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Genome-Wide Identification of the Genes of the Odorant-Binding Protein Family Reveal Their Role in the Olfactory Response of the Tomato Leaf Miner (*Tuta absoluta*) to a Repellent Plant

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Abstract: The remarkable biological and evolutionary adaptations of insects to plants are largely attributed to the powerful chemosensory systems of insects. The tomato leaf miner (*Tuta absoluta*) is a destructive invasive pest with a global distribution that poses a serious threat to the production of nightshade crops, especially tomatoes. Functional plants can attract or repel insect pests by releasing volatiles that interact with the olfactory system of insects, thereby reducing the damage of insect pests to target crops. However, there is limited research on the interaction between *T. absoluta* olfactory genes and functional plants. In this study, 97 members of the putative odorant-binding protein (OBP) family have been identified in the whole genome of *T. absoluta*. Phylogenetic analysis involving various Lepidopteran and Dipteran species, including *D. melanogaster*, revealed that *OBP* gene families present conserved clustering patterns. Furthermore, the Plus-C subfamily of OBP showed extremely significant expansion. Moreover, the expression levels of the *OBP* genes varied significantly between different developmental stages; that is, the highest number of *OBP* genes were expressed in the adult stage, followed by the larval stage, and fewer genes were expressed in high abundance in the egg stage. On the other hand, through a Y-tube olfactometer, we identified a functional plant—*Plectranthus tomentosa*—that significantly repels adult and larval *T. absoluta*. Finally, we screened the *OBP* genes in response to tomato and *P. tomentosa* volatiles at the genomic level of *T. absoluta* using RT-qPCR. These results laid a good foundation for controlling *T. absoluta* with functional plants and further studying olfactory genes.

Keywords: odorant-binding protein; *Tuta absoluta*; *Plectranthus tomentosa*; behavioral responses; transcriptional responses

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1. Introduction

Agricultural pests are surrounded by various volatile substances in the farmland ecosystem, including plant volatiles, insect volatiles, etc. Insects can use the complex olfactory system to identify and sense odor molecules so that insects can locate oviposition sites, mates, and threats, thus helping them to survive and reproduce [1,2]. The olfactory proteins of insects mainly include odorant-binding proteins (OBPs), olfactory receptors, gustatory receptors, and inotropic receptors. OBPs are expressed in the olfactory sensillium, which transport odor molecules to olfactory receptors on the surfaces of neurons and are the first biochemical reaction for insects to specifically recognize external odors, which is of great significance for insects to communicate with the outside world [3–5]. Further studying the function of OBPs is crucial for revealing the interactions between insects and host plants.

Insects can use the olfactory system to recognize external volatiles and then make a taxis choice or escape behavior [6]. For example, tobacco cutworm (*Spodoptera litura*) and

black cutworm (*Agrotis ipsilon*) can use OBPs to respond to a variety of sex pheromones and plant volatiles [7,8]. Plant volatiles are volatile organic chemicals (VOCs) produced by the leaves, flowers, and fruits of plants [9]. These volatile secondary substances include terpenes, alcohols, aldehydes, hydrocarbons, ketones, organic acids, and other organic compounds with a relative molecular weight of 100~300 kDa; they do not participate directly in the growth and development of plants but are the result of the interaction between plants, organisms and abiotics [10]. The insect preference for different host plants is very different, and this preference for selection is largely related to plant volatiles [11]. Functional plants refer to a class of plants that can play ecological functions in agriculture and forest ecosystems, including nectar source plants, attracting plants, associated plants, insect-repellent plants, barrier plants, deposit plants, etc. [12]. In modern biological control, functional plants are considered an important means of the ecological control of pests [13]. For example, the intercropping of high clover (*Trifolium incarnatum*) with cabbage (*Brassica oleracea*) significantly reduced the number of eggs deposited by diamondback moths (*Plutella xylostella*) in cabbage [14]; vetiver grass (*Vetiveria zizanioides*) released a large number of terpenoids, which significantly induced the transfer of female adult rice striped stem borers (*Chilo suppressalis*) from rice to vetiver to lay eggs [15]; and the insect-repellent plants basil (*Ocimum basilicum*) and citronella (*Mosla chinensis*) are effective repellents of cotton leafworm (*Spodoptera litura*), cabbage webworm (*Hellula undalis*), and flea beetle (*Phyllotreta sinuata*) from *B. oleracea* [16]. Although previous research has focused primarily on identifying repellent or attractant plants, studies exploring how insects' olfactory systems respond to these plants or their volatile components remain limited.

The tomato leaf miner *Tuta absoluta* (Meyrick) is an invasive alien pest that causes great harm worldwide and is known as the 'Ebola virus' on tomatoes (*Solanum lycopersicum* L.) [17]. *T. absoluta* has a wide range of host plants, mainly destroying nightshade crops such as tomatoes, tobacco, potatoes, etc., but also laying eggs and developing in a variety of plants in Amaranthaceae, Convolvulaceae, Legumes and Malvaceae, causing damage to host plants [18]. *T. absoluta* originated in Peru, South America, and was introduced into Europe in 2006. Currently, *T. absoluta* has invaded 103 countries and regions of the world, seriously threatening the healthy development of the world food industry [19,20]. At present, the most important control method of *T. absoluta* is still chemical control, which can effectively control the pest when the pest has just invaded. However, a large amount of irrational pesticide use has also led to increased resistance and brought serious harm to the agricultural ecosystem, including intensifying environmental pollution, causing excessive drug residues in agricultural products, destroying the balance of the ecosystem, reducing the number of natural enemies, etc. [21]. These adverse effects have led people to seek better methods of crop protection with environmental compatibility [22]. Currently, the use of functional plants to control *T. absoluta* is mainly through the toxic or repellent effects of essential oils produced by functional plants [23]. Boni et al. used plant essential oil prepared with *O. basilicum* to explore its inhibitory effect on the laying of *T. absoluta* eggs [24]. Mirella et al. studied the effects of essential oils of majoram (*Origanum vulgare*), bay (*Laurel nobilis*), *O. basilicum*, garlic (*Allium sativum*), and mint (*Mentha piperita*), etc., on the egg laying and larval repellent of *T. absoluta* [25]. However, direct application of functional plants to control *T. absoluta* has not been reported. Furthermore, the limited research on the olfactory system of *T. absoluta*, including OBPs, restricts the potential use of olfactory-based pest-control methods.

In this study, we first identified putative OBP family genes in the whole genome of *T. absoluta* by homologous comparison and domain-based HMMER comparison. We then identified a functional plant that significantly repels *T. absoluta*. Finally, real-time quantitative PCR (RT-qPCR) was used to identify genome-level OBPs in response to host tomatoes and repellent plants.

2. Materials and Methods

2.1. Insect Strains

The *T. absoluta* larvae were collected from Yuxi City, Yunnan Province, China (102°17′32″ E, 24°08′30″ N) in 2021 and raised in the laboratory for more than 15 generations. The larvae were fed tomato plants in fine nylon mesh cages (45 × 45 × 45) cm in an artificial climate box (MG-300A, Shanghai Yiheng Scientific Instrument Co., Ltd., Shanghai, China) under certain conditions of temperature (27 ± 0.5 °C), relative humidity RH (70 ± 5%), and photoperiod (L:D = 16:8 h). Adults were given a 10% sucrose solution (CAS: 57-50-1, 98%, Shanghai Yuanye Biotechnology Co., Ltd., Shanghai, China) as an additional supplement.

