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Expression profiles analysis and roles in immunity of transient receptor potential (TRP) channel genes in *Spodoptera frugiperda*

Hongai Su[1†], Qianxuan Dai[1†], Feiyue Teng[1], Ziyuan Li[1], Yixiang Qi[1*] and Yongyue Lu[1*]

Abstract

Background Transient receptor potential (TRP) ion channels play crucial roles in mediating responses to environmental stimuli, as well as regulating homeostasis and developmental processes in insects. Several members of the TRP superfamily are potential molecular targets for insecticides or repellents, indicating their research value in pest control. This study focuses on *Spodoptera frugiperda,* an important invasive pest in China known for its wide host range and strong reproductive capacity. Currently, there is a lack of molecular research on the TRP channels of the invasive pest *S. frugiperda*.

Results In this study, we identified 15 TRP family genes in *S. frugiperda*, which were classified into six subfamilies. The TRPP subfamily gene was not identified, whereas the TRPA subfamily contained the highest number of members in this insect. Real-time quantitative polymerase chain reaction (RT-qPCR) experiments revealed widespread expression of TRP channel genes across various developmental stages of *S. frugiperda*. However, TRPM and TRPML were highly expressed only in eggs. Transcripts of TRP channel genes were detected in the sensory organs of mature adults, including the mouthparts, antennae, compound eyes, legs, wings, harpagones, and ovipositors, as well as in tissues of 5th instar larvae (hemocytes, central nervous system, midgut, fat body, and Malpighian tubules). To explore the potential role of TRP channels in immunity, we detected their levels in larvae 24 h after infection with *Serratia marcescens*. The expression levels of *TRPML*, *TRPL*, and the *Pain* genes were significantly up-regulated, suggesting their important roles in immune responses to *S. marcescens*.

Conclusions The results of this study extend our knowledge of these critical sensory channels in *S. frugiperda*. This knowledge provides a basis for the future development of insecticides that target these channels, thereby promoting the safe and effective control of this key pest.

Keywords Expression profiles, *Spodoptera frugiperda*, *Serratia marcescens*, Transient receptor potential (TRP) channels

Background

Transient receptor potential (TRP) channels are key proteins in insects, essential for sensing changes in the external environment and regulating physiological processes such as vision [1], olfaction [2], auditory sensation [3], taste [4], temperature sensing [5], and mechanical sensation [6, 7]. Based on primary amino acid sequence homology, the TRP family is divided into seven subfamilies listed as follows: TRP-Canonical (TRPC), TRP-Ankyrin (TRPA), TRP-No

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mechanoreceptor potential C (TRPN), TRP-Vanilloid (TRPV), TRP-Melastatin (TRPM), TRP-Mucolipin (TRPML), and TRP-Polycystin (TRPP). Moreover, TRP channel families can be classified into two groups according to their differences in sequences and topologies: Group 1 contains the TRPC, TRPA, TRPN, TRPV and TRPM subfamilies, while Group 2 consists of the remaining TRPP and TRPML subfamilies [8].

In recent studies, a number of TRP channel members have been identified as molecular targets for insecticides or repellents. For example, TRPV subfamily members, including Nanchung (Nan) and Inactive (Iav) channels, are activated by pymetrozine, pyrifluquinazon, and afidopyropen, causing coordination dysfunction in adult insects, ultimately leading to death [9–11]. TRPA subfamily members, including the Water with (Wtrw) channel, can be activated by afidopyropen, pymetrozine, and the endogenous agonist, nicotinamide [12]. Furthermore, silencing TRPM subfamily channels significantly reduced the repellent activity of L-menthol and menthoxypropanediol (MPD) against *Tribolium castaneum* [13]. Additionally, TRP1, a homolog of the TRPC subfamily channel, TRPgamma (TRPγ), can be directly activated by natural repellents such as, citronellal, citronellol, and camphor in *Mesobuthus martensii* [14]. Thus, it is important to determine the sequences and understand the properties of TRP channels for effective pest control strategies.

Although numerous TRP channels have been identified and characterized in various insects [15–19], the functional roles of TRP channels in the agricultural pest *Spodoptera frugiperda* remains limited. This insect, also known as the fall armyworm, is a common polyphagous agricultural pest [20, 21], that can affect approximately 353 plant species across 76 different families [22, 23]. The wide host range, strong fertility, rapid migration, and adaptability to diverse habitats of *S. frugiperda* contribute to its characteristic large-scale, concentrated outbreaks over short periods of time, making prevention and control challenging. The damage caused by *S. frugiperda* is mainly due to its larvae feeding on crop leaves, which affects plant photosynthesis, ultimately reducing crop yield and causing economic losses [24]. To reduce production losses, chemical control is widely used in agricultural as the simplest and most convenient method for large-scale application. However, *S. frugiperda* has developed resistance to various insecticides, including lambda-cyhalothrin [25], organic phosphate esters [26], chlorpyrifos [27], chlorantraniliprole [28], lufenuron [29], and spinosad [30], due to the long-term overuse of chemical pesticides. Therefore, it is necessary to develop new prevention and control technologies, such as strategies based on biological control.

The use of organisms and microorganisms to manage pests is an essential component of integrated pest management. Among bacteria suitable for pest management, *Serratia marcescens* (Enterobacterales: Enterobacteriaceae) is a Gram-negative bacterium widely distributed in natural environments [31]. *S. marcescens* produces a variety of exoenzymes, including chitinase, which hydrolyze and destroy the surface and periplasmic structures of insects, eventually causing death. This trait renders the bacterium highly pathogenic to numerous agricultural and forestry pests [32–35]. When *S. marcescens* infects the fall armyworm, the larvae show a strong immune response [36], which reduces their survival rate, development rate and adult emergence rate [37, 38]. *S. marcescens* infection in other insects leads to upregulation of immune-related genes in *Spodoptera exigua* [39]. Additionally, it affects growth, development, and reproduction in *Mythimna separata* (Walker) and *Spodoptera litura* (Fab.) [40, 41].

In this study, we identified 15 TRP channels in *S. frugiperda* and investigated their expression patterns across different developmental stages and in multiple tissues of both larvae and adult *S. frugiperda*. In addition, we analyzed the immune response of *S. frugiperda* TRP channels following injection of the conditionally pathogenic bacterium, *S. marcescens*. Our characterization of TRP channels in *S. frugiperda* lays the groundwork for future functional studies and may contribute to the development of innovative pest control methods.

