Functional Identification and Characterization of Leucokinin and Its Receptor in the Fall Webworm, *Hyphantria cunea*

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Neuropeptides function as central neuromodulators and circulating hormones that modulate insect behavior and physiology. Leucokinin (LK) is an intercellular signaling molecule that mediates many physiological and behavioral processes. However, the functions of LK associated with environmental stress and feeding behavior in the fall webworm, *Hyphantria cunea*, is little known. Our primary objective is to understand the function of LK and LK receptor (LKR) neuroendocrine system in *H. cunea*. In the present study, the results showed that LK/LKR are expressed at different developmental stages and in various tissues of *H. cunea*. A candidate receptor–ligand pairing for LK was identified in the larval transcriptome of *H. cunea*. In a heterologous expression system, the calcium assay was used to demonstrate that LKR is activated by HcLKs in a dose-dependent manner, with 50% effective concentration (EC$_{50}$) values of 8.44–90.44 nM. Knockdown of HcLK and HcLKR by microinjecting target-specific dsRNA leads to several effects in *H. cunea*, including feeding promotion, increase in resistance to desiccation and starvation stress, and regulation of water homeostasis. The transcript levels of HILP2 (except in the LK knockdown group), HILP5, and HILP8 increased, whereas those of HILP3, HILP4, and HILP6 decreased; HILP1, HILP2 (in the LK knockdown group), and HILP7 gene expression was not influenced after LK and LKR knockdown. Variations in mRNA expression levels in insulin-like peptide genes in the knockdown larvae suggest an essential role of these genes in survival in *H. cunea*. To our knowledge, the present study is the first comprehensive study of LK and LKR – from gene to behavior – in *H. cunea*.

Keywords: G-protein-coupled receptor, *Hyphantria cunea*, leucokinin, RNA interference, gene function

INTRODUCTION

As the central neuromodulators and circulating hormones, neuropeptides orchestrate insect behavior and physiology. The complex hormonal and neuronal regulatory mechanisms maintain the metabolic homeostasis, which balance the food intake, energy expenditure, and nutrient storage in insects (Murphy and Bloom, 2006; Baker and Thummel, 2007; Leopold and Perrimon, 2007; Woods and D’Alessio, 2008; Teleman, 2010; Dalamaga et al., 2013;
de Araujo et al., 2013; Vogt and Bruning, 2013). Mechanisms of feeding and metabolism have been explored in depth in *Drosophila melanogaster* (Baker and Thummel, 2007; Itskov and Ribeiro, 2013; Owusu-Ansah and Perrimon, 2014; Padmanabha and Baker, 2014), and it is known that food ingestion and metabolic homeostasis are mediated by several peptide hormones (Wu et al., 2003, 2005; Melcher and Pankratz, 2005; Géménard et al., 2006; Bharucha et al., 2008; Al-Anzi et al., 2010; Cognigni et al., 2011; Hergarden et al., 2012; Söderberg et al., 2012; Itskov and Ribeiro, 2013). Insect food ingestion is associated with a balance of water and ions (Coast et al., 2002; Dow and Davies, 2006; Dow, 2009). Thus, it is likely that insect diuretic hormones collaborate with the hormones released after food intake to regulate satiety, metabolism, and energy reallocation.

Kinins (leucokinins) in insects have a highly conserved C-terminal pentapeptide sequence – Phe-Xaa-Xbb-Trp-Gly-NH₂, where Xaa represents Tyr, His, Ser, or Asn; Xbb may be Ala but is generally Ser or Pro (Holman et al., 1990, 1999). Insect leucokinins (LKs) are multifunctional peptides acting as neurohormones and neurotransmitters, which regulate diuresis, sleep, metabolism, response to ionic stress, food intake, and taste responsiveness (Terhzaz et al., 1999; Radford et al., 2002; Al-Anzi et al., 2010; Cognigni et al., 2011; López-Arias et al., 2011; Kwon et al., 2016; Zandawala et al., 2018a,b; Yurgel et al., 2019). In *D. melanogaster*, LK acts *in vitro* on stellate cells of the renal tubules to trigger fluid secretion, which is produced by a small set of neurons and neurosecretory cells in the central nervous system (CNS; de Haro et al., 2010). Leucokinins aid fluid excretion by increasing the secretion of primary urine by the Malpighian tubules and contracting the hindgut. Together with insulin signaling, the LK neuropeptide regulates stress tolerance and metabolism in *D. melanogaster* (Zandawala et al., 2018a).