2.2. Search and Identification of Odorant-Binding Protein Family Gene in T. absoluta

The whole-genome sequence genome assembly ZJU_Tuta_1.1 (GenBank assembly number: GCA_029230345.1), GFF annotation file, total CDS sequence, and total protein sequence file of *T. absoluta* were downloaded from the NCBI website (https://www.ncbi.nlm.nih.gov/datasets/genome/GCA_029230345.1/, accessed on 13 June 2023). Putative odorant-binding protein (OBP) family genes in *T. absoluta* were identified using two approaches: homologous comparison by BLAST with TBtools [26] and domain-based HMMER comparison with BITACORA [27]. The final identification results were combined with the results of these two software, and the incomplete annotation sequences were filtered.

2.3. Phylogenetic Analysis of OBP Genes in T. absoluta

The predicted full-length protein sequences of the OBP family genes of fruit fly (*Drosophila melanogaster*, 51 members) were downloaded from Flybase (http://flybase.org/, accessed on 20 July 2023), and the predicted full-length protein sequences of the OBP family genes of silkworm (*Bombyx mori*, 20 members), fall armyworm (*Spodoptera frugiperda*, 15 members), cotton bollworm (*Helicoverpa armigera*, 20 members), and cabbage caterpillar (*Pieris rapae*, 19 members) were downloaded from the NCBI website. Sequence IDs for each OBP used for phylogenetic analysis are listed in Supplemental Table S1. After aligning with MAFFT software (v7.520) [28], these sequences were used for phylogenetic analysis using IQ-TREE software (v2.0) [29]. The bootstrap operation was performed 1000 times. The optimal amino acid replacement model was LG+G. Genes from the same insect are represented by the same colors. The branch colors represent the Classic (yellow), Plus-C (blue), Minus-C (green), and Dimer (purple) subfamilies, according to Rondon et al. [30].

2.4. Developmental Stage-Specific Expression Profile of TaOBPs

T. absoluta is a holometabolous insect that has egg, larva (divided into four instars), pupa, and adult stages throughout its 30–35-day life cycle [31]. The developmental stage-related BioProject (PRJNA291932) of *T. absoluta* (including six developmental stages: Egg (SRR2147323), Larvae stage 1 (SRR2147324), Larvae stage 2 (SRR2147319), Larvae stage 3 (SRR2147320), Larvae stage 4 (SRR2147321) and Adult (SRR2147322)) was retrieved and downloaded from the NCBI SRA database (https://www.ncbi.nlm.nih.gov/sra, accessed on 1 August 2023). Expression levels of all genes of the OBP family in *T. absoluta* were calculated using kallisto [32], and expression levels were displayed by heat map based on the TPM value of the gene. Image GP [33] was used to analyze the expression of *OBP* genes identified in different development stages of *T. absoluta*.

2.5. Determination of the Taxis Behavior of T. absoluta to Plectranthus tomentosa

The behavior response of *T. absoluta* to *Plectranthus tomentosa* was measured using a Y-tube olfactometer according to a previous study [34,35], with some modifications. A schematic of the Y-tube was provided in our previous study [35]. For experiments with host tomatoes as controls, the odor source of the control arm was tomato, and the odor source of the treatment arm was the *P. tomentosa* plant. For experiments with pure air as control, the odor source of the control arm was pure air, and the odor source of the treatment arm was the *P. tomentosa* plant. Fresh tomato plants and *P. tomentosa* plants were placed in the glass

jar of the odor source (the pot was wrapped in tin foil). The flow rate of the two arms of the Y-tube was adjusted to 20 mL/min, and the air was pumped for 10 min before each measurement so that the arms of the flavor source of the Y-tube were filled with volatile information substances. For testing, thirty larvae of the first day of the third instar or fifteen eclosion females and fifteen males within three days of *T. absoluta* were released from the finger tube to the base of the Y-tube, and the Y-tube was placed in a black blackout box to eliminate the effect of light on the behavioral response of *T. absoluta*. After 15 min, the behavioral response of *T. absoluta* to the odor source was observed and recorded. After each measurement, the clean Y-tube was replaced and then the next test was performed. After every three tests, the orientation of the two arms of the Y-tube was changed to eliminate the error caused by the position. Bioassays were performed in a blackout chamber illuminated by an incandescent lamp (18 W, 4500 lux light intensity) above the Y-tube. The response rate is equal to the number of *T. absoluta* selected on one side of the plant divided by the total number of *T. absoluta* used in one experiment.

2.6. Induction Treatment of T. absoluta Larvae and Adults by Plant Volatiles

The induction treatment of *T. absoluta* by plant volatiles was tested using a directed airflow apparatus based on previous methods [35] with some modifications. Fresh tomato or repellent plants were placed in the glass jar of the odor source (the pot was wrapped in tin foil), and air purified by activated carbon and moistened by distilled water was continuously injected by an air compressor (QC-1S; Beijing Municipal Institute of Labour Protection, Beijing, China), with the gas flow adjusted to 20 mL/min. The breathable box containing the third instar larvae or adult of *T. absoluta* was placed in the glass jar of the odor source and treated for 6 h. For larvae, five homogeneous larvae of the first day of the third instar were used for each treatment. For adults, ten females and ten males within three days of emergence were used for each treatment. Each experiment was carried out with five biological replicates. In the blank control group, no plants were placed in the glass jar of the odor source; that is, the insects were treated with pure air.

2.7. Total RNA Extraction and Reverse Transcription

After exposure to volatiles for 6 h, *T. absoluta* larvae and adults were collected for subsequent experiments. Total RNA from every five larvae was extracted with the Eastep™ Universal RNA Extraction Kit (catalog number: LS1030, Promega Corporation, Madison, WI, USA). For adults, the antennae and part of the head tissue connecting to the antennae were cut off with a thin blade under an anatomical mirror and quickly transferred to liquid nitrogen, and the tissues of every 10 females and 10 males were mixed into one sample for total RNA extraction. After measuring the concentration and confirming the integrity of the sample, 1 μg of total RNA was reverse transcribed using the GoScript Reverse Transcription System (catalog number: A5001, Promega Corporation, Madison, WI, USA).

2.8. Real-Time Quantitative PCR (RT-qPCR) Analyses of TaOBPs

The levels of mRNA expression of OBP family genes in *T. absoluta* were measured using the SYBR Green dye method. Real-time quantitative PCR (RT-qPCR) was performed with the qPCR Master Mix (catalog number: BM60304S, Baimeng Medical Co., Ltd., Fuzhou, Fujian, China) in the LightCycler480 system (Roche Diagnostics, Indianapolis, IN, USA). The 10 μL reaction system consisted of 5 μL 2 × qPCR Master Mix, 0.2 μL (10 μM) for each primer, and a 4.6 μL cDNA template. The amplification conditions were 95 °C for 30 s, 40 cycles of 95 °C for 5 s, 60 °C for 15 s, and 72 °C for 20 s. Each reaction was performed in triplicate with two technical replicates. Relative gene expression levels were calculated with the $2^{-\Delta\Delta CT}$ method [36] by normalizing with the reference of *T. absoluta* EF-1α (MZ054826) and GADPH (MZ054823). The primers were synthesized by Sangon Biotech Co., Ltd. (Shanghai, China), and all primer sequences are listed in Supplemental Table S2.

