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Functions of keratin-associated protein genes in reproduction of *Nilaparvata lugens*

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ABSTRACT

As a notorious rice pest, *Nilaparvata lugens* (Stål) (Hemiptera: Delphacidae) inflicts severe damage owing to its exceptionally high reproductive potential. Previous ovarian transcriptome analyses have revealed that the insect Keratin-associated protein (*KAP*) gene is potentially implicated in vitelline secretion and eggshell sclerotization, and it holds promise as a novel target for the suppression of pest reproduction. In this study, *KAP5-2* (LOC111045913, XP_039288177.1) and *KAP5-4* (LOC111045916, XP_022187086.1) genes' function were studied by RNA interference technology. The results showed that gene silencing significantly suppressed female fecundity, and the pre-oviposition period extended from 3 days to 4 days. The total number of eggs laid within 10 days was significantly reduced, with only 113.2 and 101.3 eggs in the ds*KAP5-2* and ds*KAP5-4* groups, respectively, representing decreases of 49.3 % and 56.0 % compared to the control group. In addition, the hatching rates of offspring were significantly reduced by 60.1 % and 63.78 %, with many malformed unhatched eggs remaining. Molecular mechanism studies revealed that silencing *KAP* genes led to significant down-regulation of vitellogenin (Vg) and its receptor (VgR, vitellogenin receptor). Additionally, it affected the expression of key genes in the juvenile hormone (Juvenile Hormone Acid Methyltransferase (*JHAMT*), Methoprene-tolerant (*Met*)) and nutrient signaling pathways. This study indicates the critical role of *KAP* genes in regulating female reproductive processes and embryonic development in *N. lugens*, thereby providing a theoretical foundation for the rational design of eco-friendly insecticides targeting these *KAP* genes.

1. Introduction

The brown planthopper, *Nilaparvata lugens* (Stål), is classified under Delphacidae within the order Homoptera. This insect is globally recognized as a monophagous pest of rice (Shentu et al., 2016). It exhibits characteristics such as an extended adult lifespan, phototaxis, geotaxis, and the capability for long-distance migration. Its habitat spans across rice-growing regions in eastern, southern, and southeastern Asia, as well as in the South Pacific islands and Australia (Plh et al., 2023; Gu et al., 2024). *N. lugens* possesses characteristic piercing mouthparts, which it uses to penetrate rice stems and leaf sheaths. It then accesses the phloem to feed on sucrose-rich sap (Sōgawa, 1982; Wang et al., 2008; Shangguan et al., 2018). The insect absorbs nutrients from the vascular bundle by secreting saliva to create a protective sheath and causes symptoms such as wilting, lodging and even death of the rice plant (Beament, 1946). Additionally, the excretion of honeydew by the insect promotes mold growth on the rice plant, which further complicates the situation and poses a significant threat to rice farming in Asia (Bottrell and Schoenly, 2012). Chemical pesticides currently remain the primary method for controlling *N. lugens* populations in agriculture (Tang et al., 2022). However, this approach is associated with challenges such as pesticide residues, ecological pollution, and limited long-term effectiveness (Sun et al., 2018; Plh et al., 2023). Moreover, the emergence of resistance in *N. lugens* populations due to metabolic adaptations or target mutations resulting from improper pesticide use is a growing concern (Sun et al., 2018; Plh et al., 2023). Consequently, there is an urgent need to develop environmentally friendly, safe and targeted strategies for controlling this pest.

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The egg represents the initial stage of the insect life cycle and is notably vulnerable (Beament, 1946; Sim et al., 2023). The eggshell serves as the primary protective barrier. It typically consists of the vitelline membrane, inner chorion, and outer chorion. This structure effectively mitigates water loss and microbial intrusion while facilitating gas exchange. Certain species exhibit the capacity to produce antibacterial proteins thereby augmenting their defensive capabilities (Margaritis, 1985a, 1985b; Waring, 2000; Cavaliere et al., 2008). Vitellogenin Receptor (VgR)-mediated endocytosis transports vitellogenin (Vg) to mature oocytes in a process known as vitellogenesis, which is pivotal for insect reproduction. This process may play an important role in the development of insect ovaries and embryos (Guo et al., 2025; Pereira et al., 2025). Recent research has highlighted the significant involvement of juvenile hormone (JH), ecdysteroids, and nutritional signaling pathways in regulating vitellogenesis (Wu et al., 2021). Many female insects secrete adhesive substances around their eggs to enhance attachment to favorable environments, which increases egg survival rates (Zheng et al., 2021). Egg gelatin, a crucial substance, plays a significant role in this process. Comparative analysis of egg shell and egg gel proteins from various insect groups revealed a remarkable diversity and uniqueness in their protein sequences (Sim et al., 2023). Consequently, targeting essential proteins associated with eggshell formation is anticipated to specific and efficient management of pest populations.