The fall webworm *Hyphantria cunea* Drury (Lepidoptera: Noctuidae), a worldwide forest pest that originated in North America, was first reported in China in 1979 (Rong et al., 2003; Zhang et al., 2008). To alleviate the damage caused by *H. cunea*, various control strategies have been developed, such as natural predation, microbial intervention, and insecticide usage (Beckage, 2008). Because neuropeptides are regulators of critical life processes in insects and are highly specific, they are the potential targets in the development of green insecticides. The present study aims to understand the neuroendocrine pathways regulating the key physiological processes in pest insects for screening the potential analogs. The leucokinin signaling system has been studied in several other insect species; however, localization and functional roles of leucokinin in *H. cunea* remain unknown.

In this study, we first investigated the function of the LK ligand and receptor signaling system in *H. cunea*. Subsequently, we determined the transcript levels of the LK and LK receptor (LKR) genes under starvation to examine whether this signaling system was affected by the feeding behavior of *H. cunea*. LK gene knockdown via RNAi was used to further examine the potential relationship between LK signaling and the feeding behavior of *H. cunea*. We demonstrate that LK signaling regulates starvation stress and feeding.

**MATERIALS AND METHODS**

**Insects**

*Hyphantria cunea* eggs and artificial diets were obtained from the Research Institute of Forest Ecology, Environment and Protection, Chinese Academy of Forestry (Beijing, China). Eggs were incubated at 25°C until hatching, and larvae were fed on artificial diets in 250 ml transparent plastic bottles, which were maintained at 25 ± 1°C with a 16:8 h light:dark photoperiod.

**Molecular Cloning and Plasmid Construction**

Reverse transcription PCR was initially used to validate the sequences of *H. cunea* LK and LKR transcripts from the *H. cunea* genome database. The LK and LKR genes were cloned using the following thermal conditions: 94°C for 3 min; followed by 35 cycles of 94°C for 30 s, 60°C for 30 s, and 72°C for 1 min; then a final extension at 72°C for 10 min. The PCR product was sub-cloned into pMD18-T vector (TaKaRa, Japan) and then verified sequences. The primers used for the PCR cloning of *H. cunea* leucokinin receptor (*HcLKR*) are presented in Table 1. The PCR products were directly cloned into the pcDNA-3.1-myc-His vector. The recombinant vectors were verified by sequencing.

**Analysis of LK and LKR**

The deduced amino acid sequences of LK and LKR orthologs were obtained from GenBank using BLAST searches (blastx and tblastx). Multiple alignment of the amino acid sequences was performed using the ClustalX2 program and BioEdit. A phylogenetic tree was constructed using the neighbor-joining (NJ) method in MEGA 5.0 with 1,000 bootstrap replicates (Tamura et al., 2011). Signal peptides were predicted using Signal P 4.1 Server (Nové-Josserand et al., 2002), and transmembrane domains were predicted using TMHMM server v2.02 (Sonhammer et al., 1998). The presence of N-glycosylation sites in predicted protein sequences was assessed using NetNGlyc 1.0, and the generation of sequence logos for the C-terminal motifs of LK proteins was created by Weblogo (Crooks et al., 2004).

**Cell Culture and Transfection**

The human embryonic kidney 293 (HEK293) cell line was cultivated in Dulbecco’s modified Eagle medium supplemented with 10% fetal bovine serum (FBS) and 4 mM L-glutamine (Invitrogen) at 37°C in a humidified incubator containing 5% CO₂. HEK293 cells were transfected with LKR cDNA plasmid constructs using Effectene transfection reagent (Qiagen) according to the manufacturer’s instructions. Two days after transfection,

1http://www.cbs.dtu.dk/services/NetNGlyc/
stably expressing cells were selected by the addition of 800 mg/L G418.