2.9. Data Analysis

Statistical analysis of the data was performed using IBM SPSS Statistics 27 (SPSS Inc., Chicago, IL, USA). In the Y-tube olfactory-selection experiment, the χ^2 test was used to determine whether the theoretical distribution of H_0 was assumed to be 50:50 between the two odor sources in the *T. absoluta* behavior-selection experiment. The χ^2 and p values were calculated (*, $p < 0.05$; **, $p < 0.01$), and those who did not make a choice were not included in the selection rate. Different letters indicate significant differences ($p < 0.05$) following the use of Tukey's honest significant difference multiple-range test. All data are presented as mean ± standard error (SE).

3. Results

3.1. Annotation and Identification of Genes from the Odorant-Binding Protein Family in T. absoluta

According to homologous gene analysis, intersection analysis was performed on the odorant-binding protein (OBP) family genes of *T. absoluta* identified by homologous comparison and domain-based recognition, and a total of 97 *OBP* genes were identified (Table 1). Molecular characteristics analysis showed that the average code sequence length was 571, the longest *TabsOBP20* CDS length was 1158 bp, and the shortest *TabsOBP63* CDS length was 261 bp (Table 1). The average number of exon fragments was 4, with TabsOBP72 containing the highest number of fragments at 9 exons (Table 1).

Table 1. Identification and characteristics of the *TaOBPs*.

Gene Name	Genome Identifier	Chr.	Locus Starting	Ending	CDS (bp)	No of Exons	No of TMSs
TabsOBP01	Tabs020023.1	chr1	20,474,931	20,476,482	483	4	0
TabsOBP02	Tabs003327.1	chr1	22,042,224	22,045,788	405	4	0
TabsOBP03	Tabs020567.1	chr1	22,052,312	22,054,427	402	4	0
TabsOBP04	Tabs001544.1	chr1	24,733,631	24,739,562	768	5	0
TabsOBP05	Tabs020984.1	chr2	6,902,003	6,930,209	774	5	0
TabsOBP06	Tabs001496.1	chr2	6,933,200	6,936,252	750	6	0
TabsOBP07	Tabs011682.1	chr2	6,937,697	6,941,224	789	5	0
TabsOBP08	Tabs011532.1	chr2	6,944,108	6,949,332	735	5	0
TabsOBP09	Tabs001649.1	chr2	6,966,595	6,982,926	750	5	0
TabsOBP10	Tabs003077.1	chr3	10,972,251	10,992,148	753	5	0
TabsOBP11	Tabs004058.1	chr4	7,306,416	7,306,929	402	2	0
TabsOBP12	Tabs012945.1	chr4	7,307,857	7,309,328	426	2	0
TabsOBP13	Tabs014664.1	chr4	8,793,786	8,795,957	588	5	0
TabsOBP14	Tabs017562.1	chr4	10,087,502	10,091,190	651	4	0
TabsOBP15	Tabs018202.1	chr4	10,265,535	10,282,898	771	6	0
TabsOBP16	Tabs012221.1	chr4	10,349,993	10,352,269	702	4	1
TabsOBP17	Tabs000652.1	chr4	10,360,251	10,364,950	477	6	0
TabsOBP18	Tabs000441.1	chr9	5,168,187	5,198,937	555	6	0
TabsOBP19	Tabs006266.1	chr10	15,096,649	15,106,364	798	6	0
TabsOBP20	Tabs007711.1	chr12	15,334,109	15,335,900	1158	5	0
TabsOBP21	Tabs006148.1	chr14	8,101,391	8,108,160	312	3	0
TabsOBP22	Tabs006809.1	chr14	8,109,187	8,117,805	327	3	0
TabsOBP23	Tabs014933.1	chr15	1,281,831	1,284,781	561	5	0
TabsOBP24	Tabs011481.1	chr15	5,249,011	5,251,111	444	5	0
TabsOBP25	Tabs001448.1	chr15	5,256,732	5,261,756	552	5	0
TabsOBP26	Tabs015427.1	chr15	7,017,169	7,026,005	738	5	1
TabsOBP27	Tabs004992.1	chr15	7,048,260	7,058,579	681	5	0
TabsOBP28	Tabs021922.1	chr15	7,069,040	7,080,090	732	5	1
TabsOBP29	Tabs016278.1	chr15	7,313,748	7,321,387	768	5	0
TabsOBP30	Tabs003939.1	chr15	7,330,368	7,333,804	852	5	0
TabsOBP31	Tabs012068.1	chr15	7,334,821	7,338,351	771	5	0
TabsOBP32	Tabs003951.1	chr15	7,339,501	7,342,294	780	5	0
TabsOBP33	Tabs019705.1	chr15	7,348,269	7,352,529	777	5	0
TabsOBP34	Tabs010627.1	chr15	7,363,776	7,375,218	753	5	0
TabsOBP35	Tabs017381.1	chr15	7,391,770	7,405,770	690	5	0
TabsOBP36	Tabs002475.1	chr15	13,600,553	13,615,475	726	5	0
TabsOBP37	Tabs010825.1	chr15	13,619,535	13,625,215	729	5	0
TabsOBP38	Tabs019998.1	chr15	13,634,512	13,639,054	717	5	1
TabsOBP39	Tabs014722.1	chr15	13,749,598	13,752,428	726	5	0
TabsOBP40	Tabs021282.1	chr15	19,271,022	19,286,596	729	5	0
TabsOBP41	Tabs017578.1	chr15	19,308,964	19,326,115	711	6	0
TabsOBP42	Tabs008070.1	chr15	19,340,229	19,355,389	942	6	0
TabsOBP43	Tabs021817.1	chr16	2,311,543	2,313,131	504	3	0
TabsOBP44	Tabs020287.1	chr16	2,314,295	2,317,205	621	3	0
TabsOBP45	Tabs021019.1	chr16	2,318,859	2,320,133	489	3	0