Keratin-associated proteins (KAP or KRTAP) were initially identified in mammalian hair. They are essential for the formation of pliable hair alongside keratin intermediate filaments (KIF) (Khan et al., 2014). Consequently, thereby influencing the distinct characteristics of hair, fur, wool, and feathers across diverse animal species. (Litman & Stein, 2023). Despite the absence of conventional mammalian keratin and hair structures in insects, a direct ortholog of the mammalian KAP family is absent (Kumar *et al.*, 2022). Nevertheless, some insect structural proteins are rich in cysteine (Cys) or glycine (Gly). These proteins show sequence homology to mammalian KAP. They are often annotated as "keratin-associated protein-like" (Hua et al., 2016). These proteins have been identified in *Drosophila melanogaster* (Waring, 2000; Cavaliere et al., 2008), *Aedes aegypti* (Li and Li, 2006), *Blattella ger manica* (Irles & Piulachs, 2011), and *Cimex lectularius* (Sim et al., 2023). Their expression has been documented in the epidermis or ovaries of insects. Relevant studies have shown that Cimex lectularius *KAP* genes may be involved in secreting cement proteins around eggs. These genes contribute to oviposition lubrication and prevent egg desiccation. Additionally, they are likely involved in the final cross-linking of eggshell proteins (Sim et al., 2023). We also identified several cysteine-rich genes annotated as "keratin-associated protein-like" in our prior transcriptomic analysis of *N. lugens* ovary (Liu et al., 2025a). These genes exhibited significant expression changes when *TRE* or *TPS* was inhibited, with *KAP5-2* and *KAP5-4* showing notable alterations under both conditions. These genes have been preliminarily associated with eggshell structure formation, indicating a potential role in insect reproduction. However, the precise functions of these genes in N. lugens egg development and hatching remain unclear. Their overall impact on the reproductive cycle of N. lugens is also not well understood.

This study aims to employ RNAi technology to suppress the *KAP* gene in *N. lugens* females with a specific emphasis on elucidating the influence of the *KAP* gene on the reproductive processes and embryo development of this insect species (Irles & Piulachs, 2011; Mehlhorn et al., 2021). This study investigates the mechanisms of *KAP* gene-regulated reproduction and embryonic development in *N. lugens*. It assesses the gene's effects on ovary development, egg production, hatching rates, and expression of key reproductive regulatory genes. Ultimately, these findings are anticipated to furnish a theoretical foundation for the advancement of novel environmentally friendly pesticides.

2. Materials and methods

2.1. Plant and insect sources

The commonly used rice variety Taichung Native 1(TN1) was used as the host plant for the *N. lugens*, The experimental population of *N. lugens* females was continuously reared in the laboratory for over 60 generations. Both the rice plants and the *N. lugens* were maintained in an artificial climate chamber. Insects were maintained under controlled environmental conditions of 27 ± 1 °C, 65 ± 5 % relative humidity, and a photoperiod of 18:8 (L:D) h.

2.2. Determination of target genes

In the previous transcriptome studies, we found that inhibiting either *TRE* or *TPS* would affect multiple *KAP* genes (screening criteria: $|\log2(\text{FoldChange})| > 1.5$, $P < 0.05$). Among them, the two genes that were simultaneously affected were *KAP5-2* and *KAP5-4*. we selected these two genes for subsequent microinjection and further in-depth investigations of their functions and molecular mechanisms in this study.

2.3. dsRNA synthesis

Five to ten adult insects were randomly selected. Total RNA was extracted using the Trizol method following tissue disruption with an automatic sample rapid grinder. Using the extracted RNA as a template, first-strand cDNA was synthesized with the PrimeScript RT reagent Kit With gDNA Eraser (Takara, Japan). Gene-specific primers (Table 1) were employed for PCR amplification with the synthesized cDNA as the template. The PCR products were subjected to agarose gel electrophoresis for verification. After confirmation of the correct bands, the target bands were excised from the gel and purified. The purified products were then used directly as templates. In vitro transcription and dsRNA synthesis were carried out using the T7 RiboMAX™ Express RNAi System. After the completion of the reaction, DNase I was added to degrade the DNA template. Finally, the dsRNA product was purified using an appropriate purification method, quantified by spectrophotometry, and stored at − 80 °C for future use.

2.4. Microinjection of N. lugens and post-injection rearing

Fifth-instar nymphs were collected from the experimental population and reared under controlled conditions. Subsequently, newly emerged female adults were selected in accordance with the methods reported by Liu *et al.*(Liu et al., 2010; Liu et al., 2018). Under a stereomicroscope (Leica, Germany), microinjection was performed on the insects using a TransferMan 4r microinjector (Eppendorf, Hamburg, Germany) after they were anesthetized with CO_2. Three experimental injection groups were set up: the ds*GFP* group, the ds*KAP5-2* group, and the ds*KAP5-4* group, with the ds*GFP* group serving as the control. For all groups, the concentration of the injected dsRNA was 4000 ng/μL. A volume of 100 nL was injected per insect, as reported by Zhao L *et al.* (Zhao et al., 2016) and Tang B *et al.* (Tang et al., 2017). Injection sites were determined on the lateral cuticle of the thorax, between the mid and hind legs. Three independent biological replicates were conducted, with 20 insects injected in each replicate. Injected insects were reared in transparent plastic tubes under the aforementioned conditions.