Results

Identification and sequence analysis of TRP channels in *S. frugiperda*

Fifteen TRP channel genes were identified in *S. frugiperda* that exhibited sequence homology to known *Drosophila* TRP channel sequences (Fig. 1 and Table 1). Phylogenetic analysis categorized these channels into subfamilies: three TRPC, seven TRPA, one TRPN, two TRPV, one TRPM, and one TRPML subfamily members. Interestingly, no TRPP subfamily members were identified in *S. frugiperda* (Fig. 1 and Table 1). These 15 *S. frugiperda* TRP channel genes were classified into six subfamilies and divided into two groups based on structural specificity and homology (Fig. 1 and Table 1). Group 1 included five subfamilies: TRPC (*SfruruTRP*, *SfruTRPL*, *SfruTRPγ*), TRPA (*SfruTRPA1*, *SfruTRPA*5, *SfruPain*, *SfruPyx1*, *SfruPyx2*, *SfruWtrw1*, *SfruWtrw2*), TRPN (*SfruNompC*), TRPV (*SfruIav*, *SfruNan*), and TRPM (*SfruTRPM*). Group 2 comprised only *SfruTRPML* of the TRPML subfamily. Sequence analysis identified six transmembrane domains in all *S. frugiperda* TRP channels (Table 1). Most group 1 TRP proteins had 1 to 29 N-terminal ankyrin repeat (AR) domains, except for the TRPM

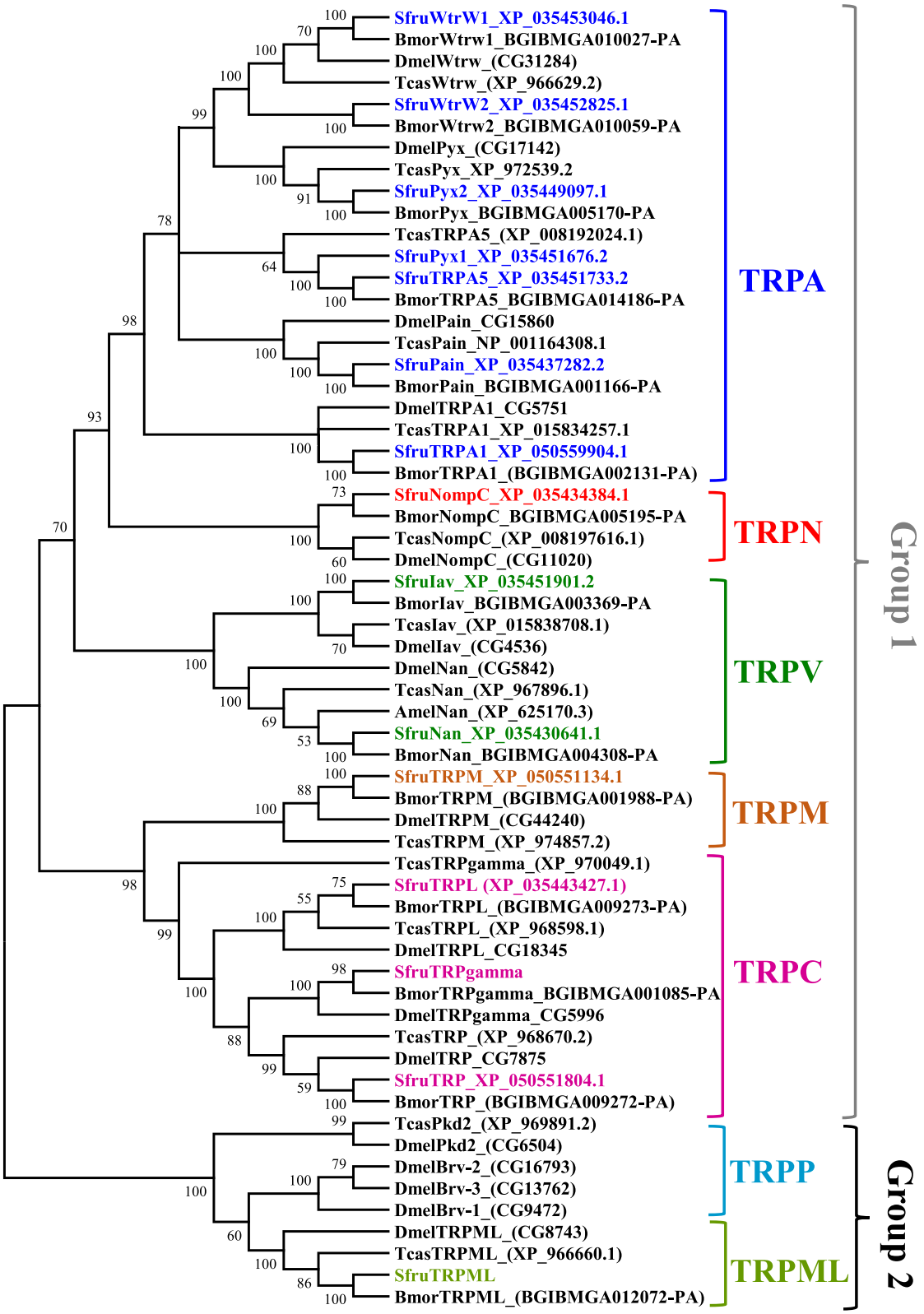

Fig. 1 Phylogenetic analysis of TRP channels in *Spodoptera frugiperda* and other insects. The tree was constructed using the software MEGA 6.06 with 1000 bootstrap replicates based on the Maximum Likelihood method. The numbers on branch nodes denote levels of bootstrap support. Species abbreviations are Sfru, *Spodoptera frugiperda*, Bmor, *Bombyx mori*, Dmel, *Drosophila melanogaster*, Tcas, *Tribolium castaneum*