Table 1. *Cont.*

Gene Name	Genome Identifier	Chr.	Locus Starting	Ending	CDS (bp)	No of Exons	No of TMSs
TabsOBP46	Tabs016106.1	chr16	2,322,267	2,324,749	492	3	0
TabsOBP47	Tabs020787.1	chr16	2,327,768	2,328,714	480	3	0
TabsOBP48	Tabs019694.1	chr16	2,329,157	2,330,090	492	3	0
TabsOBP49	Tabs008083.1	chr16	2,685,303	2,710,260	501	3	0
TabsOBP50	Tabs015113.1	chr16	2,998,204	2,999,676	372	3	0
TabsOBP51	Tabs018547.1	chr16	5,453,277	5,462,809	1464	4	0
TabsOBP52	Tabs002572.1	chr16	10,456,814	10,458,608	477	3	0
TabsOBP53	Tabs012705.1	chr16	10,473,039	10,473,978	375	2	0
TabsOBP54	Tabs011469.1	chr16	10,482,872	10,483,856	381	2	0
TabsOBP55	Tabs006705.1	chr16	10,487,715	10,489,717	387	3	0
TabsOBP56	Tabs011723.1	chr16	10,494,905	10,496,370	393	3	0
TabsOBP57	Tabs017738.1	chr16	10,504,939	10,506,577	465	4	1
TabsOBP58	Tabs004569.1	chr16	10,517,393	10,525,941	390	2	0
TabsOBP59	Tabs016148.1	chr16	10,526,599	10,527,497	369	2	0
TabsOBP60	Tabs002679.1	chr16	10,530,590	10,531,705	360	2	0
TabsOBP61	Tabs016850.1	chr16	10,533,210	10,534,500	384	2	0
TabsOBP62	Tabs006415.1	chr16	10,547,657	10,549,207	396	4	0
TabsOBP63	Tabs000522.1	chr16	10,554,299	10,556,471	261	3	0
TabsOBP64	Tabs011176.1	chr16	10,560,330	10,561,033	366	2	0
TabsOBP65	Tabs021726.1	chr16	10,562,757	10,563,449	363	2	0
TabsOBP66	Tabs016239.1	chr16	10,569,165	10,570,123	363	2	0
TabsOBP67	Tabs008941.1	chr16	10,571,905	10,572,952	378	2	0
TabsOBP68	Tabs020095.1	chr16	10,654,204	10,658,840	753	6	0
TabsOBP69	Tabs012728.1	chr16	11,298,069	11,299,744	378	2	0
TabsOBP70	Tabs003652.1	chr16	11,407,380	11,408,982	378	2	0
TabsOBP71	Tabs009636.1	chr17	4,447,737	4,453,664	441	5	0
TabsOBP72	Tabs013199.1	chr17	4,461,601	4,476,794	906	9	0
TabsOBP73	Tabs009535.1	chr17	4,479,265	4,484,324	273	4	0
TabsOBP74	Tabs001054.1	chr17	4,487,417	4,489,144	330	4	0
TabsOBP75	Tabs013797.1	chr17	4,498,725	4,501,477	366	4	0
TabsOBP76	Tabs006184.1	chr17	4,504,823	4,507,643	366	4	0
TabsOBP77	Tabs003612.1	chr17	4,522,428	4,526,310	453	5	0
TabsOBP78	Tabs003709.1	chr17	4,527,188	4,531,697	435	6	0
TabsOBP79	Tabs021931.1	chr17	4,535,639	4,539,656	480	5	0
TabsOBP80	Tabs014350.1	chr17	4,540,637	4,544,986	462	5	1
TabsOBP81	Tabs018171.1	chr17	4,548,567	4,556,919	450	5	0
TabsOBP82	Tabs016909.1	chr17	4,557,328	4,563,313	435	5	0
TabsOBP83	Tabs011627.1	chr17	4,566,965	4,576,767	432	5	0
TabsOBP84	Tabs005154.1	chr17	4,930,978	4,938,967	699	5	1
TabsOBP85	Tabs000196.1	chr18	10,446,352	10,450,296	420	6	0
TabsOBP86	Tabs016953.1	chr20	2,752,027	2,757,025	453	4	0
TabsOBP87	Tabs019360.1	chr20	3,696,624	3,697,265	642	1	1
TabsOBP88	Tabs003259.1	chr20	5,312,448	5,312,897	450	1	0
TabsOBP89	Tabs015434.1	chr20	5,314,670	5,315,573	342	2	0
TabsOBP90	Tabs007749.1	chr20	5,328,286	5,336,568	582	5	0
TabsOBP91	Tabs014543.1	chr20	8,925,425	8,931,904	660	3	0
TabsOBP92	Tabs014416.1	chr21	1,643,734	1,656,139	747	6	0
TabsOBP93	Tabs006243.1	chr21	12,411,678	12,418,079	657	5	0
TabsOBP94	Tabs003544.1	chr21	12,420,711	12,425,861	588	4	0
TabsOBP95	Tabs015492.1	chr21	12,628,571	12,631,367	459	4	0
TabsOBP96	Tabs017882.1	chr21	14,305,708	14,309,043	1083	3	0
TabsOBP97	Tabs017358.1	chr29	7,179,442	7,199,087	633	6	0

TMSs: Transmembrane Segments.

3.2. Phylogenetic Analysis and Chromosomal Location of OBP Genes in T. absoluta

To explore the evolutionary relationship of members of the OBP family in insects, parts of putative *OBP* genes from six representative species of two insect orders (Lepidoptera: Bm, *Bombyx mori*; Ha, *Helicoverpa armigera*; Ta, *Tuta absoluta*; Pr, *Pieris rapae*; Sf, *Spodoptera frugiperda*; Diptera: Dm, *Drosophila melanogaster*) were identified and phylogenetically analyzed (Figure 1). Phylogenetic trees of multiple species show that the *OBP* gene families present conserved clustering patterns in different Lepidoptera insects and even in Diptera *D. melanogaster*, since most of the *OBP* genes in these six insects were clustered (Figure 1). Furthermore, members of *T. absoluta* OBP family grouped in a large clade consisting of 56 members, suggesting that those *OBP*s may play a synergistic role in olfaction, and the remaining 41 members were scattered in other clades (Figure 1). For the OBP protein subfamilies, four subfamilies can also be distinguished in *T. absoluta*, i.e., 28 Classic OBPs, 11 Minus-C OBPs, 56 Plus-C OBPs, and 2 Dimer OBPs (Figure 1). The Plus-C subfamily has the largest number of members in *T. absoluta* and shows significant expansion compared to other insects.