2.5. Gene expression level detection

Seventy − two hours after injection, surviving female insects were randomly sampled from each experimental group. Three biological replicates were established per group, with each biological replicate consisting of three technical replicates. Total RNA was extracted using TRIZOL® reagent (Invitrogen, Carlsbad, CA, USA) in strict accordance with the manufacturer's instructions. The integrity and purity of the

Table 1
Primer sequences for dsRNA synthesis.

Gene Name	Forward Primer Sequence (5′-3′)	Reverse Primer Sequence (5′-3′)	Target Gene
ds*KAP5-4*	CTTTTCGCTGCTTTCTTCGC	GCTCCTTGATGATGGTGGTGT	LOC111045916
ds*KAP5-2*	CCCGCCTCAAAATTCCTC	AGCCACAACCACCACAACC	LOC111045913
ds*GFP*	AAGGGCGAGGAGCTGTTCACCG	CAGCAGGACCATGTGATCGCGC	U55763.1
T7 promoter	GGATCCTAATACGACTCACTATAGG		/

RNA samples were evaluated using 1.2 % agarose gel electrophoresis and a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). Genomic DNA removal and first-strand cDNA synthesis were then carried out using the PrimeScript™ RT reagent Kit with gDNA Eraser (TaKaRa, Kyoto, Japan). With the synthesized cDNA as the template and Actin as the internal reference gene (Jiang et al., 2015), RT − qPCR amplification was carried out (Liang et al., 2020; Tang et al., 2025b). RT-qPCR reactions were conducted using TB Green® Premix Ex Taq™ (TaKaRa, Kyoto, Japan) on a Bio − Rad CFX96™ real − time PCR system (Bio − Rad, Hercules, CA, USA). The reaction mixture was composed of 5 μL TB Green Premix Ex Taq, 0.4 μL each of forward and reverse primers, 1 μL cDNA template, and ddH_2O to make up a final volume of 10 μL. The relative expression levels of the target genes (*Vg*, *VgR*, and key genes in the reproductive regulation network) in the surviving female insects were determined using the $2^{-\Delta\Delta CT}$ method(Livak and Schmittgen, 2001).

2.6. Assessment of reproductive capacity in N. lugens

Injected female insects were mated with untreated males at a 1:2 (female:male) ratio and cultured in feeding tubes. Fifteen to thirty pairs were established per treatment group. The pre-oviposition period, oviposition period, total number of eggs laid, and female longevity were monitored and documented until death. Ten days after the onset of oviposition, rice seedlings containing eggs were extracted and cultured separately. The number of hatched nymphs and unhatched eggs was tallied to precisely calculate the overall egg hatch rate. Additionally, unhatched egg phenotypes were documented under a stereomicroscope on day 10 post-oviposition. Additionally, on day 3 post-injection, females were subjected to dissection to observe and photograph intact ovaries. Nine female adults were randomly sampled from each group for ovarian dissection. Imaging was carried out with a Leica EZ4 HD stereomicroscope (20 × magnification) using LAS EZ software. Ovarian developmental stages were classified according to the grading criteria proposed by Lu *et al.* (Lu et al., 2011). Based on oocyte maturity, color, and number within the ovarioles, the adult ovaries were classified into five distinct stages: milky transparent (Grade I), vitellogenic (Grade II), mature (Grade III), egg-laying (Grade IV), and late egg-laying (Grade V).

2.7. Statistical analysis

Before statistical analysis, all measured data were evaluated for normality and homogeneity of variances. Experimental data are reported as mean ± standard error of the mean (mean ± SEM) from three independent biological replicates. Statistical analyses were conducted using IBM SPSS Statistics 20 software. Differences between groups were assessed for statistical significance using Student's t − test ($P < 0.05$ was considered statistically significant, denoted by*; $P < 0.01$ was considered highly statistically significant, denoted by**). All experimental groups were individually compared with the control group. Herein, the significance level was set at $\alpha = 0.05$. Graphs were generated using GraphPad Prism 10.

3. Results

3.1. Inhibition of target gene expression by RNAi

According to our previous transcriptome data, silencing either *TPS* or *TRE* genes led to a significant downregulation of *KAP5-2* and *KAP5-4* expression (screening criteria: $|\log_2(\text{FoldChange})| > 1.5$, $P < 0.05$). This suggests that the trehalose metabolism pathway may participate in insect cuticle development by regulating the expression of cuticular structural genes. To test this hypothesis, we applied validamycin treatment, which resulted in a significant upregulation of *KAP5-4* expression, further supporting its regulation by trehalose metabolic signals. Based on these findings, we selected differentially expressed *KAP* genes, including *KAP5-2* and *KAP5-4*, for subsequent functional investigation (Fig. 1A-C). To validate their functions, we silenced *KAP5-2* and *KAP5-4* separately by microinjecting gene-specific ds*RNA* into female adults of the brown planthopper (Fig. 1D, E). At 72 h post-injection, the silencing efficiencies for the target genes reached 92.5 % and 90.3 %, respectively, showing a significant difference compared to the ds*GFP*-injected control group. This confirmed the success of the RNAi experiment, thereby enabling subsequent phenotypic analysis.