**Intracellular Calcium Assay**

To investigate the interaction between the LKR and LKs in *H. cunea*, the response of the LKR to chemically synthesized LKs was examined using the Ca$^{2+}$ imaging assay. A fluorescent Ca$^{2+}$-sensitive probe, Fura-4/AM (Beyotime, Shanghai, China), was used to detect the intracellular cytosolic calcium signals according to the manufacturers' instructions. In brief, HEK293 cells stably expressing LKR were washed twice with phosphate-buffered saline and were suspended at 5 × 10$^6$ cells/ml in Hanks’ balanced salt solution. The cells were then loaded with 2 μl Fura-4/AM for 20 min and washed twice with HBSS buffered medium. Then, cells were stimulated with 0.1 and 1 μM HcLKs (HcLK-1, HcLK-2, and HcLK-3) chemically synthesized by Sangon Biotech Co., Ltd. (Shanghai, China). Each 96-well plate was transferred into a Multi-Mode Microplate Reader (Varioskan Flash Beckman XL-70 F; Thermo Fisher Scientific Inc. Waltham, MA) to monitor the Fluo-4 fluorescence. The excitation wavelength was 485 nm, and fluorescence emission was detected at 520 nm. Various concentrations of receptor ligands were added when Fluo-4 fluorescence had reached a stable value at 520 nm. Various concentrations of receptor ligands were added when Fluo-4 fluorescence had reached a stable value at 520 nm.

**RNA Interference**

A 463-bp dsRNA representing the *H. cunea* LK-encoding gene sequence and a 505-bp dsRNA representing the *H. cunea* LKR-encoding gene sequence were synthesized using the MEGAscript T7 high-yield transcription kit (Ambion) according to the manufacturers’ protocol. The dsRNA was purified with phenol/chloroform followed by ethanol precipitation. The dsRNA of the enhanced green fluorescent protein gene (pEGFP-N1 plasmid as template, WP_031943942.1, 507-bp dsRNA) was microinjected into the penultimate posterior abdominal section of individual seventh instar *H. cunea* larvae using an injection needle (MICROLITERTM #65 with 33-gauge needle, Hamilton Co., Reno, NV, United States) under ice anesthesia (Sun et al., 2016). Control *H. cunea* larvae were microinjected with the EGFP dsRNA. Microinjected *H. cunea* larvae were allowed to recover for 2h at room temperature and then reared on an artificial diet under a 16:8 h light: dark photoperiod at 25 ± 1°C. After 72 and 96h, LK and LKR mRNA levels in the dsRNA-treated seventh instar *H. cunea* larvae were measured by qRT-PCR technology.

**Bioassays**

To measure water content, the larvae treated with dsEGFP, dsLK, and dsLKR for 48h were dehydrated at 80°C until a constant weight. Ten *H. cunea* larvae were weighed before and after dehydration using a Mettler MT5 analytical microbalance (Columbus, OH, United States). Water content was calculated as the difference between the fresh and dry weight. Each replicate contained 10 *H. cunea* larvae, and the experiment was performed in triplicate.

To study survival under dessication and starvation, the *H. cunea* larvae treated with dsRNA were kept in empty vials or vials containing cotton ball with sterile water, respectively. Ten *H. cunea* larvae were used per replicate, and the experiment was performed in triplicate. The survival was recorded every 24h until all the *H. cunea* larvae were dead. The vials were placed in an incubator at 25 ± 1°C under normal photoperiod conditions (16:8 h light: dark).

**Food Intake Assay**

On day of the seventh instar stage, *H. cunea* larvae were microinjected with dsRNA (LK and LKR dsRNA or EGFP...
dsRNA) and then returned to transparent plastic vials and starved for 24 h. After a subsequent 4-day feeding period, the appetite of the larvae was checked by measuring the amount of artificial diet eaten by individual larvae during 24 h. The weight of the artificial diet was measured before and after *H. cunea* larva feeding. Three biological replicates were included for each experiment, and for each biological replicate, 10 *H. cunea* larvae were kept in transparent plastic vials. The vials were placed in an incubator at 25°C under normal photoperiod conditions (16:8 h light: dark).