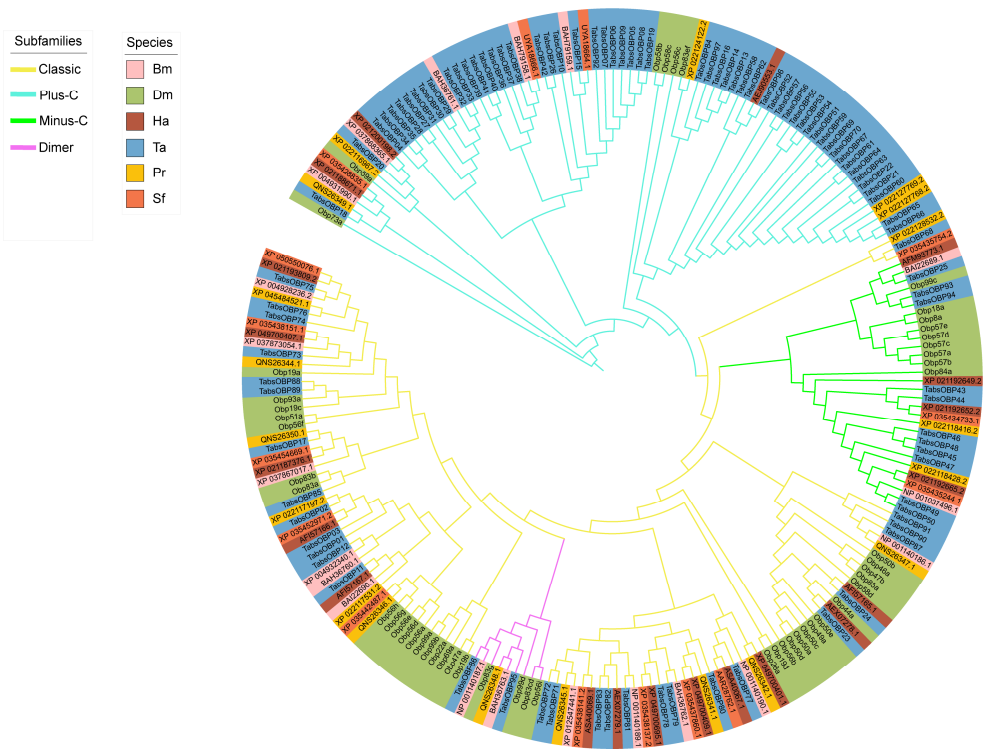

Figure 1. Phylogenetic relationship among OBP family members from six different insect species belonging to two orders. Bm, *Bombyx mori*; Dm, *Drosophila melanogaster*; Ha, *Helicoverpa armigera*; Ta, *Tuta absoluta*; Pr, *Pieris rapae*; Sf, *Spodoptera frugiperda*. Genes from the same insect are represented by the same colors. Branch colors represent the Classic (yellow), Plus-C (blue), Minus-C (green), and Dimer (purple) subfamilies.

The *OBP* genes identified in *T. absoluta* were located and analyzed on chromosomes (Figure 2). The results showed that the *OBP* genes of *T. absoluta* were widely distributed in 15 autosomes (chr1, chr2, chr3, chr4, chr9, chr10, chr12, chr14, chr15, chr16, chr17, chr18, chr20, chr21, and chr29) of the total 29 chromosomes, and the largest number of *OBP*s was on found on chromosome 16 (28 *OBP*s), followed by chromosome 15 (20 *OBP*s) and chromosome 17 (14 *OBP*s). The remaining *OBP* genes were randomly distributed on other chromosomes (Figure 2).

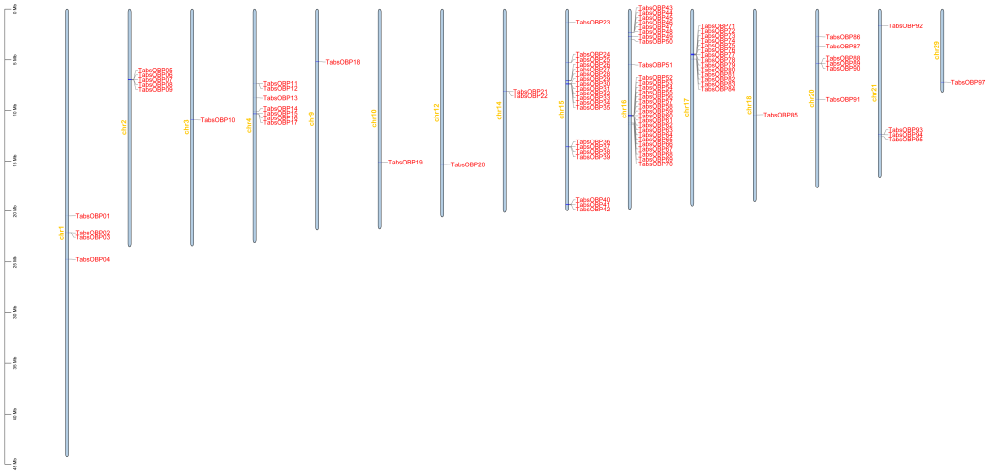

Figure 2. Chromosome localization of *OBP* genes in *T. absoluta* genome. All the identified *OBP* genes were mapped onto the *T.absoluta* chromosomes using TBtools software (v0.665) and BITACORA software (v1.3) based on the genome annotation document. The length of each chromosome is indicated by the sale on the left.

3.3. Developmental Stage-Specific Expression Profile of TaOBPs

Using transcriptomic data (BioProject: PRJNA291932), the expression levels of all genes in the OBP family were determined at the six developmental stages of *T. absoluta* (egg, first, second, third and fourth instar larvae, and adult). The results showed that the *TaOBP* genes showed very different abundances of expression at different stages of developmental (Figure 3). In the egg stage, *OBP53*, *OBP65*, and *OBP41* showed high abundance (TPM value > 500). In the first instar larvae, *OBP67*, *OBP54*, *OBP40*, *OBP57*, *OBP03*, *OBP65*, *OBP37*, *OBP26* and *OBP80* showed high abundance. In the second instar larvae, *OBP54*, *OBP67*, *OBP65*, *OBP56*, *OBP57*, *OBP40* and *OBP03* showed high abundance. In the third instar larvae, *OBP65*, *OBP54*, *OBP67*, *OBP57*, *OBP56*, *OBP66* and *OBP40* showed high abundance. In the fourth instar larvae, *OBP65*, *OBP54*, *OBP67*, *OBP57*, and *OBP66* showed high abundance. In the adults, *OBP67*, *OBP60*, *OBP77*, *OBP54*, *OBP46*, *OBP58*, *OBP45*, *OBP12*, *OBP89*, *OBP55*, and *OBP62* showed a high abundance (Figure 3 and Supplemental Table S3).

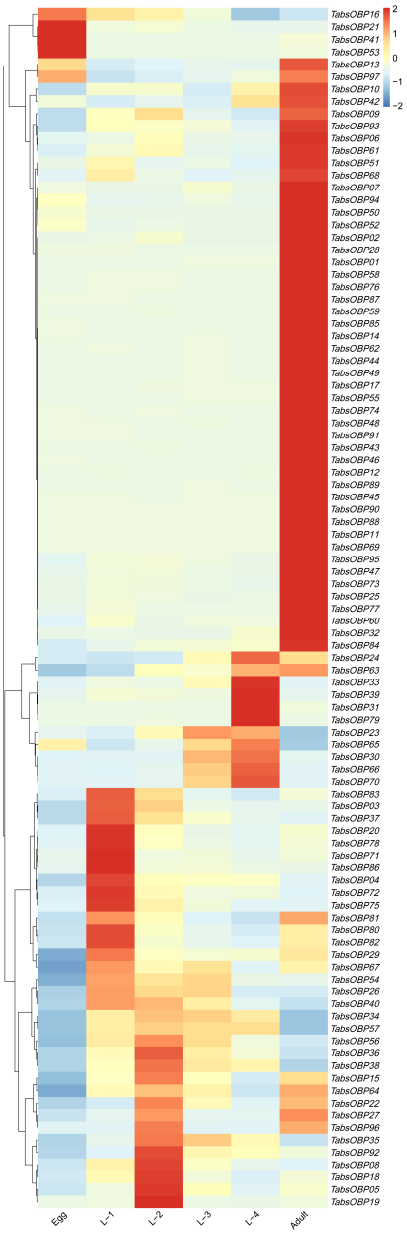

Figure 3. Heatmap of the expression level of *OBP* genes in different developmental stages of *TaOBPs*. L-1, L-2, L-3, and L-4 represent the first, second, third, and fourth instar larvae, respectively. The heat map is the expression after Z-transformation.