3.2. Effect of dsKAP treatment on the development of N. lugens ovaries

The ovaries of *N. lugens* females were sampled 3 days (72 h) after the injection for morphological observation, ovary grading and the number of mature egg grains. Morphological observation of the ovaries of *N. lugens* females revealed that, compared with the control group, the ds*KAP5-2* and ds*KAP5-4* groups exhibited an overall developmental delay, a decreased number of ovarian tubules, and a reduction in the number of egg grains (Fig. 2A). The results of ovarian grading and the number of mature egg grains were consistent with the morphological observation that on day 3 after dsRNA injection, most of the ovaries in the ds*GFP* group entered the stage of Grade III, and most of the ovaries in the two treatment groups were in the stage of Grade I and II (Fig. 2B). In addition, on day 3 after dsRNA injection, the number of mature oocytes in the ds*KAP5-2* and ds*KAP5-4* groups decreased by 58.92 % and 72.03 %, respectively (Fig. 2C); the relative expression of *Vg* gene and *VgR* gene was also extremely significantly reduced. The relative expression of *Vg* and *VgR* genes also decreased significantly (Fig. 2D, E). This indicates that the silencing of *KAP* genes significantly decreased the number of mature eggs in the ovaries of *N. lugens* females, which in turn inhibited the reproduction of *N. lugens*.

3.3. Statistics of N. lugens preoviposition period and egg production after dsKAP treatment

After the injection of ds*GFP*, ds*KAP5-2* and *dsKAP5-4*, the preoviposition period of *N. lugens* in each group were about 3d, and 4d (Fig. 3A), which was significantly prolonged in the experimental group compared with the control group ($P < 0.05$), which corresponded to the development of the ovary. The mean total egg production for the ds*GFP*, ds*KAP5-2*, and ds*KAP5-4* treatment group was 223.1, 113.2, and 101.3, respectively (Fig. 3B). These results suggest a significantly reduced total egg production in the *N. lugens* treated with ds*KAP5-2* and ds*KAP5-4* compared to the ds*GFP* group ($P < 0.01$), by 49.3 % and 56.0 %, respectively.

