SmosOBP21c	1 MKIVLMVLGVVGLSLAALEIPEH
SmosOBP21d SmosOBP20	1LAAPLKVMRKAQLAESGVDENIVNQSK
SmosOBP22	1
SmosOBP9	1MKFFLCILVLSFATMQELVVAKRGPFDPNMCCKVENSEPDSATHEAMMKLLDECKKELGI
SmosOBP23	1CKIG-AEGQAKYDEKVQQLMQQC <mark>I</mark> QELDL
SmosOBP3	1MKFLLCALLIALASAHEPPGLFEYTKCCKIQISAATTQF <u>I</u> DSMTKLEDECKQELGE
SmosOBP7	1WSKTISCLVFLSICGACLAGMTVEQVKSAETIRNVCQPKSKLSDVVNKMNE
SmosOBP17 SmosOBP19	1VELTQMMTSFRVQEQAQTGASDDLLDGINV
SmosOBP18	1
SmosOBP4	
SmosOBP6	1RQVADKVAALCFAVICSVAAVTDEQRQVADKVAKDASEASENGLSADEVQKIRT
SmosOBP16	1 MSSTKLFIAMAVILFLGCMQCCSAAGAEKQVAAAASGEIPASAPAKAVDDNTDQLDQEAIMQM <mark>G</mark> NESFRTSMEYLDELNS
SmosOBP5	52 W_NEENDEVTONT VATE AVAILANDED AVAILAND TO THE TOTAL AVAILAND TO THE TOTAL AVAILAND
SmosOBP1	52 W-NFPNDEKTQCYIKCIFGKMGLFNEKDGFNVEHL-VKQLG-QGKNETIIRPEVVKCADKN 60 G-NRTNSEATQCFAKCIFTKLGMFNETNGFNED-SSIDRFRVAMVA-KEDNIPSIREIVVKCASKK
SmosOBP2	53 R-KTPSTRAGKEVHAELGERIGVMKMNOVDVESTVAVADMAFDGDSRKVOMARELANDEAG
SmosOBP13	49 K-KLPSTTPAKEMHACISEKIGFMKNNKIDVEGNIALAKKVFDNDAAKIQTATDIANECTG
SmosOBP8	54 K-KPATSPTGKCHRACMHETFGTMKDNKFNPDGFMAMIKMSTDGDEAKMKIAQEVVKDCAD
SmosOBP14	54 H-EVPKTTTCKGLLSGSQEKLGILVDGKLSIDGLKALGAKKHEGNDKALATFNEIVAECEN
SmosOBP10 SmosOBP15	52 H-KLPNGPNGKGFLAGINEKTGVILDGKINGDMLKANILKENGGNEKAAGSVNEMVAECQG
SmosOBP12	54 H-EVPRTTTGKCLLSCSQEKLGILVDGKLSIDGLKALGAKHEGNDKATATFNE VAECEN
SmosOBP11	21 GDEIFEDDKLKGYMDGLLQEKGFIMPDGKIDFVSLHESFNE-DKEIHFTFIHMIRRGLY
SmosOBP21a	52 G-NLPDVPKLG GYILG FFEHAGMIEEDGTIHFNDVLHLLSPSLAETAKYVSEE G KT
SmosOBP21b	52 G-NLPDVPKLG <mark>CYILC</mark> FFEHAGMIEEDGTIHFNDVLHLLSPSLAETAKYVSEECKT
SmosOBP21c	52 G-NLPDVPKLGCYILCLEHCKMIEDDGTIHFNDVMHLLLPSTAETVRYVTKEST
SmosOBP21d SmosOBP20	52 G-NLPDVPKLGCYILCLEHCKMIEDDGTIHFNDVMHLLLPSTAETVRYVTKEST
SmosOBP22	22 G-NLPDI - PELREVVLELMENSGI IDDAGVVDFSKIVHIFTSMKETTFOATNDGGT
SmosOBP9	52 G-HLSDA - PGMCG VINCLMEHSGMVEEDGRIH FDDIMHLLIP ETRETVIKVVNECQT 22 G-NLPDI - PEIRG VVLCLMEHSGI DDAGVVD FSKIYLLFTP SMKETFQDATNDCGT 61 DGEGKKKHEPFHGI IECVSKKLNVINADGTINEA EFTNIVK-KMATASYQQAVAEDVAKCIAEAKKATATDNGPKDSK 29 ANQTEDKQREGFVC VVECVGKKVNVIDANKNLIEE GLRNFVINEMALEPHQKVVAEQTIKKCTEELKTPS PETNG
SmosOBP23	29 ANQTEDKQREGFV <mark>G</mark> VVE <mark>G</mark> VGRKVNVIDANKNLIEEGLRNFVINEMALEPHQKVVAEQTIKK <mark>G</mark> TEELKTPSPETNG