Table 1 TRP channels identified from *S. frugiperda*

Subfamily	Gene name	Genomic Sequence ID	NCBI accession no.(Transcripts)	Length (amino acids)	Protein region identified (TM)	Number of ankyrin repeats (AR)	CG no. of the *D. melanogaster* orthologue	Sequence identity between *S. frugiperda* and *D. melanogaster*
Group- 1 TRPs								
TRPC	SfruTRP	NW_023337121.1	XM_035587337.1	1078	TM1 - 6	1	NM_001276161.1	72%
	SfruTRPL	NW_023337121.1	XM_035587534.1	1007	TM1 - 6	2	NM_165694.3	69%
	SfruTRPγ	NW_023337111.1	XM_035575546.1	1048	TM1 - 6	2	NM_001144358.3	73%
TRPA	SfruTRPA1	NW_023337107.1	XM_035582162.1	978	TM1 - 6	9	NM_001104084.5	73%
	SfruTRPA5	NW_023337130.1	XM_035595840.2	1118	TM1 - 6	13	–	–
	SfruPain	NW_023337116.1	XM_035581389.1	946	TM1 - 6	8	–	–
	SfruPyx1	NW_023337130.1	XM_035595783.1	1113	TM1 - 6	13	–	–
	SfruPyx2	NW_023337127.1	XM_035593204.1	922	TM1 - 6	9	NM_167813.2	67%
	SfruWtrw1	NW_023337131.1	XM_035597153.1	972	TM1 - 6	9	NM_001300285.1	68%
	SfruWtrw2	NW_023337131.1	XM_035596932.1	1009	TM1 - 6	10	NM_001300285.1	62%
TRPN	SfruNompC	NW_023337113.1	XM_035578494.1	1575	TM1 - 6	29	NM_078759.4	69%
TRPV	Sfrulav	NW_023337130.1	XM_035596008.1	1362	TM4 - 6	5	NM_132125.2	73%
	SfruNan	NW_023337110.1	XM_035574748.1	846	TM1 - 6	5	NM_001274904.1	72%
TRPM	SfruTRPM	NW_023337114.1	XM_035578922.1	1725	TM1 - 6	0	NM_001299519.1	70%
Group- 2 TRPs								
TRPML	SfruTRPML	NW_023337123.1	XM_035589037.1	602	TM1 - 6	0	NM_140888.4	70%

subfamily. Conversely, no AR domains were detected at the N-terminus of the group 2 TRPML subfamily protein (Table 1). BLASTP analyses of protein sequence alignments showed that all *S. frugiperda* TRP channels exhibited a high level of sequence identity (> 62%) with their counterparts in *Drosophila melanogaster* (Table 1).

TRP channel transcript levels in different *S. frugiperda* developmental stages

Next, we performed RT-qPCR analyses to detect TRP channel expression patterns at different stages of *S. frugiperda* development, including eggs, larvae, pupae, and adults (Fig. 2). The results showed that *SfruTRP* was highly expressed in 1-day-old, with lower expression in 3-day-old adult males (Fig. S1 A). *SfruTRPL* was primarily expressed in 6th instar larvae and pupae, while its expression was low at other stages (Fig. S1B). *SfruTRPγ* transcripts were generally detected at all stages tested, with the highest expression levels observed in pupae and 1-day-old adults (Fig. S1 C). *SfruTRPA1* was abundantly expressed in 1st instar larvae, with significantly higher levels compared toother stages (Fig. S1D). *SfruTRPA5*, which is specific to the Lepidoptera, was detected in pupae, 1-day-old adults, and 3-day-old males (Fig. S1E). *SfruPain* was more highly expressed in adults compared to other developmental stages (Fig. S1 F). Both *SfruPyx* splice forms were abundantly expressed in 3-day-old males (Fig. S1G and H). *SfruPyx1* was also expressed in pupae, while *SfruPyx2* showed additional expression in 1-day-old adult males. Both transcripts encoding Wtrw molecules were highly expressed in 1-day-old and 3-day-old adult males (Fig. S1I and J). In contrast to *SfruWtrw1*, *SfruWtrw2* was also expressed in 1st to 4th instar larvae (Fig. S1 J). Similar to *SfruTRP*, high *SfruNompC* transcripts levels were detected in 1st instar larvae, as well as 1- day-old adults and 3-day-old males (Fig. S1 K). *SfruIav* was widely expressed across all tested stages, particularly at the pupal stage (Fig. S1L). In contrast, *SfruNan* was mainly highly expressed in 1st and 2nd instar larvae (Fig. S1M). Unlike most other TRP channels, which are predominantly expressed in adult stage insects, *SfruTRPM* and *SfruTRPML* were almost exclusively highly expressed in eggs (Fig. S1 N and O).

Tissue distribution of TRP channels in *S. frugiperda* adults

To explore the possible roles of TRP channels in *S. frugiperda*, RT-qPCR analyses were performed to determine their relative transcription levels in various external sensory organs of adults, including mouthparts, antennae, compound eyes, legs, wings, harpagones, and ovipositors (Fig. 3). Both *SfruTRP* and *SfruTRPL* were highly expressed in compound eyes (Fig. S2 A and B), whereas *SfruTRPγ* exhibited high expression in all tissues except the wings and ovipositors (Fig. S2 C). *SfruTRPA1* was