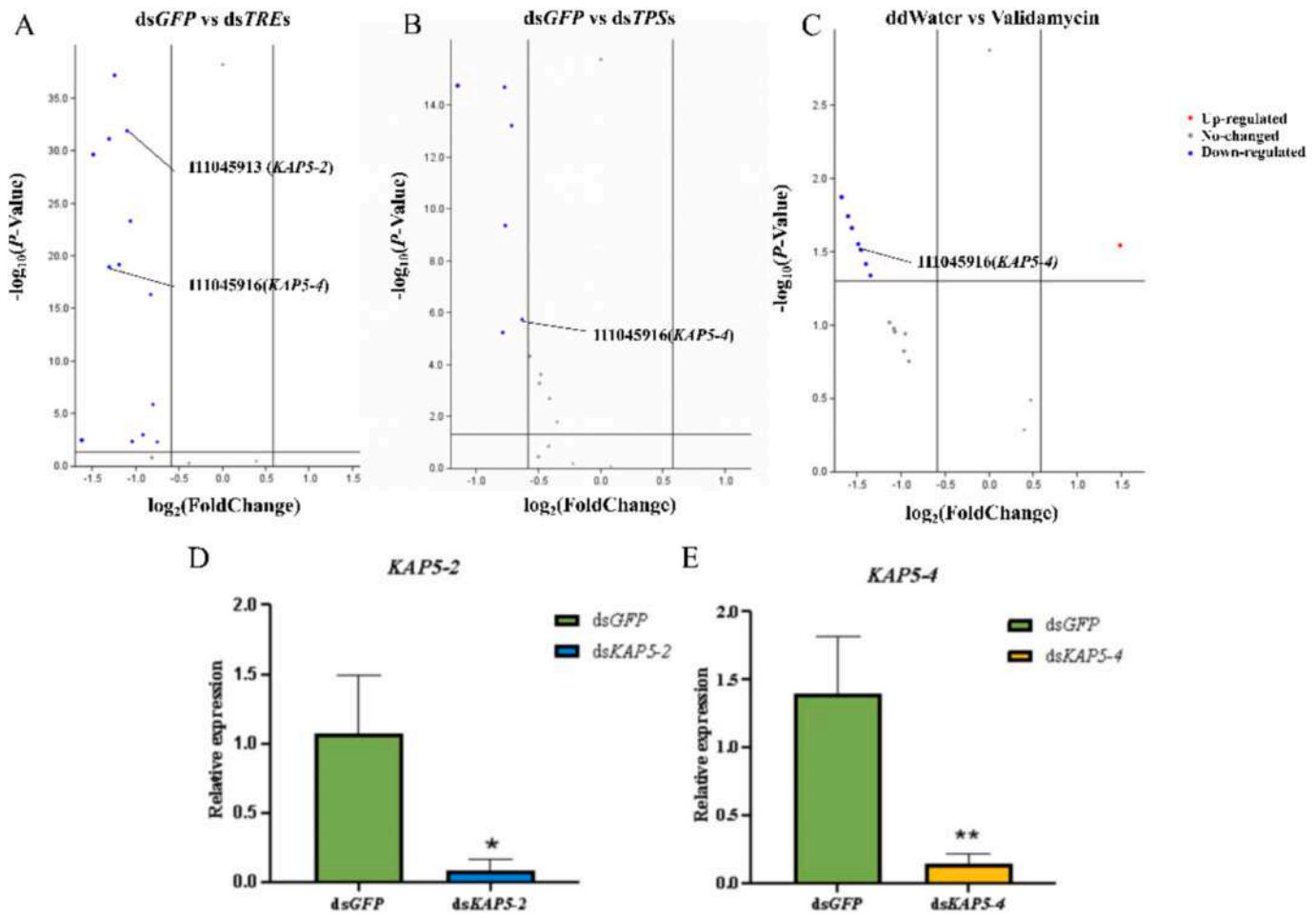

Fig. 1. Screening of *KAP* genes and validation of RNAi silencing efficiency. In transcriptomic analysis of *N. lugens* ovaries under conditions of *TRE* and *TPS* inhibition, reproduction-associated *KAP* genes were identified. Comparisons are shown between the following groups: (A) ds*GFP* vs. ds*TREs*, (B) ds*GFP* vs. ds*TPSs*, and (C) dd (control) vs. Validamycin. (D, E) Validation of the RNAi silencing efficiency for (D) *KAP5-2* and (E) *KAP5-4* (n = 3). Data are presented as mean ± SD. Statistical significance was determined by an independent-samples *t*-test, and asterisks represent comparisons with the dsGFP control group. "*", $P < 0.05$; "**", $P < 0.01$.

3.4. Hatching of N. lugens after dsKAP treatment

Compared with the control group, *N. lugens* first-fledged females treated with ds*KAP5-2* and ds*KAP5-4* showed a highly significant decrease ($P < 0.01$) in the hatching rate of the offspring, with a decrease of 60.1 % in the ds*KAP5-2* group; and a decrease of 63.78 % in the ds*KAP5-4* group (Fig. 4A), as well as the occurrence of deformities such as the inability to hatch in time during the egg incubation process (Fig. 4B). The experimental data indicated that ds*KAP5-2* and ds*KAP5-4* could effectively reduce the hatching rate of *N. lugens*.

3.5. RT-qPCR detection of relative expression of key genes of the N. lugens reproduction regulatory network after dsKAP treatment

The effect of RNAi was tested by taking the *N. lugens* females on the 3rd day after the injection of ds*KAP5-2* and ds*KAP5-4*. According to the results of RT-qPCR, the relative expression of two reproduction-related genes, *JHAMT* and *Met*, decreased significantly ($P < 0.05$) after injection of ds*KAP5-2* into female *N. lugens* compared with that of the ds*GFP* group (Fig. 5E, F); the relative expression of two reproduction-related genes, *InR2* and *Met*, decreased significantly ($P < 0.05$) after ds*KAP5-4* treatment (Fig. 5B, F); the relative expression levels of the remaining genes in the experimental group did not change significantly, which are consistent with the previous results of reduced ovary development and egg production.

4. Discussion

N. lugens is a notorious pest that severely impacts rice production, posing a grave threat to global food security (Lu et al., 2013; Wei et al., 2024). In recent years, RNA interference (RNAi) technology has emerged as a promising tool for pest control due to its various advantages, with the key determinant being the selection of target genes (Irles & Piulachs, 2011; Sim et al., 2023; Niebres and Alviar, 2023; Silva et al., 2024; Toprak, 2025). In the previous transcriptome studies, we found that inhibiting either *TRE* or *TPS* would affect multiple *KAP* genes (screening criteria: $|\log2(\text{FoldChange})| > 1.5$, $P < 0.05$). Among them, the two genes that were simultaneously affected were *KAP5-2* and *KAP5-4*. we selected these two genes for subsequent microinjection and further in-depth investigations of their functions and molecular mechanisms in this study.(Liu et al., 2025a). In this study, the silencing of the *KAP5-2* and *KAP5-4* genes in *N. lugens* via RNAi exerted a significant impact on ovarian development. Phenotypic analysis disclosed delayed ovarian development, a reduced number of ovarioles, and a significant decrease in the number of mature eggs after gene silencing. Thus, these results indicate that *KAP* genes play a crucial role in normal ovarian development. Considering that insect egg maturation requires the synthesis and deposition of structural eggshell proteins (Lu et al., 2022), and that KAP proteins are typically cysteine-rich — implying potential cross-linking capacity and structural functions (Hua et al., 2016; Sim et al., 2023) — we postulate that silencing *KAP* genes may disrupt the synthesis or assembly of eggshell proteins, thereby impairing normal egg