3.4. Behavioral Responses of *T. absoluta* to *Plectranthus tomentosa*

The behavior response of the adult or larvae of *T. absoluta* to *Plectranthus tomentosa* was measured using a Y-tube olfactometer. The results of the behavior-selection experiment showed that in the tomato control group, *P. tomentosa* had a significant repellent effect on both adults (Figure 4A, 66.7% chose tomatoes vs. 20.0% chose *P. tomentosa*, $p = 0.02$) and larvae (Figure 4B, 60.0% vs. 23.3%, $p = 0.04$) of *T. absoluta*.

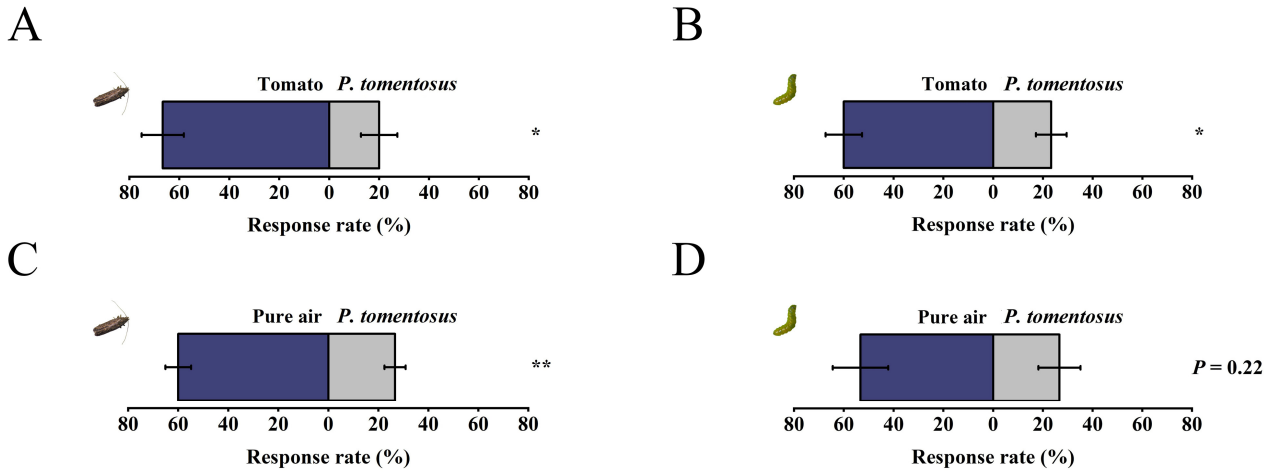

Figure 4. Behavioral responses of *T. absoluta* to *P. tomentosa*. (**A**) Taxis behavior of adult in tomato control group; (**B**) Taxis behavior of larvae in tomato control group; (**C**) Taxis behavior of adult in pure air control group; (**D**) Taxis behavior of larvae in pure air control group. * $p < 0.05$; ** $p < 0.01$.

To further confirm the effect of *P. tomentosa* on the sexual behavior of *T. absoluta* and exclude the attraction effect of tomato volatiles, we conducted a selection experiment with pure air as the control group. The results indicated that the volatiles of *P. tomentosa* alone could also significantly repel adults of *T. absoluta* (Figure 4C, 60.0% vs. 26.7%, $p < 0.01$), and the degree of repelling was reduced in larvae (Figure 4D, 53.3% vs. 26.7%, $p = 0.22$).

3.5. Transcriptional Responses of TaOBPs to Tomatoes and P. tomentosa

OBP proteins are key proteins used by insects to recognize volatile external signals. To further study the olfactory response mechanism of *T. absoluta* to volatile compounds from the most suitable host plant tomato and the repellent plant *P. tomentosa*, after being exposed to volatiles of tomatoes or *P. tomentosa* for a while, transcriptional expression levels of *OBP* genes were determined throughout the genome of *T. absoluta* using real-time quantitative PCR (RT-qPCR).

The results showed that compared to the control exposed to pure air, the treatment of tomato volatiles induced the up-regulation (fold change >1.5) of 10 *OBP* genes in adult *T. absoluta* (Figure 5). More obviously, 20 *OBP* genes were up-regulated in adults after exposure to the volatiles of *P. tomentosa*, and the expression ratio was higher than that induced by volatiles of tomatoes (Figure 5). Among them, *OBP09*, *OBP19*, *OBP22*, *OBP23*, *OBP24*, *OBP29*, *OBP37*, *OBP40*, *OBP41*, *OBP46*, *OBP58*, *OBP60*, *OBP65*, *OBP77*, *OBP85* and *OBP92* showed significantly higher regulation than the pure air group or the tomato volatiles group (Figure 6, $p < 0.05$).

However, in larvae, only a relatively small number of *OBP* genes were affected by volatiles of tomatoes and *P. tomentosa*, and the amplitude of gene up-regulation was significantly smaller than that of the adults (Figure 7). That is, the expression levels of *OBP3* and *OBP24* were down-regulated after exposure to volatiles of *P. tomentosa* compared to the control exposed to pure air (Figure 7).

