Biochemical and Biophysical Research Communications 444 (2014) 164–170

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

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

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

Zhong-Jun Gong, Jin Miao, Yun Duan, Yue-Li Jiang, Tong Li, Yu-Qing Wu *

Institute of Plant Protection, Henan Academy of Agricultural Sciences, Key Laboratory of Crop Pest Control of Henan Province, Key Laboratory of Crop Integrated Pest Management of the Southern of North China, Ministry of Agriculture of the People's Republic of China, Zhengzhou, PR China

ARTICLE INFO

Article history:
Received 9 December 2013
Available online 16 January 2014

Keywords:
Sitodiplosis mosellana
Odorant binding protein
Real-time PCR
Transcriptome
Expression profile

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.

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*.

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

* Corresponding author. Address: Institute of Plant Protection, Henan Academy of Agricultural Sciences, Zhengzhou 450002, PR China.
E-mail address: yuqingwu36@hotmail.com (Y.-Q. Wu).

0006-291X/$ - see front matter © 2014 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.bbrc.2014.01.036

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 antennae, male 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

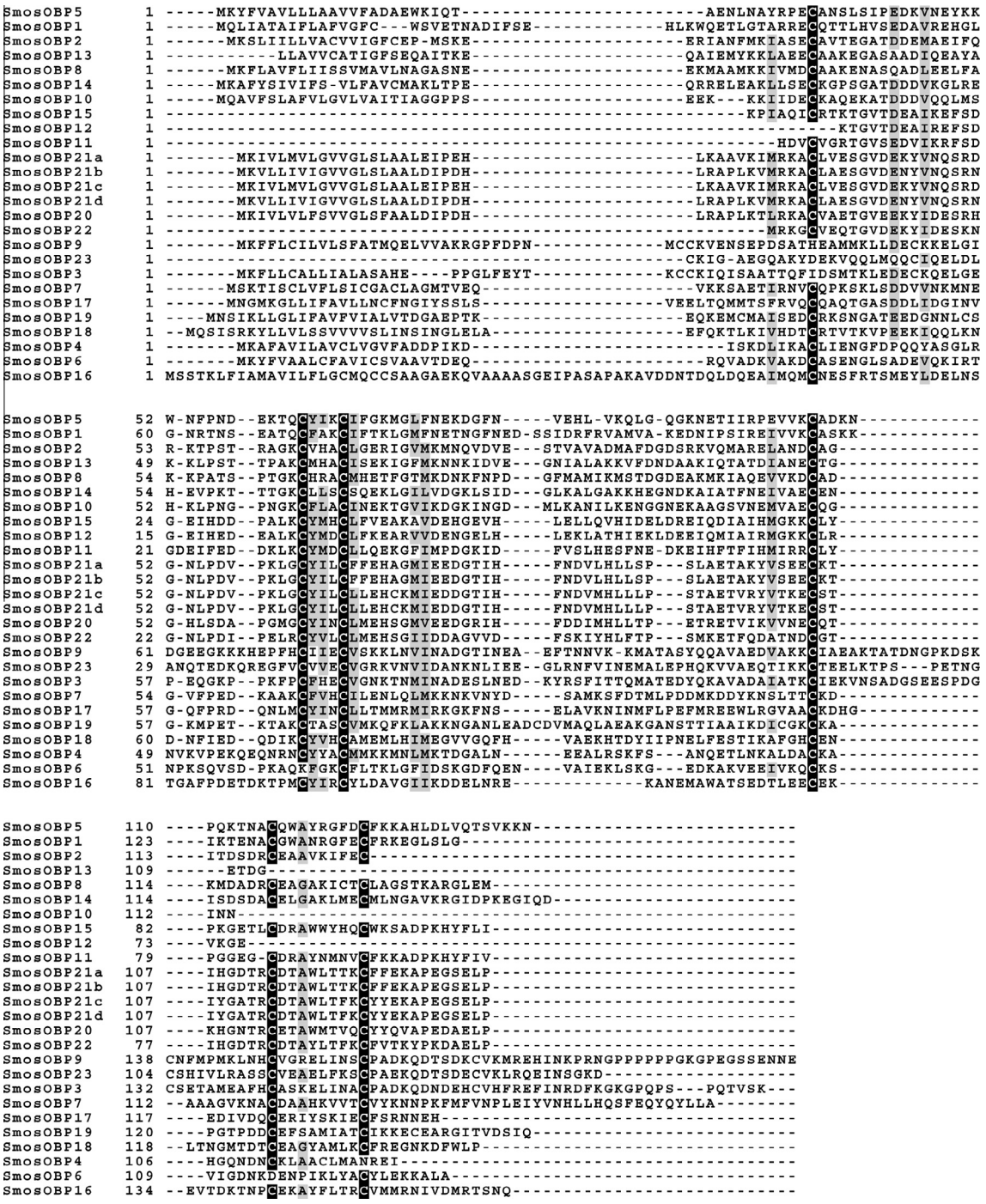

Fig. 1. Alignment of deduced amino acid sequences of *S. mosellana* OBP family members. The alignment was performed using Clustal X 1.83 and their homologous areas were marked by the Boxshade 3.21 program. GenBank accession numbers are available in Table S2.