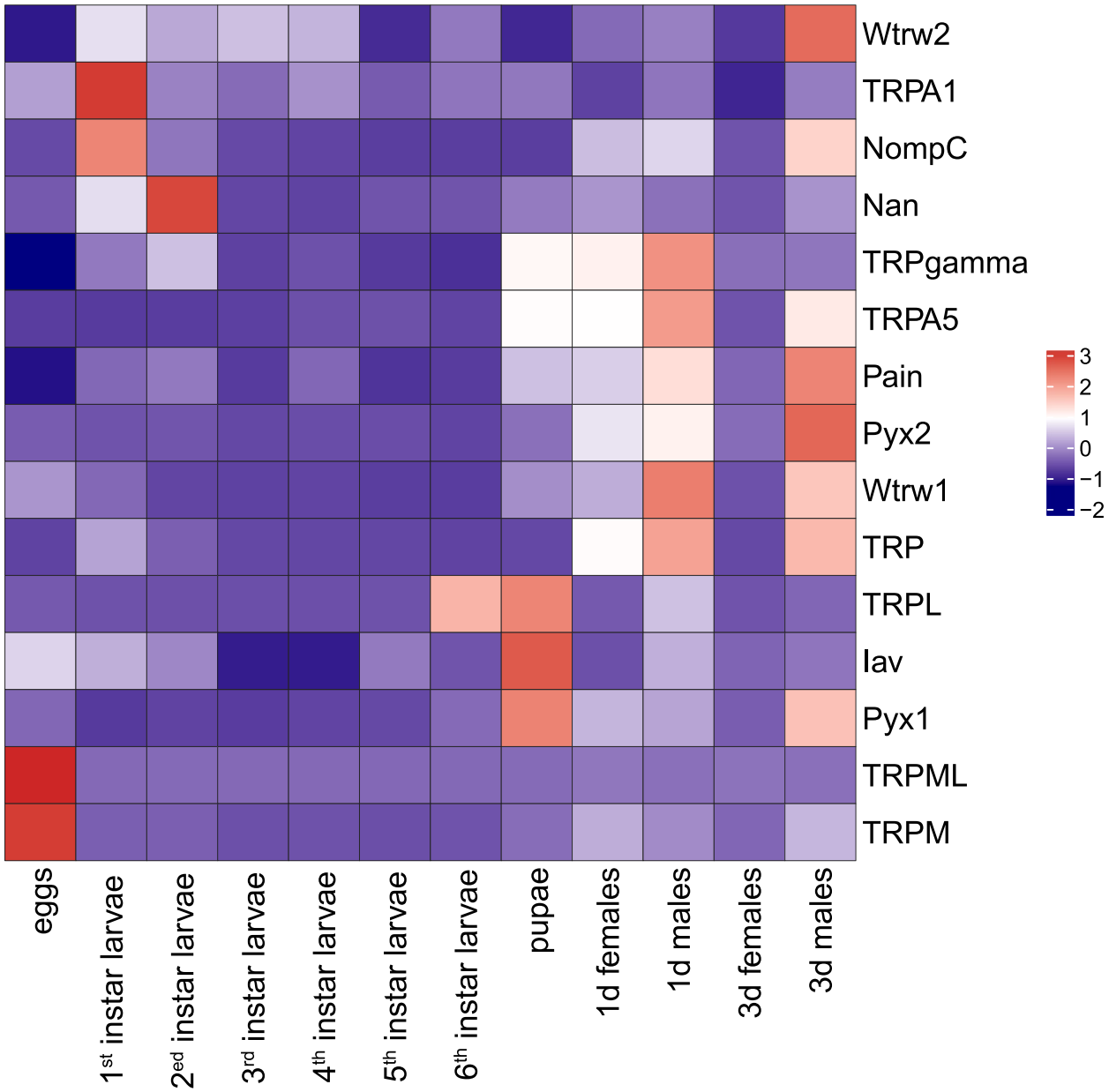

Fig. 2 Relative expression level of TRP channels in different developmental stages of *Spodoptera frugiperda*, including eggs (1 to 2 h), larvae (1 st, 2nd, 3rd, 4 th, and 5.[th] instar), pupae, immature adult male and female insects (1-day-old), and mature adult male and female insects (3-day-old)

mainly expressed in mouthparts, antenna, and compound eyes (Fig. S2D). Meanwhile, *SfruTRPA5* expression levels were high in most tissues, except for the compound eyes and ovipositors (Fig. S2E). Similar to *SfruTRPA1*, transcripts of *SfruPain* were abundantly detected in the mouthparts, antennae, and compound eyes (Fig. S2 F). *SfruPyx1* expression levels were highest in the compound eyes (Fig. S2G), while *SfruPyx2* showed peak expression in the mouthparts (Fig. S2H). *SfruWtrw1* was highly expressed in the antennae (Fig. S2I), whereas *SfruWtrw2* was hardly detectable in this tissue (Fig. S2 J). The antennae displayed the highest levels of *SfruNompC*, *SfruIav*, *SfruNan*, *SfruTRPM*, and *SfruTRPML* mRNAs (Fig. S2 K-O). In addition, *SfruNompC* and *SfruTRPM* showed high transcription levels in the mouthparts (Fig. S2 K and N), while *SfruIav* was expressed at higher levels in the compound eyes (Fig. S2L). Beyond the antennae, *SfruTRPML* mRNA expression levels were also increased in the mouthparts, legs, harpagones, and ovipositors, but were lower in the compound eyes and wings (Fig. S2O).

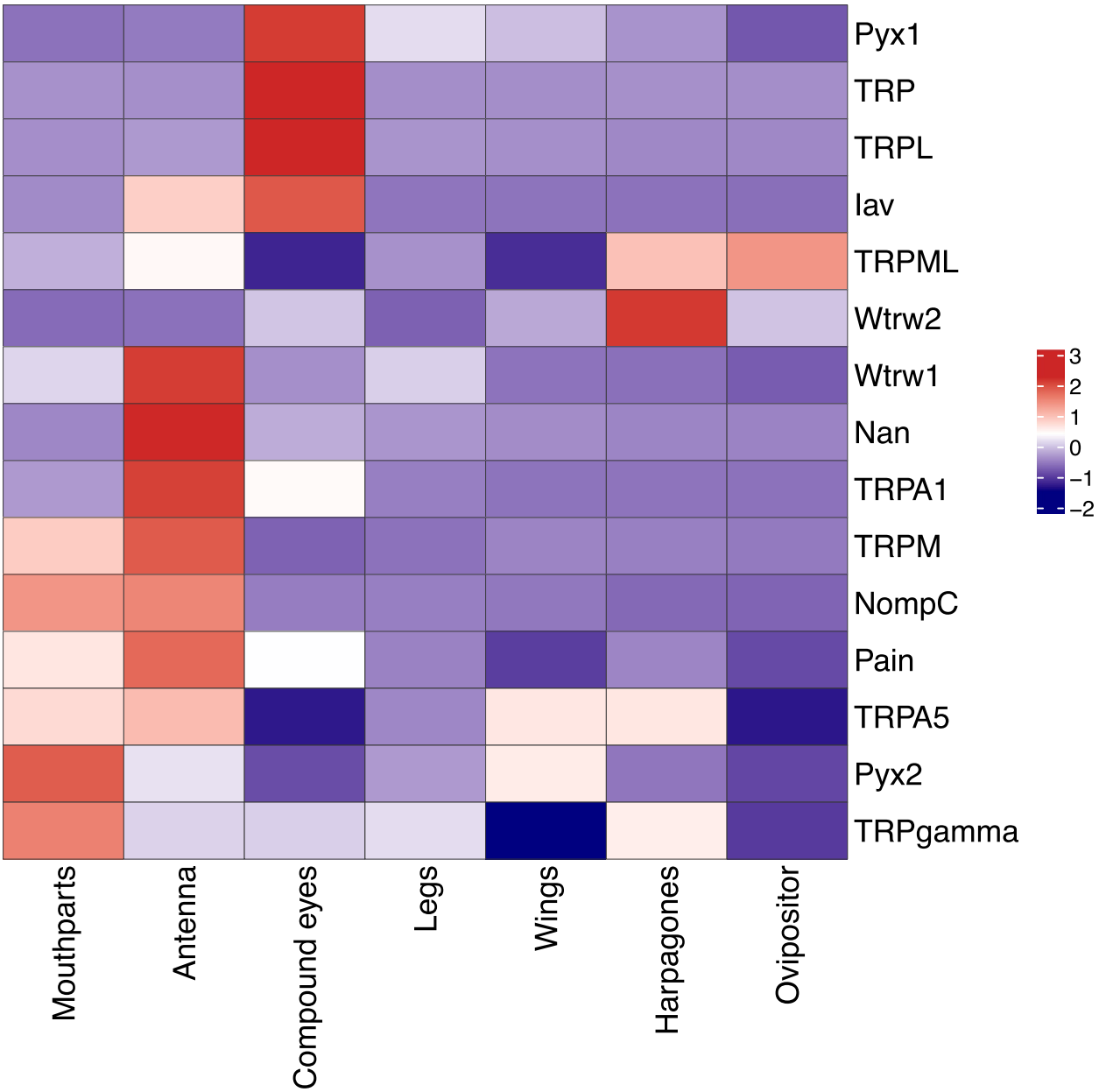

Fig. 3 TRP channels relative expression levels in mature adult *Spodoptera frugiperda* various tissues, including mouthparts, antennae, compound eyes, legs, harpagones, ovipositors