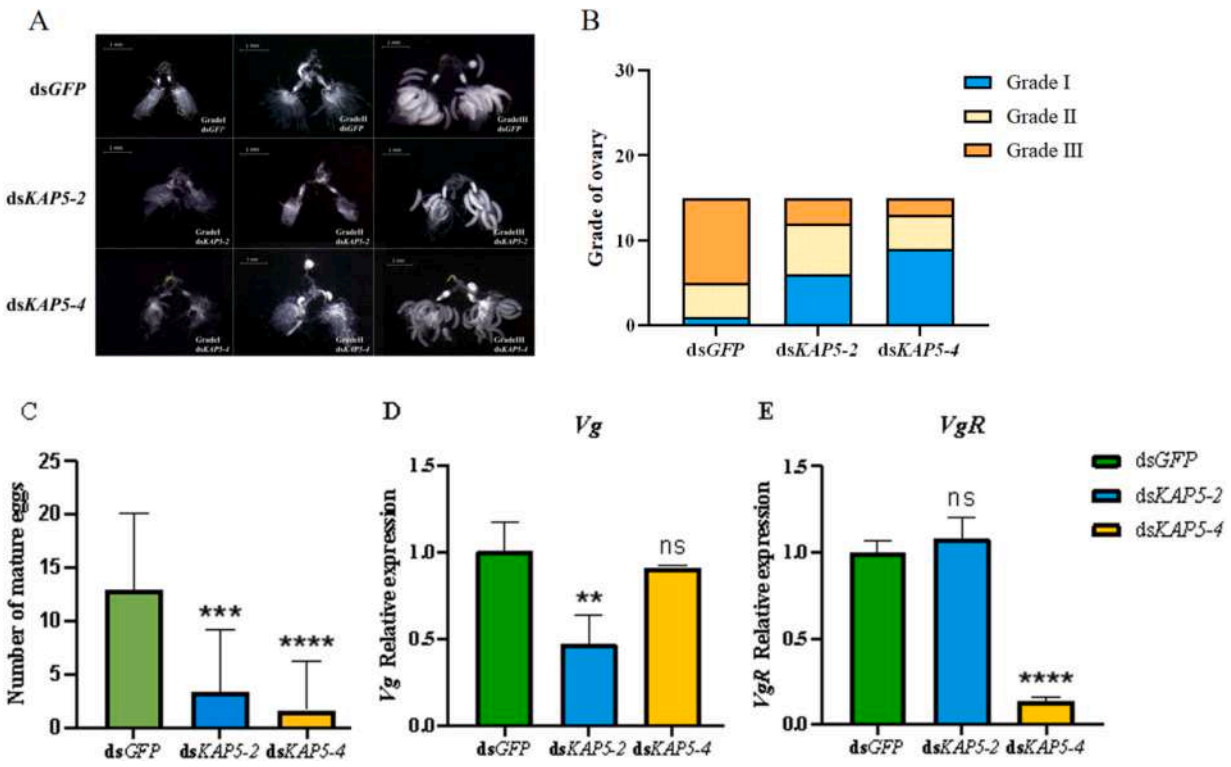

Fig. 2. Effects of ds*KAP* treatment on ovarian development in *N. lugens*. Morphology of Class I-III ovaries at day 3 post-injection (16x magnification) (A). Statistical analysis of ovary grading at day 3 post-injection (n = 15) (B). The number of mature eggs per female at day 3 post-injection (n = 13) (C). Relative expression levels of the *Vg* (D) and *VgR* (E) genes in females at day 3 post-injection (n ≥ 3). Differences were analyzed by an independent-samples *t*-test, and all significance levels denote comparisons with the dsGFP group. "ns" means no significant difference between the data of this group and the control group. "**", $P < 0.01$; "****", $P < 0.0001$.

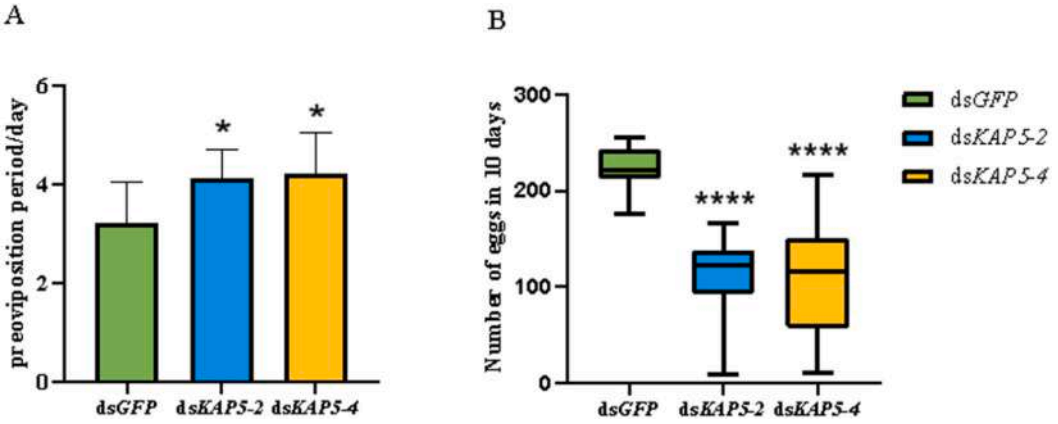

Fig. 3. Effects of ds*KAP* treatment on the preoviposition period and total number of eggs in 10 days of *N. lugens*. The preoviposition period (A) and total number of eggs laid within 10 days (B) post-injection are shown (n = 9). Statistical significance was assessed using an independent-samples *t*-test relative to the dsGFP control group. "*", $P < 0.05$; "****", $P < 0.0001$.

development and maturation (Wang et al., 2025).