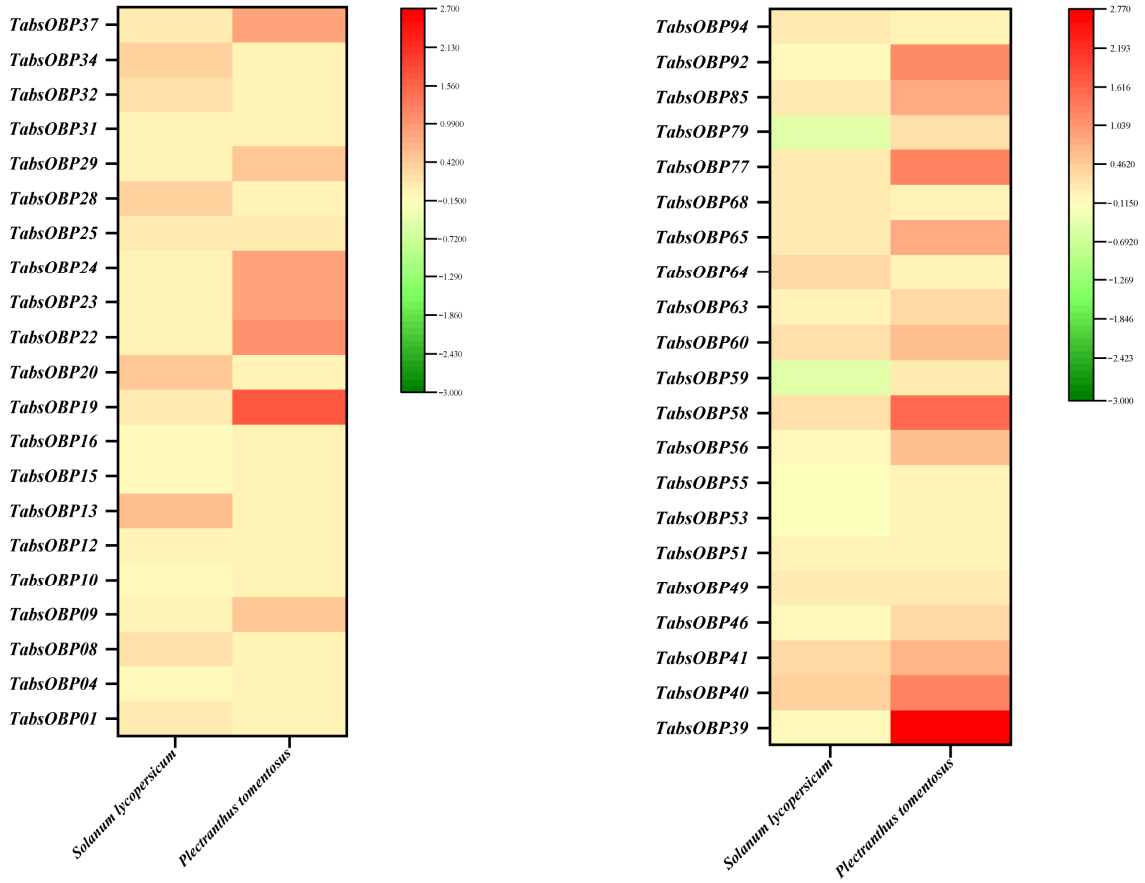

Figure 5. Transcriptional responses of *TaOBPs* in adult *T. absoluta* to tomatoes and *P. tomentosa*. The amount of gene expression in the heat map is the amount of tomato volatiles treated or *P. tomentosa* volatiles divided by the amount of expression treated with pure air. The logarithmic conversion of the relative expression value with base 10 was performed.

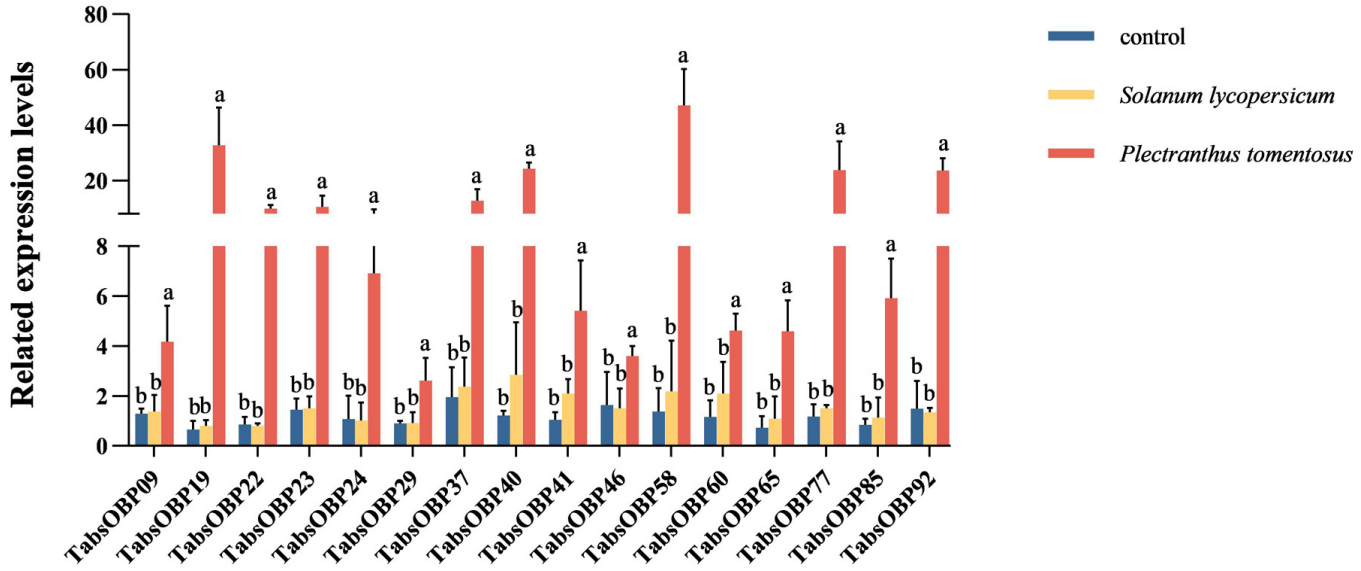

Figure 6. Significantly up-regulated *OBPs* in adult *T. absoluta* after exposure to *P. tomentosa* volatiles. Different letters indicate significant differences ($p < 0.05$) by using Tukey's honest significant difference multiple-range test. All data are presented as the mean ± standard error (SE).

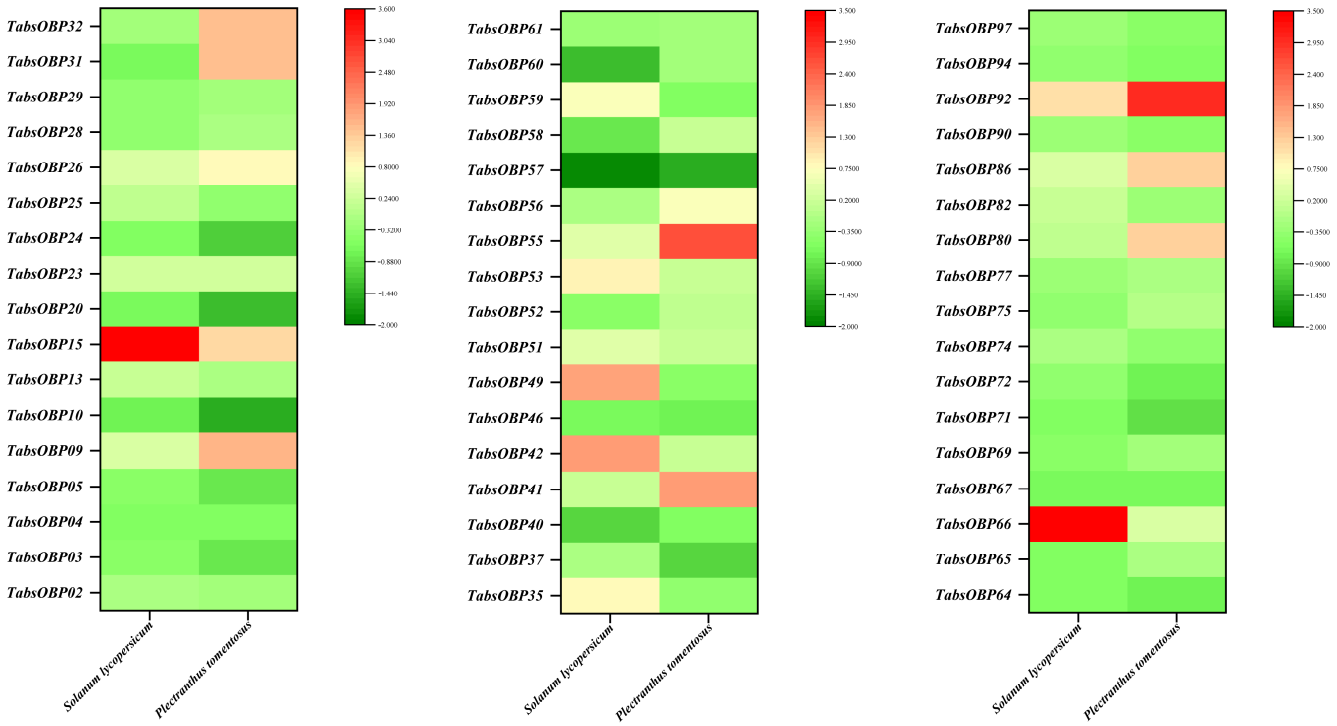

Figure 7. Transcriptional responses of *TaOBPs* in larvae of *T. absoluta* to tomatoes and *P. tomentosa*. The amount of gene expression in the heat map is the amount of tomato volatiles treated or *P. tomentosa* volatiles divided by the amount of expression treated with pure air. The relative expression value was homogenized.