Tissue distribution of TRP channels in *S. frugiperda* larvae

The internal tissues of insect larvae, including the hemocytes, central nervous system (CNS), midgut, fat body, and Malpighian tubules (MT), play crucial roles in maintaining homeostasis. To investigate the role of TRP channels in larval internal tissues, we examined their relative transcription levels, revealing distinct expression patterns (Fig. 4). *SfruTRP* was highly expressed in the CNS, with low expression levels detected in other tissues (Fig. S3 A). Transcription levels of *SfruTRPL* were higher in the MT than in other tissues (Fig. S3B). Abundant transcripts of *SfruTRP*, *SfruTRPA1*, *SfruPain*, *SfruWtrw1*, *SfruNompC*, and *SfruIav* were detected in the CNS. However, their expression levels were low in other tissues (Fig. S3 C, D, F, H, J, K). High levels of *SfruTRPA5* and *SfruPyx2* transcripts were detected in hemocytes, while their expression was minimal in other tissues (Fig. S3E, H). Moreover, *SfruIav* transcripts were highly expressed in the CNS, hemocytes, and fat body (Fig. S3 K). Similar

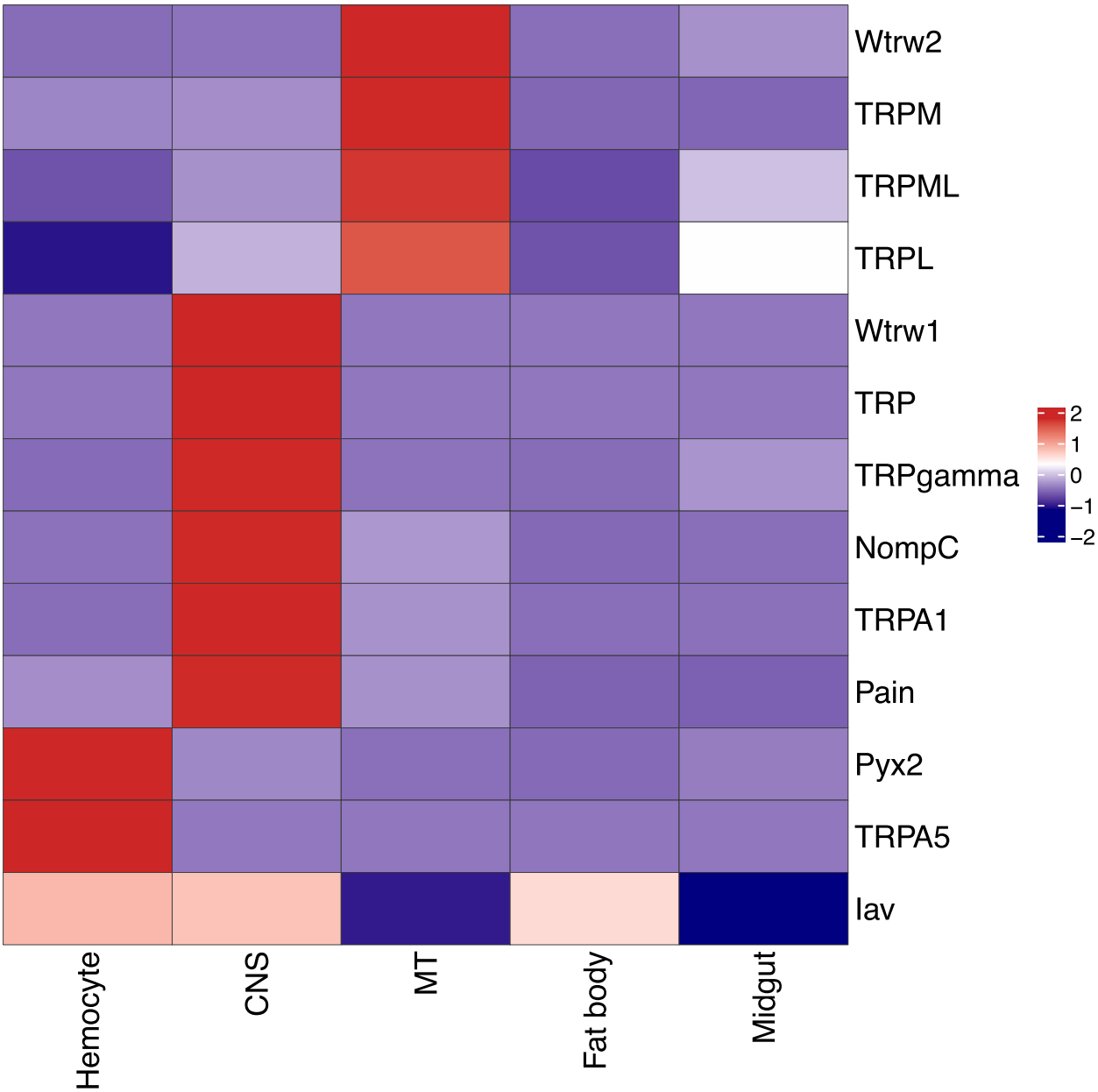

Fig. 4 TRP channels relative expression levels in larval *Spodoptera frugiperda* various tissues, including hemocytes, central nervous system (CNS), midgut, fat body, and Malpighian tubules (MT)

to *SfruWtrw*, *SfruTRPML* transcripts were also highly expressed in the MT (Fig. S3M).