Moreover, silencing *KAP* genes markedly prolonged the preoviposition period and reduced the total number of eggs laid within 10 days, suggesting their potential role in regulating reproductive behavior. This result aligns with findings in *Cimex lectularius*, where gene knockdown of the *KAP5-10L* gene also led to a significant decrease in fecundity (Sim et al., 2023). Silencing *KAP* genes also markedly decreased the hatching rate of offspring. In a similar vein, gene knockdown of *KAP5-10L* in *Cimex lectularius* reduced egg hatchability, with unhatched eggs displaying shrunken and malformed phenotypes. It is postulated that this defect could originate from impaired secretion of egg glue proteins, leading to egg desiccation and disruption of embryogenesis (Pan et al., 2023). We additionally observed abnormal embryonic phenotypes during hatching in *KAP*-silenced *N. lugens*; however, the specific mechanisms require further investigation. Taken together, these data suggest that *KAP* genes are potentially involved not only in ovarian development and oogenesis but also in embryonic development and eggshell function. Evidence in support of their functional diversity includes the involvement of the keratin-associated protein gene *KAP19-2* in phosphine resistance and developmental regulation in *Tribolium castaneum*, and the potential role of KAP proteins in facilitating microbial colonization at wound sites in arthropods (Che et al., 2021), suggesting that *KAP* may influence insect physiology through multiple pathways.

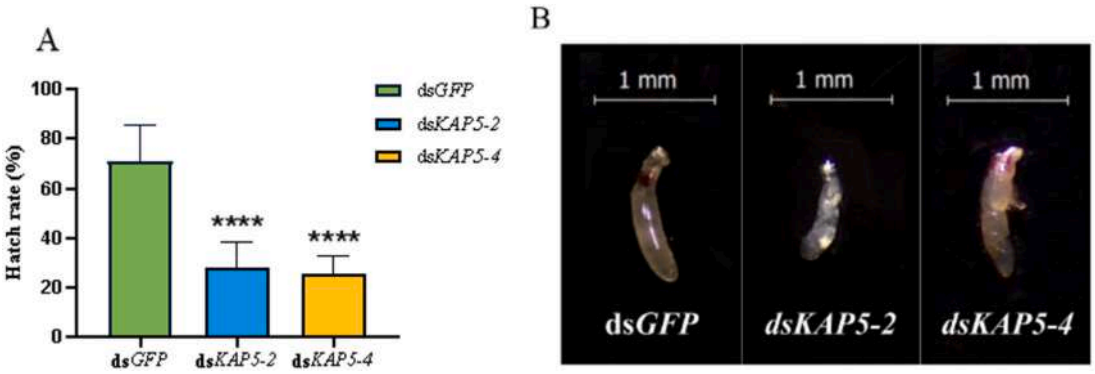

Fig. 4. Influence of ds*KAP* silencing on the hatching rate and offspring phenotype of *N. lugens*. Hatching rate (A) and representative non-hatching phenotypes (B) of *N. lugens* offspring. Data are presented as mean ± SEM. The hatching rate (A) was analyzed by an independent-samples *t*-test compared to the dsGFP group (n = 9 independent biological replicates). "****", $P < 0.0001$.

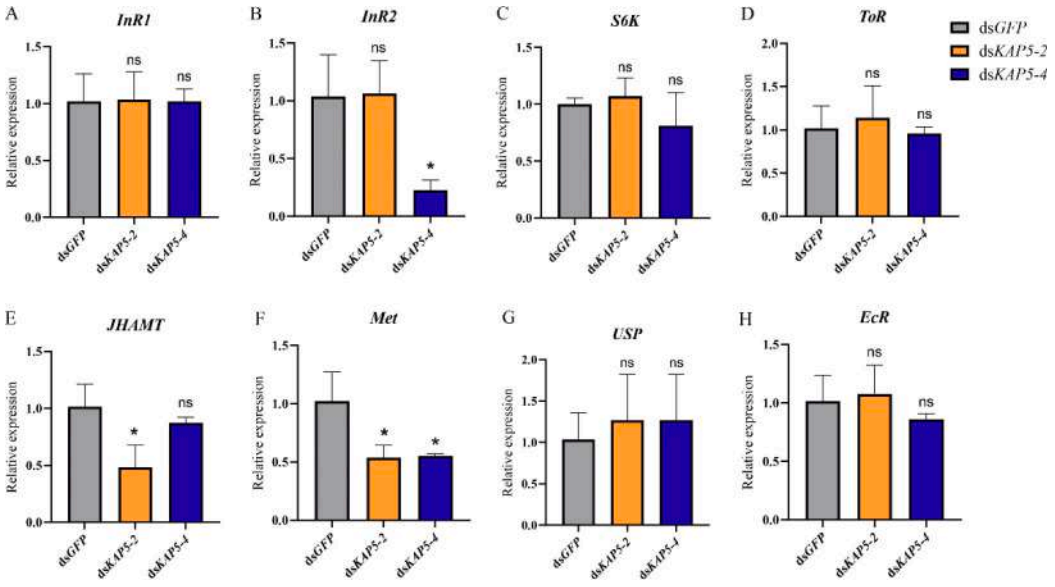

Fig. 5. The relative expression levels of the reproduction-related genes *InR1*, *InR2, S6K*, *ToR*, *JHAMT*, *Met*, *USP* and *EcR* in *N. lugens* on the third day after ds*KAP5-2* and ds*KAP5-4* treatments. Data are presented as mean ± SEM. Gene expression was analyzed by an independent-samples *t*-test relative to the dsGFP control group (n = 3). "ns" means no significant difference between the data of this group and the control group. "*", $P < 0.05$.