4. Discussion

As *Tuta absoluta* is a devastating global quarantine pest, its comprehensive control has become one of the hotspots of plant protection research in recent years [21]. As a promising ecological control measure, the 'push and pull' strategy based on functional plants has become a new method, playing an increasingly important role in the comprehensive control of many agricultural pests [37]. However, the direct application of functional plants to the control of *T. absoluta* is rarely reported. In this study, we identified *Plectranthus tomentosa*, which significantly repels adult and larval *T. absoluta* through a Y-tube olfactometer (Figure 4). *P. tomentosa* is a perennial shrub herb of the genus Spicularia in the Labiaceae family. It is known as 'touch fragrance' because it emits a comfortable aroma after touching, which has the effects of being refreshing, repelling mosquitoes, and reducing swelling [38]. Sun et al. have demonstrated that *P. tomentosa* has strong repellent effects against *Tetranychus kanzawai* [39]. Next, GC-MS combined with an olfactory tube experiment can be used to screen and identify specific compounds with repellent effects in *P. tomentosa*, and the potential of using *P. tomentosa* and its volatiles to control *T. absoluta* can be further verified through an envelope experiment and greenhouse experiment. In addition to the 'push and pull' strategy, agricultural control (such as the selection of resistant varieties) [40], cultivation management control (including reducing nitrogen fertilizer use) [41], biological control [42], physical control, and other applications have been applied to the prevention and control of *T. absoluta* [43]. Continued research on functional plants and the 'push and pull' strategy holds significant promise for improving the ecological control of *T. absoluta*.

In general, insects use olfactory-related proteins, such as odorant-binding proteins (OBPs), to make taxis between plants [6]. Here, we first identified 97 OBPs at the genome-wide level of *T. absoluta* (Table 1). We identified 97 *OBP* genes in *T. absoluta*, which is a significantly higher number compared to other reported insects (51 in *Spodoptera frugiperda*, 43 in *Bombyx mori*, 49 in *Manduca sexta*, 51 in *Heliconius melpomene*, 32 in *Danaus plexippus*) [44]. This significant expansion of *OBP* genes in *T. absoluta* suggests a potential role in its rapid invasion and

extensive host adaptation capabilities. The multispecies phylogenetic tree reveals conserved clustering patterns in *OBP* gene families across Lepidoptera and even in Diptera, as the *OBP* genes of these five insects are mostly clustered together (Figure 1). In addition, a branch was clustered by 56 OBP members, suggesting that these OBPs may work synergistically in olfaction (Figure 1). Furthermore, the Plus-C subfamily of OBPs has the largest number of members (56 OBPs) in *T. absoluta* and shows significant expansion compared to other insects (Figure 1). The Plus-C subfamily of OBPs in *Drosophila* shows a striking level of divergence, with many of these sequences lacking the typical hydrophobic ligand-binding domain of OBPs [30]. The extremely significant expansion of the Plus-C subfamily may be involved in the rapid adaptation of *T. absoluta* to the host plant and the external odor environment, thus facilitating the rapid expansion and spread of *T. absoluta*.

The highest number of these *OBP* genes were highly expressed in the adult stage, followed by the larval stage, suggesting their crucial role in olfactory selection in both stages of life (Figure 3). Our study revealed that *P. tomentosa* exerts repellent effects on both adults and larvae of *T. absoluta*, as evidenced by tests using tomato and pure air as controls (Figure 4). To pinpoint specific *OBP*s responding to plant volatiles, we identified several potential *OBP*s in *T. absoluta* through RT-qPCR. In adults, sixteen *OBP*s were significantly up-regulated after exposure to *P. tomentosa*, with the *TabsOBP39* gene showing a remarkable 588-fold increase (Figure 6). We hypothesized that these genes may be involved in the regulation of the repellent behavior of adult insects.

However, compared to adults, a small number of *OBP* genes in larvae were affected by volatiles of tomatoes and *P. tomentosa*, with a markedly lower amplitude of up-regulation (Figure 7). For a long time, studies on the olfactory system of insects have focused mainly on adult insects. However, Lepidoptera pests damage crops mainly at the larval stage, and so far, studies on the olfactory system of larvae have been very scarce [45]. In recent years, researchers have found that olfactory-related proteins are not only expressed in the larval stage of Lepidoptera insects but also play an important role in the selection of insect food [45,46]. For example, research has shown that *Spodoptera littoralis* larvae are significantly attracted to Z9, E11-14:Ac, a major component of adult sex pheromones—a discovery that could inform new pest-control strategies [46]. Only two *OBP* genes were significantly decreased in larvae, which may be consistent with the results of taxis behavior. The repellent effect of *P. tomentosa* on larvae was lower than that of adults, and there was no significant difference between the control group and the air control group (Figure 4D). *T. absoluta* was able to use those OBPs to identify external volatiles, leading to trend- or escape-selection behavior. In the future, we can further study how the key OBP recognizes the external volatiles, how to further regulate the signaling pathway of insects, and finally produce selection behavior.

5. Conclusions

In summary, we identified 97 putative members of the OBP family from the whole genome of *T. absoluta*. We then described the basic characteristics, phylogenetic analysis, and the developmental stage-specific expression profile of these gene family members. On the other hand, we identified the functional plant *P. tomentosa*, which significantly repels *T. absoluta*, and further pinpointed key *OBP* genes in *T. absoluta* that respond to the induction of volatiles of *P. tomentosa*. These findings provide preliminary information on the response mechanism of *T. absoluta* to tomato plants and repellent plants such as *P. tomentosa*. This research improves our understanding of the mechanisms of olfactory response in *T. absoluta* and offers potential avenues for environmentally sustainable pest-control strategies.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agronomy14010231/s1, Table S1: The sequence IDs for each OBP used for phylogenetic analysis; Table S2: Primers used in the qRT-PCR analysis; Table S3: Developmental stage-specific expression profile of *TaOBPs* based on TPM value.

Author Contributions: Conceptualization, Z.S. and F.G.; methodology, Z.S., R.M., D.L., C.P. and S.W.; software, Z.S.; formal analysis, Z.S. and Y.C.; investigation, Z.S., R.M., D.L., C.P. and S.W.; writing—original draft preparation, Z.S. and R.M.; writing—review and editing, Z.S. and F.G.; project administration, Z.S. and F.G.; funding acquisition, Z.S. and F.G. All authors have read and agreed to the published version of the manuscript.

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Data Availability Statement: Data are contained within the article.

Conflicts of Interest: The authors declare no conflicts of interest.

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