Effect of *S. marcescens* infection on the expression of TRP channels

To investigate the response of TRP channels to *S. marcescens* infection, we monitored the expression of the 15 TRP genes in insect hemocytes. The bacteria were injected into the mid-region of 5 th instar *S. frugiperda* larvae, and gene expression was analyzed 24 h post-injection. Only seven TRP genes were expressed 24 h after inoculation with *S. marcescens*, while the levels of the other genes were barely detectable. *SfruTRPML* gene expression increased 2.64-fold 24 h post-infection (Fig. 5A), while *SfruTRPL* levels were significantly up-regulated by 2.68-fold (Fig. 5B). In addition, *SfruPain* expression increased 1.48-fold compared to wild-type (WT) controls (Fig. 5C). Since *SfruTRP5* and *SfruPyx2* were highly expressed in hemocytes, we speculated that they might play a role in immune responses. However,

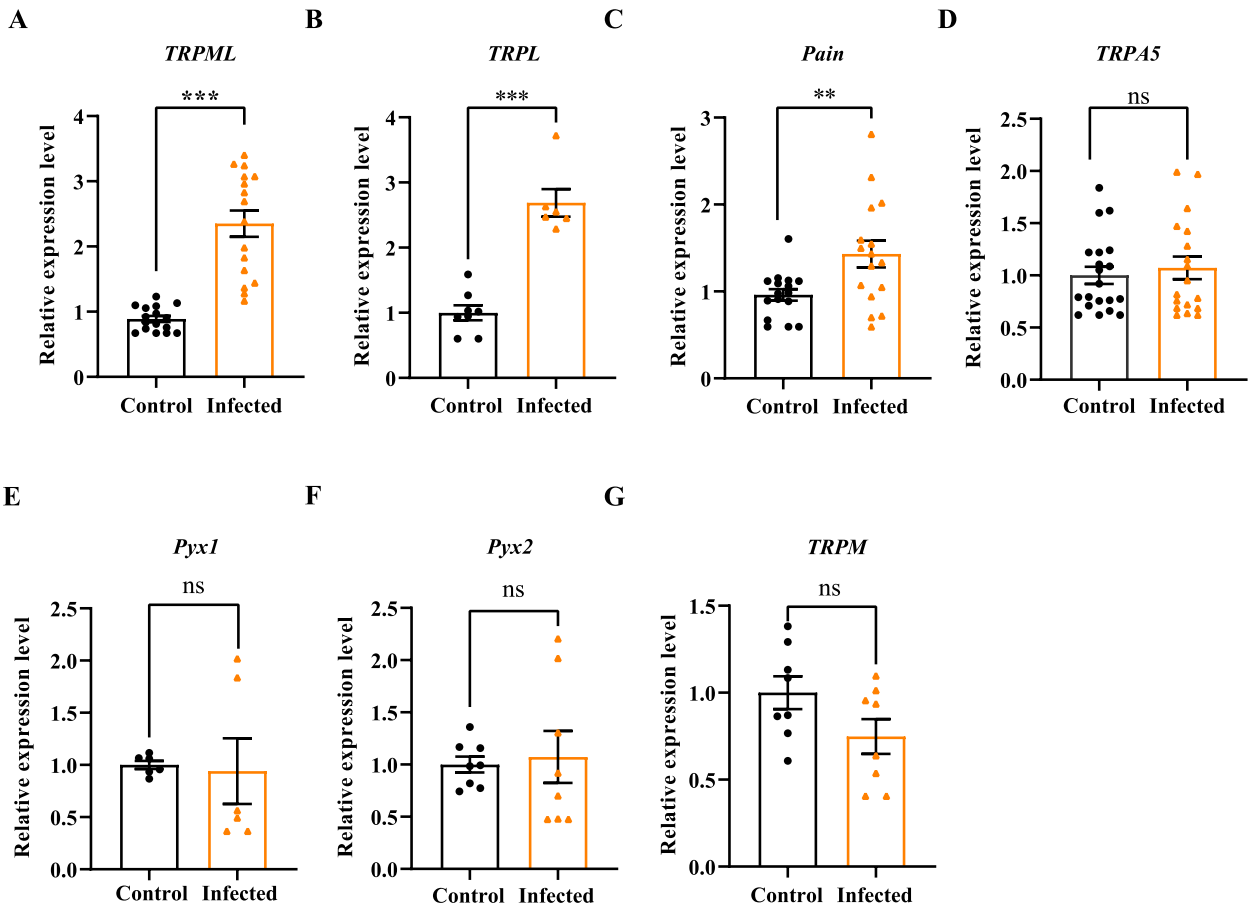

Fig. 5 The relative expression levels of TRP channels in the hemocytes larval *Spodoptera frugiperda* 24 h after injection with *Serratia marcescens*. Error bars represent standard error. "ns" indicates no significant difference, while asterisks indicate values significantly different from control values determined from unpaired *t* -test (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$)

after injecting the bacteria, no significant differences in the expression levels of these genes were observed compared to WT controls (Fig. 5D). Furthermore, *SfruPyx1* and *SfruTRPM* expression levels showed no significant changes following *S. marcescens* injection (Fig. 5E–G).

Discussion

In this study, we identified 15 TRP channels in *S. frugiperda*, which were classified into six subfamilies, TRPC, TRPA, TRPN, TRPV, TRPM, and TRPML, based on structural and phylogenetic analyses. However, TRPP, considered the oldest subfamily, was not found in *S. frugiperda*, suggesting functional compensation by other TRP channels or alternative pathways in this insect. Among all TRP channels subfamilies, TRPA exhibits the highest diversity in arthropods [42], consistent with our finding that the TRPA subfamily in *S. frugiperda* has more members than other TRP subfamilies. In addition, the gene encoding TRPA5 is widely found in Lepidoptera, Isoptera, and Blattodea [17], although its function has not been reported yet. In this study, the gene encoding TRPA5 was detected in *S. frugiperda*, along with two *Wtrw* genes (*SfruWtrw1* and *SfruWtrw2*). Multiple Wtrw transcripts are widely present only in the Lepidoptera, which may be related to their specific habitats and life histories [17].

Except for *SfruTRPM* and *SfruTRPML*, which were almost exclusively expressed in egg, all TRP channels in this study were detected across all developmental stages of *S. frugiperda*. These results indicate that different TRP channels may be involved in distinct physiological processes, offering opportunities for the development of insecticides targeting specific life cycle stages. *S. frugiperda* TRPM was only highly expressed during the egg stage and was almost undetectable in other tissues. This situation also occurs in the *Drosophila* and *B. dorsalis*, where this gene is crucial for $Mg^{2+}$and Zn^{2+} homeostasis [43, 44]. Unlike other *TRPML* genes, which are highly expressed in the gut and MT [16, 17], *S. frugiperda* TRPML was not detected in these tissues, but like TRPM,

it was highly expressed during the egg stage. Hence, this gene may be necessary for the early development of *S. frugiperda*.