**Quantitative Real-Time Reverse Transcription PCR**

The RNA was extracted from *H. cunea* eggs, first to seventh instar larvae, pupae, adults, and tissue samples using the RNeasy Mini Kit (Qiagen, Valencia, CA, United States). The tissues – head, silk glands, midgut, epidermis, testis, ovary, Malpighian tubules, and fat body – were collected from larvae on day 1 of the seventh instar stage. cDNA was synthesized using the total RNA (0.5 μg) and the PrimeScript® RT Reagent Kit with gDNA Eraser (Perfect Real Time, TaKaRa, Japan), according to the manufacturer’s protocol. The mRNA levels of *LK*, *LKR* and insulin-like peptide (*ILP*) genes were assessed using RT-qPCR with a SYBR Green kit (Toyobo, Osaka, Japan) and MJ Opticon™ machine (Bio-Rad, Hercules, CA, United States). The reaction mixture (20 μl) was composed of SYBR Green real-time PCR Master Mix (10 μl; Toyobo), nuclease-free water (7 μl), gene-specific primers (1 μl, 0.5 μM; Table 1), and cDNA template (2 μl; equivalent to 50 ng of total RNA). RPL13 and EF-1α were used as internal reference genes (Sun et al., 2019). The conditions for RT-qPCR reactions were as follows: 1 cycle at 95°C for 30 s, followed by 45 cycles at 95°C for 12 s, 60°C for 30 s, 72°C for 40 s, and 82°C for 1 s for plate reading. The purity of the amplified products was analyzed by melting curve analysis. qRT-PCR was performed in using independent biological repeats in triplicate to ensure the reproducibility of the results. The expression levels of the clones were calculated using the $2^{-ΔΔCt}$ method (Livak and Schmittgen, 2001).

**Statistical Analysis**

Statistical analysis was performed using SPSS (v17.0, SPSS Inc., Chicago, Illinois). One-way ANOVA was performed using Prism 8.0 (GraphPad Software, La Jolla, CA, United States). Value of $p < 0.05$ was considered to indicate statistical significance for all experiments performed in the present study.

**RESULTS**

**HcLK and HcLKR Analyses**

The sequences of *LK* and *LKR* genes were identified using transcriptome and genome analysis (Sun et al., 2019; Wu et al., 2019). The *LK* gene contains a 1,014 bp open reading frame (ORF), which encodes a signal peptide (23 residues). The three mature peptide sequences comprise six (YFSWPWamide, HcLK-1), seven (VRFSPWamide, HcLK-2), and eight (KVKFSAWGamide, HcLK-3) amino acid residues, respectively. The mature peptide cleavage site is a combination of lysine (K) and arginine (R) and has an amidation site “G” (Figure 1A). LK proteins from *H. cunea* and other insects showed very high sequence similarity (Figure 1B). Phylogenetic analysis revealed that HcLK and LKs from other insect species were clustered in a single group and that HcLK is most closely related to the *Danaus plexippus* plexippus homologs (Figure 1C).

The full-length HcLKR cDNA consists of 2,186 nucleotides; the predicted ORF encodes 485 amino acids (Figure 2A). The ORF contains an ATG initiation codon, an upstream 608 bp 5' untranslated region (UTR), and a termination (TAA) codon followed by a 120 bp 3' UTR (Figure 2A). The HcLKR protein contains the characteristic seven transmembrane domains (TM, Figure 1B, TMHMM 2.0 server), with a typical signature of rhodopsin-like G protein-coupled receptor (GPCR; Figure 2A). The amino acid residues at positions 49–73, 82–104, 120–142, 163–179, 215–238, 266–291, and 306–331 represented TMI, TMII, TMIII, TMIV, TMV, TMVI, and TMVII, respectively. The predicted three-dimensional model of HcLKR showed a characteristic structure, with seven TM segments with α-helices (TM-I to TM-VII) linked by three intracellular and three extracellular loops, an extracellular amino terminus, and an intracellular carboxy terminus (Figure 2B). Likewise, the Pfam analysis predicted seven transmembrane passes, and six conserved cysteine residues in the N-terminal extracellular domain. Six potential N-glycosylation sites were predicted for the N-terminal extracellular domain (NetNGlyc 1.0 server). Multiple amino acid sequence alignment between HcLKR and other LKRs showed high overall amino acid homology in the seven transmembrane domains (Figure 2B).

**Developmental and Tissue-Specific Expressions of HcLK and HcLKR**

The tissue-specific and developmental mRNA profiles of HcLK and HcLKR in *H. cunea* were quantified using RT-qPCR (Figure 3). Compared with that at the egg stage, the transcript level of HcLK in the first instar larvae was the highest (1.81-fold that in eggs) and that in the seventh instar larvae was the lowest (0.47-fold that in eggs). The expression level of HcLK in the hindgut was 24.45-fold of that in the head (Figures 3A,B). The expression of HcLK in silk gland, foregut, Malpighian tubules, testis, and ovary was 0.24-fold, 0.74-fold, 0.52-fold, 1.87-fold, and 0.38-fold of that in head tissue, respectively, and did not differ significantly. The HcLKR expression in the first and fifth larval stages was similar but significantly higher than that at other instar stages ($p < 0.05$, Figure 3C). Compared with that in the head, the transcript level of HcLKR in the hindgut was the highest (32.96-fold that in the head and that in the fat body was the lowest 0.0004-fold that in the head). The HcLKR expression in the epidermis, silk gland, foregut, Malpighian tubules, ovary, and testis was 0.0004–1.16-fold that in the head (Figure 3D).