Vitellogenin (Vg) is a crucial protein in the reproduction of *N. lugens*, with its expression significantly upregulated after adult emergence (Shen et al., 2019). Disruption of Vg expression leads to female sterility and abnormal oocyte development. The Vg receptor (VgR) is also highly expressed after emergence, and inhibition of its function impedes the accumulation of Vg in the ovaries, thereby reducing fecundity (Lu et al., 2015). In the present study, injection of *dsKAP* significantly downregulated the expression of both Vg and its receptor VgR in females, consistent with the observed reduction in fecundity. Specifically, silencing *KAP5-2* gene suppressed Vg expression, while silencing *KAP5-4* genes resulted in a marked decrease in VgR levels, suggesting that these two genes may act through a complementary mechanism to jointly regulate vitellogenesis.

The expression of Vg and VgR is known to be regulated by juvenile hormone (JH), 20-hydroxyecdysone (20E), and nutrient signaling pathways. Disruption of key genes in these pathways can result in embryonic developmental failure or reduced hatching rates, collectively contributing to the reproductive regulatory network in *N. lugens* (Jin and Lin, 2014; Wu et al., 2021; Luo et al., 2021; Chen, 2023). In this study, silencing either *KAP5-2 gene* or *KAP5-4 gene* downregulated the expression of *Met*, while *dsKAP5-2* also reduced the transcript level of *JHAMT*. *Met* functions as a JH receptor in JH signal transduction, and *JHAMT* serves as a key rate-limiting enzyme in JH biosynthesis, both of which play important regulatory roles in normal reproduction of *N. lugens* (Cui et al., 2021). In addition, silencing *KAP5-4* significantly decreased the expression of insulin-like receptor 2 (*InR2*). Two insulin-like receptors, *InR1* and *InR2*, have been identified in this insect: *InR1* activates the PI3K/Akt pathway, whereas *InR2* acts as a negative regulator of this signaling cascade (Xue et al, 2021; Xu et al, 2015). Previous studies have demonstrated that RNAi-mediated downregulation of *InR2* impairs reproduction in *N. lugens*(Tang et al., 2025). The present results further suggest that the regulatory role of *KAP* genes is linked to both the JH and nutrient signaling pathways, with potential implications for reproductive process modulation. This conclusion is supported by the observed significant decline in egg-laying and defective ovarian development. This observation is consistent with findings concerning *TRE* and the nuclear protein Akirin in *N. lugens*; silencing of these genes likewise resulted in decreased *Vg* expression and disruption of JH and nutrient signaling pathways (Luo et al., 2022; Gou et al., 2024; Tang et al., 2025). Intriguingly, analogous to the phenotypes observed after *KAP* gene silencing, silencing *TRE* also led to abnormal chitin deposition in the eggshell and embryonic eye-spot inversion (Luo et al., 2022; Liu et al., 2025b). Considering the presence of eggshell defects and hatching abnormalities in *KAP*-silenced individuals, future research should delve

deeper into the potential regulatory function of *KAP* genes in eggshell chitin metabolism. Moreover, while silencing the cytochrome *P450* gene *CYP303A1* significantly reduced offspring hatchability and delayed embryonic eye development (Wang, 2024), it did not affect female fecundity or the expression of key reproductive signaling genes such as *Vg*, unlike the effects of *KAP* silencing. This distinction underscores the central and potentially multifaceted role of *KAP* genes within the reproductive regulatory network of *N. lugens*.

In summary, through functional screening, our study uncovered the indispensable roles of the *KAP5-2* and *KAP5-4* genes in ovarian development, oogenesis, and embryonic survival in the *N. lugens*. Silencing these genes disrupted chorion formation, was associated with the downregulation of *Vg* expression, and perturbed the hormonal and nutritional signaling network, ultimately leading to reproductive failure. Future research ought to center on elucidating the precise molecular functions of KAP proteins in chitin deposition and protein cross-linking within the eggshell, which would offer a more robust theoretical foundation for developing novel *KAP*-targeted RNAi-based eco-friendly control strategies.

CRediT authorship contribution statement

Yi Zhang: Visualization, Validation, Software, Data curation, Conceptualization. **XinYi Zhang:** Visualization, Validation, Software, Data curation, Conceptualization. **Fan Zhong:** Visualization, Validation. **Ye Han:** Visualization. **Liwen Guan:** Visualization. **Qixuan Mao:** Visualization, Software. **LiYuHan Hua:** Validation. **Busheng Liu:** Visualization. **Min Zhou:** Visualization. **Hong Yang:** Visualization, Conceptualization. **Yan Wu:** Visualization. **Bin Tang:** Writing – review & editing, Visualization, Validation, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

Data will be made available on request.

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