Studies in *Drosophila* have shown that TRP and TRPL channels are involved in phototransduction [1, 45]. *SfruTRP*, *SfruTRPL*, and *SfruTRPγ* were expressed at high levels in the compound eyes, implying that they are important for photoreception. In addition to compound eyes, we detected *SfruTRPγ* in the mouthparts, antennae, and legs of *S. frugiperda*. In other insects, TRPγ is also highly expressed in these tissues, and participates in coordinated locomotion, with insects lacking *DmTRPγ* unable to properly coordinate leg locomotion [17, 46]. The proprioceptive neurons are distributed in the appendage joints of *Drosophila*, including the legs and wings [47]. TRPA1 is involved in temperature sensation and avoidance of noxious heat, aversive odorants and tastants, non-volatile irritants, strong lights, and mechanical stimuli [48]. The presence of olfactory, auditory, and gravitational sensory organs in insect antennae [48], along with the high expression of TRPA1 in *S. frugiperda* antennae, suggests that TRPA1 may contribute to these functions. Similar to *Pieris rapae* [17], the Pain channel is widely distributed in *S. frugiperda*, which may be important for the sensation of gravity, as well as the avoidance of noxious heat, mechanical stimulation, and dry environments [48]. *Pyx* is involved in the gravity sensing in *Drosophila* [6], while Wtrw detects dry air and mosquito repellent [12, 49]. The widespread distribution of taste receptor neurons in the labellum, wings, legs, and ovipositors of female *Drosophila* contributes to food selection and aversion behaviors [50], which is consistent with our findings of relatively high levels of *SfruPyx2* and *SfruNompC* expression in the mouthparts of *S. frugiperda*. The dominant expression of *Nan* and *Iav* in antennae has been previously reported [11]. We also observed higher expression of these genes in *S. frugiperda* gut samples. Nan and Iav may play a critical role in gravity, sound sensation, and feeding in *S. frugiperda*. Pymetrozine and pyrifluquinazon disrupt mechanosensation and chordotonal organ functions by activating the Nan-Iav channel complex, thereby impairing gravity perception, sound perception, and feeding behavior in insects [11, 48]. Previous studies have shown that *TRPM* channels mediate the repellent responses of *Tribolium castaneum* to l-menthol and MPD. These findings suggest that these TRP channels may play a key role in insect perception and response to insecticides [13]. The distribution of *SfruTRPM* in *S. frugiperda* antennae and mouthparts suggests a similar role in this insect. Our data show high expression of *SfruTRPM* in *S. frugiperda* MT, suggesting a functional similarity to *D. melanogaster*. *Drosophila* TRPM impacts noxious cold sensation and gentle touch mechanical sensation [51], and we found that it is highly expressed in the mouthparts and compound eyes of *S. frugiperda*. These genes play a role in temperature perception and avoidance of harmful stimuli. Therefore, inhibitors targeting these channels can affect the response capability of the *S. frugiperda* to environmental changes, thereby reducing its adaptability.

Regarding larval tissues, our data showed that several TRP channels (*SfruTRP*, *SfruTPRγ*, *SfruTRPA1*, *SfruPain*, *SfruWtrw1*, *SfruNompC*, and *SfruIav*) were detected in the CNS. We hypothesize that these genes play significant roles in sensory perception, motor control, behavior regulation, endocrine modulation, as well as learning and memory in *S. frugiperda* larvae. TRPM is essential for Mg^{2+} and Zn^{2+} homeostasis, and it has been reported that knocking out this channel in *D. melanogaster* leads to shortened MT and results in larval growth arrest during the larval stage [44]. Furthermore, TRPML is involved in locomotion, autophagy, and apoptotic cell clearance [48]. *SfruTPRL*, *SfruWtrw2*, *SfruTRPM* and *SfruTRPML* are highly expressed in MT, suggesting functional roles in this tissue. Although *SfruTPRA5* and *SfruPyx2* are highly expressed in blood cells, their functions have been rarely studied.

In our study, transcript levels of *SfruTRPML*, *SfruTRPL*, and *SfruPain* were found to be significantly higher in *S. frugiperda* after being infected by the Gram-negative bacterium, *S. marcescens*, suggesting their involvement in immune response functions. Similar immune-related expression of *SfruTRPML* has also been observed in *Drosophila*, suggesting that expression of TRPML in hemocytes is essential for antibacterial immune responses [52]. TRPL belongs to the TRPC subfamily and is mainly involved in photoreception transduction in insects, which has rarely been reported in the context of insect immunity. However, TRPC subfamily molecules contribute to Ca^{2+} signaling in immune cells of other organisms [53]. Other TRP channel subfamilies have a wide range of functional roles in inflammation and immunity. TRPM2 channels are widely expressed in immune cells [54], where they are essential for regulating cation balance in inflammatory environments [55]. TRPV4, a member of the vanilloid (TRPV) subfamily of TRP channels, forms a widely expressed mechanosensitive channel which can also be found in many (innate) immune cells. It plays a regulatory role in increasing proinflammatory cytokine expression induced by bacterial lipopolysaccharides [56, 57]. Collectively, this study identifies three *TRP* genes activated by *S. marcescens*, providing preliminary evidence for their potential as target genes for insecticides. However, other members did not exhibit a response regarding immune function; these genes may still play significant roles in additional

important physiological processes requiring further investigation. Therefore, they remain viable research targets for developing insecticides.

Conclusions

In summary, the gene expression results suggest that TRP channels may be involved in immune responses, warranting further investigation into their specific roles and mechanisms. Our study expands knowledge of these functions, as well as key sensory pathways in *S. frugiperda*, laying the foundation for new strategies to control these important pest insects.

Materials and methods

Insects

S. frugiperda larvae used in this study were collected from Qilin North Farm, South China Agricultural University, and raised in an incubator. The insects had been cultured for more than 10 generations. Individual *S. frugiperda* were reared at 26 °C ± 1℃ and 75% ± 1% relative humidity, with a photoperiod cycle of 16-h light and 8-h dark. Collected eggs were soaked in a 5% formaldehyde solution for 20 min, rinsed with water for 5 min, dried, and placed in a 90 mm petri dishes sealed with film. Hatched larvae were fed an artificial diet described by Li et al. [58]. Then, 3rd to 5th instar larvae were transferred to a new 150 mm petri dish, with approximately 10 larvae per dish. Fresh artificial feed, composed of bean powder 100 g, wheat bran 80 g, yeast powder 26 g, casein 8 g, ascorbic acid 8 g, distilled water 500 mL, agar 26 g, choline chloride 1 g, sorbic acid 1 g, inositol 0.2 g, streptomycin 0.1 g, penicillin sodium 0.1 g, and propylparaben 2 g, was replaced daily, and feces and dead insects in the culture dishes were cleaned. After pupation, pupae were housed in homemade paper tubes (diameter: 85 mm; height: 150 mm) until adults emerged. Adults were provided with honey water (approximately 5%). Egg mass collection began 2 days after successful adult mating.