**Functional Activation of HcLKR**

The ORF of the HcLKR was inserted into the expression vector pcDNA3.1-Myc-His to construct a recombinant plasmid for
stable expression. The HcLK gene encodes a 338-amino acid polypeptide (Figure 1A), which is a precursor of three LKs – LK-1–3 (Figure 4A). Notably, HEK293 cells expressing HcLKR responded to all HcLKS at a concentration of 1 μM. The dose response of LKR to LKs was further investigated (Figure 4B). Of the three tested LKs, LK-2, and LK-3 stimulated LKR at lower concentrations, with EC₅₀ values of 28.0 and 8.44 nM, respectively, whereas LK-1 showed a lower activity (EC₅₀ values: 90.44 nM).

Functions of HcLK and HcLKR by RNAi
Considering the induction of HcLK and HcLKR mRNA expression by starvation stress, we investigated whether HcLK and HcLKR gene expression in the systemic silence plays a functional role in organismal stress tolerance employing knockdown of HcLK and HcLKR via dsRNA microinjection. The HcLK and HcLKR knockdown larvae showed ~80% lower LK and LKR mRNA levels than the control dsEGFP larvae after 96 h (Figures 5A, B). Next, the survival of H. cunea RNAi larvae was investigated following desiccation and starvation stress.

Under desiccation and starvation stress, HcLK and HcLKR RNAi larvae survived longer than control larvae (Figures 5C, D). To determine whether the difference in survival rates of larvae stems from changes in water content, the water content in H. cunea larvae microinjected with dsEGFP, dsLK, and dsLKR were assayed after 48 h of desiccation treatment. As expected, H. cunea larvae with dsLK and dsLKR silencing contained more water than those in control dsEGFP group (Figure 5E).

The expression of ILP genes in H. cunea was altered in dsLK and dsLKR larvae after 48h of starvation. Significant effects on HILP transcription were observed only for HILP2 (except dsLK treatment), HILP3, HILP4, HILP5, HILP6, and HILP8. The transcript levels of HILP2, HILP5, and HILP8 in the dsLKR larvae were significantly higher (1.31–4.42-fold) than those in the dsEGFP group. However, the transcript levels of HILP3, HILP4, and HILP6 in the dsLK larvae were significantly lower (0.33–0.52-fold) than those in the dsEGFP group (Figure 6). Complex results were also observed when LKR and LK were knocked down in H. cunea larvae, the LK signal positively regulated HILP2, HILP4, and HILP6 expression but positively regulated HILP5 and HILP8 expression and played no significant regulatory role in HILP1 and HILP7 expression (Figure 6).

HcLK and HcLKR Knockdown Promoting Feeding Behavior
Our results suggested that LK signaling is associated with starvation stress. Thus, the HcLK and HcLKR knockdown mutants were found to affect food intake over different periods. The food intake of larvae microinjected with dsHcLK and dsHcLKR after starvation for 1 day was significantly different from that of larvae microinjected with dsEGFP (Figure 7). During the feeding time tested, the food intake of dsHcLK and dsHcLKR larvae was significantly higher than that of the control dsEGFP larvae. The food intake of dsHcLK and dsHcLKR larvae on the day 1 was 1.61- and 1.62-fold higher than that of the control dsEGFP larvae, respectively (Figure 7).
4 of feeding, the food intake of dsHcLK and dsHcLKR larvae was 1.26- and 1.66-fold higher than that of the control dsEGFP larvae, respectively.

