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ARTICLE

Knockdown of *TOR* causing ovarian diapause in a genetically stable brachypterous strain of *Nilaparvata lugens*

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Abstract

Brown planthopper (BPH), *Nilaparvata lugens* (Stål) (Hemiptera: Delphacidae), is one of the most damaging pests of rice crops. BPH is a migratory insect with a delayed ovarian development in migrants classified as reproductive diapause. The molecular mechanism of reproductive diapause remains unclear, although we suspect it might be regulated by one or more nutrient signaling pathways. The target of rapamycin (TOR) pathway regulates cell growth in response to nutritional information, which raised a hypothesis that TOR mediates BPH reproductive diapause. We used a pure brachypterous strain (BS) and a predominantly macropterous strain (MS) to investigate the roles of *NITOR* in BPH reproductive diapause. We found that *NITOR* is expressed from the nymphal to adult stages, with a higher expression level of *NITOR* in BS adults at 1, 2, and 4 days posteclosion than in MS at the same time points. Injection of ds*NITOR* into BS nymphs resulted in the termination of BPH female ovary development and the retardation of nymph development. We infer that TOR signaling functions in BPH reproductive diapause by regulating the expression of *NlFoxA* and *NlVitellogenin*.

KEYWORDS
Nilaparvata lugens, ovary, reproductive diapause, TOR

1 | INTRODUCTION

The brown planthopper (BPH), *Nilaparvata lugens* (Stål) (Hemiptera: Delphacidae), is one of the most damaging and widespread insect pests in the rice growing areas of Asia (Madurangi, Ratnasekera, Hemachandra, & Senanayake, 2012). BPH is dimorphic with brachypterous (BF) and macropterous forms (MF). Macropterous adults are long-distance migrants; they can fly and escape from challenging circumstances to establish new colonies. While BF adults have no flight ability, they have a much higher fecundity than the MF (Kisimoto, 1965). Environmental cues, especially

the nutrition conditions of the host, significantly influence the wing morph of BPH. A poor nutrition condition increases the proportion of MF, while a good nutrition condition increases the proportion of BF (Johno, 1963; Kisimoto, 1956). If the habitat is favorable, most BPHs develop into BF while food consumption and fecundity are increasing (Syobu, Mikuriya, Yamaguchi, Matsuzaki, & Matsumura, 2002); under poor habitat conditions, most BPHs develop into MF to get flight ability to facilitate migration (Kisimoto, 1956).

In addition to lower fecundity, MF females have a longer preoviposition period than BF females (Kisimoto, 1965). This difference is caused by the reproductive diapause of the migratory females, whose ovarian development is delayed during migration (Chen, Cheng, Yang, & Yin, 1979). BPH wing morph is determined by *Insulin Receptor 1* and *Insulin Receptor 2* at the nymphal stage (Xu et al., 2015), indicating that reproductive diapause in the migratory females is pre-determined before adult emergence. The molecular factors involved in reproductive diapause and ovary development need to be explored.

Target of rapamycin (TOR), a classical factor responding to nutritional signaling, controls cell growth, proliferation, and tissue development (Oldham, Montagne, Radimerski, Thomas, & Hafen, 2000; Zhang et al., 2000). Knockdown of *TOR* expression can decrease the number of offspring and affect ovary development in insects (Lu, Chen, Liu, & Zhou, 2016; Maestro, Cobo, & Belles, 2009; Park, Attardo, Hansen, & Raikhel, 2006). *TOR* has been identified from *N. lugens* (*NlTOR*). Until now, there has been only one *TOR* gene identified from *N. lugens*. *NlTOR* (GenBank accession no. JQ793898) encodes 2,507 amino acid residues and has a predicted molecular weight of 283.3 kDa, has the highest sequence identity with *Blattella germanica* TOR (78%), followed by *Aedes aegypti* TOR (63%) (Lu et al., 2016).