Identification of TRP channels

The methods used to identify TRP channels in various species were similar to those reported in our previous study [16]. To search exhaustively all TRP genes in each species, we screened several types of databases including assembled genomes, reference sequence (RefSeq) database from National Center for Biotechnology Information (NCBI) (https://www.ncbi.nlm.nih.gov/refseq/) and transcriptomic data acquired from NCBI Sequence Read Archive (SRA) Databases (https://www.ncbi.nlm.nih.gov/genbank/tsa/). We obtained the genome data of *Spodoptera frugiperda*, *Bombyx mori*, *Tribolium castaneum* from Silkworm Genome database (http://silkworm.genomics.org.cn/), and Beetlebase (http://www.beetlebase.org/) respectively. Firstly, candidate *S. frugiperda* TRP genes were identified by TBLASTN searches against genome and transcriptomes with an E-value cutoff of $1e^{-5}$, using known TRP protein sequences of *D. melanogaster*. Then, candidate genes were further verified using BLASTP versus non-redundant NCBI protein sequences without species limits and with a cut-off e-value of $1e^{-5}$. The same procedure was used to identify TRP genes of *B. mori*, *T. castaneum* by a homology-based approach. Transmembrane segments were predicted using TMHMM 2.0 (http://www.cbs.dtu.dk/services/TMHMM-2.0/) and SMART (http://smart.embl-heidelberg.de/). Multiple alignments of complete amino acid sequences were performed with ClustalW2 (http://www.ebi.ac.uk/Tools/msa/clustalw2/), and the results were displayed using BioEdit (https://bioedit.software.informer.com/). Phylogenetic trees and molecular evolutionary analyses were performed using the maximum likelihood method in MEGA 6.06 software with 1000 bootstrap replicates.

Real-time quantitative PCR (RT-qPCR) analysis

S. frugiperda is a holometabolous insect, and its developmental stages include the egg, larva, pupa, and adult phases. To study the spatiotemporal distribution of TRP channels in *S. frugiperda*, samples were collected from various developmental stages, including eggs (1 to 2 h), larvae (1 st, 2nd, 3rd, 4th, and 5th instar), pupae (male: female ratio: 1:1), immature adult male and female insects (1-day-old), and mature adult male and female insects (3-day-old). In addition, we selected the important tissues required for insects to perceive environmental stimuli and maintain internal balance. Tissues, including mouthparts, antenna, compound eyes, legs, harpagones, ovipositors, hemocytes, central nervous system (CNS), midgut, fat body, and Malpighian tubules (MT) were dissected from equal numbers of male and female 3-day-old adults. Temporal distribution of gene expression was analyzed using pooled samples of eggs ($n = 100$ per pool); 1 st ($n = 20$), 2nd ($n = 10$), 3rd ($n = 5$), 4 th ($n = 3$), 5 th ($n = 3$), and 6 th ($n = 3$) instar larvae; pupae ($n = 3$); and single 1-day-old (immature adult) and 3-day-old (mature adult) male and female adult insects. Tissue distribution analysis was performed with 20 adult tissue samples included in each pool. At least three biological replicates were carried out for each experiment.

RNA was extracted using TRIzol reagent (Invitrogen, Carlsbad, CA, USA) and RNA quantities measured using a Nanodrop 2000 spectrophotometer (Thermo Scientific Inc., Bremen, Germany). Reverse transcription was then performed with 1 μg RNA samples using TransScript one-step gDNA removal and cDNA Synthesis SuperMix (TransGen Biotech, China). Synthesized cDNA served as a template for RT-qPCR, which was performed on

a Stratagene Mx3000P thermal cycler (Agilent Technologies, Wilmington, DE). Each reaction mixture contained 5 μL TB Green Premix Ex Taq II (Tli RNase H Plus) (TaKaRa Bio, Otsu, Japan), 0.4 μL of each primer (0.2 μM), and 0.8 μL of template cDNA, with sterile distilled water added to a final volume of 10 μL. Thermal cycling conditions were: 30 s at 95 °C, then 40 cycles of 95 °C for 5 s and 60 °C for 34 s. Three sample replicates were performed for each group, and no-template negative controls were included in each run, to detect possible contamination or carryover. A series of gene-specific primers were designed for RT-qPCR using the software, Primer 3 (http://bioinfo.ut.ee/primer3-0.4.0/) (Additional file 1: Table S1). Primers were used to investigate the relative expression levels of selected samples and melting curve analysis was performed between 60 °C to 95 °C for all reactions, to ensure the specificity and consistency of generated products (Fig S4). The specificity of all RT-qPCR reaction products was further established via electrophoresis on 1.0% agarose gel prior to sequencing. All experiments were performed independently at least twice, to ensure their reliability and reproducibility. Transcript levels of different genes were quantified using the $2^{-\Delta\Delta CT}$ method [59], with *SfruRPS3 A* and *SfruL17*, commonly used reference genes in the *S. marcescens*, serving as normalization genes.

Microbial infection by injection

We selected the well-recognized model pathogen, *S. marcescens*, which is widely used in the study of insect immune responses. Fifth instar larvae were dehydrated for 24 h without food and then injected with *S. marcescens* cultured in LB medium at 37 °C with shaking (200 rpm). Bacterial cultures were harvested at an optical density at 600 nm (OD600) of 1.0, corresponding to approximately 5×10^8 colony-forming units per mL. Bacterial cultures (250 mL) were pelleted by centrifugation (10 min, 4000 g) and washed twice with phosphate buffered saline (PBS). For larval systemic infection, bacterial pellets were resuspended in PBS and adjusted to OD600 = 0.025 and 1 μL bacterial solution injected into the middle part of larvae using a syringe, while larvae injected with PBS served as controls. Hemocytes were collected from larvae 24 h after injection. Pools of ten larvae were used, with three biological replicates and two independent experimental replicates conducted.

Statistical analysis

Statistical analysis of data was performed using Prism 8.0 (GraphPad Software). The *t*-test was used for unpaired comparisons between two groups of data, and one-way analysis of variance (ANOVA) followed by Tukey's multiple pairwise comparison test was applied for comparisons of three or more groups. Significance was set at $p < 0.05$. Data are presented as mean ± standard error of the mean (SEM). The heatmap analysis was performed using OmicShare tools (https://www.omicshare.com/tools/).

Supplementary Information

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Supplementary Material 1.

Supplementary Material 2.

Authors' contributions

YXQ and YYL conceived and designed the experimental plan. HAS and QXD analyzed, interpreted the experimental data, and drafted the manuscript. FYT and ZYL reared insects and participated in the experiments. All authors read and approved the final manuscript.

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Data availability

All sequence data that support the findings of this study were listed in Additional file 1: Table S1.

Declarations

Ethics approval and consent to participate

The material we used in this study is a notorious agricultural pest, the fall armyworm *Spodoptera frugiperda*. The strain in this study is a wide used laboratory strain for the oriental fruit fly functional researches. Therefore, it does not involve ethical issues and not need relevant permission.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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