**DISCUSSION**

Leukokinin, a multifunctional peptide acting as a neurohormone and neurotransmitter, is primarily synthesized in the CNS. Only a single LK gene was identified in *D. melanogaster* (Terhzaz et al., 1999). However, a single LK gene was first identified in *H. cunea*; which shares a similar typical structure of the LK family. Specifically, three putative LK proteins (LK-1–3) in *H. cunea* (HcLK-1–3) possess the general C-terminal motif sequence FxyWGamide (Veenstra et al., 1997). HcLK-1–3 showed high similarity with helicokinins 1–3 of *Helicoverpa zea*. The LKs are highly conserved between *H. cunea* and *H. zea* (Figure 1B). The HcLK genes were expressed in various tissues of *H. cunea*, especially highly expressed in the midgut and hindgut, as has been demonstrated in several insect species.
In *Grapholita molesta*, LK was predominately expressed in the gut and FB (Cheng et al., 2021), whereas in *Chilo suppressalis*, LK were predominately expressed in the CNS and gut (Xu et al., 2016). Seven transmembrane domains involved in GPCR ligand binding and receptor activation are functionally conserved in *HcLKR*, which contains amino acid motifs typical of the GPCR family (Marco et al., 2013). Moreover, the isolated *HcLKR* was highly analogous to other LK receptors in various insect species. *HcLKR* was mostly expressed in the midgut and hindgut, as previously reported in *Aedes aegypti* and *D. melanogaster* (Kwon et al., 2016; Zandawala et al., 2018a,b). This phenomenon corresponds with the main function of LK in diuresis and ion transport (Gonzalez et al., 2012). The insect hindgut is the main organ of the excretory system. The highest expression levels of *HcLK* and *HcLKR* genes in the hindgut suggest a conserved function of the LK signaling system in the regulation of diuresis and ion transport (Coast et al., 2002; Dow and Davies, 2006; Nässel and Winther, 2010).

The intracellular Ca$^{2+}$ levels were performed to determine the binding between HcLK peptides and *HcLKR* because Ca$^{2+}$ acts as a second messenger for LKR signal transduction. Pharmacological data demonstrate that *HcLKR* was strongly activated by HcLK peptides in a concentration-dependent
manner. Our results are consistent with the previously reported pharmacological characterization of LKR in *D. melanogaster* (Terhzaz et al., 1999; Radford et al., 2002).

The LK signaling system has been demonstrated to be involved in food intake, metabolism, and stress in insects (Al-Anzi et al., 2010; Liu et al., 2015; Zandawala et al., 2018a,b). Feeding or
starvation affects the expression of LK and LKR in D. melanogaster (Zandawala et al., 2018a,b). Cannell et al. (2016) showed that, in D. melanogaster, starvation increases the epithelial LKR gene expression, and Malpighian tubule stellate cell-specific knockdown of LKR significantly reduces starvation tolerance. Zandawala et al. (2018b) showed that targeted knockdown of LKR in abdominal ganglion LK neurons using the CRISPR/Cas9 technology significantly increased starvation tolerance in D. melanogaster. LKR mutation and targeted knockdown of LKR in insulin-producing cells of Drosophila altered the expression of ILPs and increased starvation resistance (Zandawala et al., 2018b). Yurgel et al. (2019) reported that the LK neuropeptide plays an essential role in the metabolic regulation of sleep. Moreover, the activity of LK neurons is modulated by feeding; decreased activity is observed in response to glucose, whereas increased activity is observed under starvation conditions. In the present study, our results showed that LK or LKR knockdown increased the water content in H. cunea and extended survival during desiccation and starvation. Under desiccation conditions, the survival rate of H. cunea larvae was improved by deletion of LK/LKR signaling, which promotes water retention. The findings confirm that the LK signaling system plays a vital role in the regulation of water homeostasis and the resistance to desiccation and starvation. The LK likely plays a regulatory role during starvation; however, its detailed functions remain to be identified. Moreover, HcLK and HcLKR knockdown increased the transcript levels of HILP2 (except in the dsLK larvae), HILP5, and HILP8 and decreased the transcript levels of HILP3, HILP4, and HILP6. However, HcLK and HcLKR knockdown had little effect on the transcript levels of HILP1, HILP7, and HILP2 (except in the dsLKR treatment group). The LK/LKR system in H. cunea could be used to control H. cunea by synthesizing leucokinin analogs. However, the potential regulatory role of LK and LKR in the transcription of ILPs in H. cunea needs to be further studied.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding author.

AUTHOR CONTRIBUTIONS

LS and CC designed the research and wrote the manuscript. HM, YG, and ZW performed the experiments and analyzed the data. CC revised the manuscript. All authors contributed to the article and approved the submitted version.

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