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Identification of Odorant Receptors through the Antennal Transcriptome of the Grapevine Pest, Lobesia botrana (Lepidoptera: Tortricidae)

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Abstract : In agriculture, grape production has great economic importance at global level, considering that in 2013 it reached 7.4 million hectares (ha) covered by plantations of this fruit worldwide. Chile is the number one exporter in the world with 800,000 tons. However, these values have been threatened by the attack of the grapevine moth, Lobesia botrana (Denis & Schiffermuller) (Lepidoptera: Tortricidae), since its detection in 2008. Nowadays, the use of semiochemicals, in particular the major component of the sex pheromone, (E,Z)-7.9-dodecadienil acetate, are part of mating disruption methods to control L. botrana. How insect pests can recognize these molecules, is being part of huge efforts to deorphanize their olfactory mechanism at molecular level. Thus, an interesting group of proteins has been identified in the antennae of insects, where odorant-binding proteins (OBPs) are known by transporting molecules to odorant receptors (ORs) and a co-receptor (ORCO) causing a behavioral change in the insect. Other proteins such as chemosensory proteins (CSPs), ionotropic receptors (IRs), odorant degrading enzymes (ODEs) and sensory neuron membrane proteins (SNMPs) seem to be involved, but few studies have been performed so far. The above has led to an increasing interest in insect communication at a molecular level, which has contributed to both a better understanding of the olfaction process and the design of new pest management strategies. To date, it has been reported that the ORs can detect one or a small group of odorants in a specific way. Therefore, the objective of this study is the identification of genes that encode these ORs using the antennal transcriptome of L. botrana. Total RNA was extracted for females and males of L. botrana, and the antennal transcriptome sequenced by Next Generation Sequencing service using an Illumina HiSeq2500 platform with 50 million reads per sample. Unigenes were assembled using Trinity v2.4.0 package and transcript abundance was obtained using edgeR. Genes were identified using BLASTN and BLASTX locally installed in a Unix system and based on our own Tortricidae database. Those Unigenes related to ORs were characterized using ORFfinder and protein Blastp server. Finally, a phylogenetic analysis was performed with the candidate amino acid sequences for LbotORs including amino acid sequences of other moths ORs, such as Bombyx mori, Cydia pomonella, among others. Our findings suggest 61 genes encoding ORs and one gene encoding an ORCO in both sexes, where the greatest difference was found in the OR6 because of the transcript abundance according to the value of FPKM in females and males was 1.48 versus 324.00. In addition, according to phylogenetic analysis OR6 is closely related to OR1 in Cydia pomonella and OR6, OR7 in Epiphyas postvittana, which have been described as pheromonal receptors (PRs). These results represent the first evidence of ORs present in the antennae of L. botrana and a suitable starting point for further functional studies with selected ORs, such as OR6, which is potentially related to pheromonal recognition.

Keywords : antennal transcriptome, lobesia botrana, odorant receptors (ORs), phylogenetic analysis
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