SmosOBP3	57 P-EQGKPPKFP@FHE@VGNKTNMINADESLNEDKYRSFITTOMATEDYOKAVADAIATK@IEKVNSADGSEESPDG
SmosOBP7 SmosOBP17	54 G-VFPEDKAAKCFVHCILENLQLMKKNKVNYDSAMKSFDTMLPDDMKDDYKNSLTTCKD 57 G-QFPRDQNLM <mark>C</mark> YINCLLTMMRMIRKGKFNSELAVKNINMFLPEFMREEWLRGVAACKDHG
SmosOBP19	57 G-KMPETKTAKTASGVMKOFKLAKKNGANLEADCDVMAOLAEAKGANSTTIAAIKDICGKCKA
SmosOBP18	60 D-NFIEDQDIKCYVHCAMEMLHIMEGVVGQFHVAEKHTDYIIPNELFESTIKAFGHCEN
SmosOBP4	49 NVKVPEKQEQNRN <mark>G</mark> YYA <mark>G</mark> MMKKMNLMKTDGALNEEALRSKFSANQETLNKALDA <mark>C</mark> KA
SmosOBP6	57 G-WHET-WARDING TASCUMKOFKLAKKNGANLEADCDUMAQLAEAKGANSTITAAIKDI GKKKA
SmosOBP16	81 TGAFPDETDKTPMCYIRCYLDAVGIIKDDELNREKANEMAWATSEDTLEECEK
SmosOBP5	110PQKTNAGQWAYRGFDGFKKAHLDLVQTSVKKN
SmosOBP1	123IKTENAGGWANRGFEGFRKEGLSLG
SmosOBP2	110PQKTNACQWAYRGFDCFKKAHLDLVQTSVKKN
SmosOBP13 SmosOBP8	
SmosOBP14	
SmosOBP10	
SmosOBP15	112 INN 82 PKGETLEDRAWWYHQEWKSADPKHYFLI- 73 VKGE 79 PGGEG-CDRAYNMNVEFKKADPKHYFIV- 107 IHGDTRCDTAWLTTKCFFEKAPEGSELP
SmosOBP12	73VKGE
SmosOBP11	79PGGEG- OPAINMNVFFKKADPKHYFIV
SmosOBP21a SmosOBP21b	107IHGDTRCDTRWLTTRCFFERAFEGSELP 107IHGDTRCDTAWLTTRCFFERAFEGSELP
SmosOBP21D SmosOBP21c	107 IYGATRODTAWITFKOYYEKAPEGSELP
SmosOBP21d	107IYGATRCDTAWLTFKCYYEKAPEGSELP
SmosOBP20	107KHGNTREETAWMTVQEYYQVAPEDAELP
SmosOBP22	77IHGDTRCDTAYLTFKCFVTKYPKDAELP
SmosOBP9 SmosOBP23	138 CNFMPMKLMHGVGRELINSGPADKODTSDKCVKMREHINKPRNGPPPPPGKGPEGSSENNE
SmosOBP23 SmosOBP3	104 CSHIVLRASSCVEAELFKSCPAEKQDTSDECVKLRQEINSGKDPOTVSK 132 CSETAMEAFHCASKELINACPADKODNDEHCVHFREFINRDFKGKGPOPSPOTVSK
SmosOBP3 SmosOBP7	112 AAAGVKNAGDAAHKVVTGVYKNNPKFMFVNPLETYVNHLLHOSFEGYGYLLA
SmosOBP17	117EDIVDQCERIYSKIECFSRNNEH
SmosOBP19	117EDIVDQCERTYSKIE FSRNNEH 120PGTPDDCEFSAMIATCIKKECEARGITVDSIQ 118LTNGMTDTCEAGYAMLKEFREGNKDFWLP
SmosOBP18	118 LINGMIDIGEAGYAMLKEFREGNKDFWLP
SmosOBP4 SmosOBP6	106HGQNDNCKLAACLMANREI
SmosOBP6 SmosOBP16	109VIGDNRDERFIRLYAGYLERKALA

Fig. 1. Alignment of deduced amino acid sequences of *S. mosellana* OBP family members. The alignment was performed using Clustal X 1.83 and their homologous areas were marked by the Boxshade 3.21 program. GenBank accession numbers are available in Table S2.

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