There have been some reports on the function of *NlTOR* in BPH reproduction system development. Knockdown of *NlTOR* in wild-type nymphs at second-, third-, fourth-, and fifth-instar stages resulted in poor development of accessory glands in wild-type males, and no offspring were produced when normal females mated with *NlTOR* RNA interference (RNAi) males (Zhuo et al., 2017). Injection of ds*NlTOR* into newly emerged brachypterous females decreased the fecundity of BPH (Zhai et al., 2015). These reports demonstrated that *NlTOR* plays important roles in BPH reproduction system development; however, whether *NlTOR* can activate BPH reproduction diapause during the nymphal stage remains unknown.

After 5 years of successive selection, a BPH strain with predominantly MF (MS, ~85% MF) and a BPH strain with pure BF (brachypterous strain (BS), 100% BF) were constructed in our lab. In the current study, we compared the expression profiles of *NlTOR* between MS and BS and injected ds*NlTOR* into BS nymphs to investigate whether the depletion of ds*NlTOR* could terminate the development of female ovary and delay the development duration of nymphs. This is the first report on the roles of *NlTOR* in the reproductive diapause of BPH.

2 | MATERIALS AND METHODS

2.1 | Insects

BPH populations were maintained on TN1 (Taichuang Native 1, a BPH-susceptible rice variety) at Hubei Insect Resources Utilization and Sustainable Pest Management Key Laboratory, College of Plant Science and Technology, Huazhong Agricultural University, China. A BPH strain with predominantly MF (MS, ~85% MF) and a BPH strain with pure BF (BS, 100% BF) were constructed in our lab according to Morooka and Tojo (1992) with some modifications. BS is genetically stable. Insects were maintained under the condition of $28 \pm 1°C$, 14:10 h light:dark, and $70 \pm 5\%$ RH.

2.2 | cDNA cloning of *NlTOR*

The expressed sequence tags (EST) sequence of *NlTOR* was obtained from the BPH transcriptome of our lab. This sequence has 100% identity with *NlTOR* (GenBank accession number of JQ793898.1) deposited in the NCBI Database. cDNA of *NlTOR* was constructed from total RNA of BPH fourth-instar nymphs. The primers used for cDNA cloning are *NlTOR*-F/R (Table 1). The cloning procedure followed that of Liu et al. (2015).

TABLE 1 List of primers

GenBank accession number	Name of primer	Primer sequences	Length of PCR product
JQ793898.1	NlTOR-F	GATCGGCATGAGGGAGGGAGACA	6,768 bp
	NlTOR-R	CGACGACGGTACACTGCGTTTGG	
	ds1NlTOR-F	TAATACGACTCACTATAGGACCAGTGAAATGCTCGTAAACA	550 bp
	ds1NlTOR-R	TAATACGACTCACTATAGGCCAGGTGCAGGTAATCGTCCAG	
	ds2NlTOR-F	TAATACGACTCACTATAGGTTGACGGTCACTCACTACTGCA	492 bp
	ds2NlTOR-R	TAATACGACTCACTATAGGGCTCTTTGTTTCGTCCCATACC	
U76561	dsGFP-F	TAATACGACTCACTATAGGGTAAACGGCCACAAGTTCAG	400 bp
	dsGFP-R	TAATACGACTCACTATAGGTCGGCCATGATATAGACGTT	
EU179846.1	qPCR-NlActin1-F	CCAACCGTGAGAAGATGACC	256 bp
	qPCR-NlActin1-R	GATGTCACGCACGATTTCAC	
JQ793898.1	qPCR-NlTOR-F	AACGCCATGGAGGTGACAGG	143 bp
	qPCR-NlTOR-R	ATGAGGCGCCAGTTGAGCAG	
KY827832	qPCR-NlE74B-F	AACAACATAATAGGCACAGTC	175 bp
	qPCR-NlE74B-R	GGAATGGCGAAGAAGTATC	
FJ263049.1	qPCR-NlEcR-F	GCCAGAAAGTACGACGTGAA	234 bp
	qPCR-NlEcR-R	TTGGATCTTCTCCACCTTCC	
19JF345255	qPCR-NlFoxA-F	GCGGAGGTTATGTTGTGTTGTA	193 bp
	qPCR-NlFoxA-R	CTGAGCCTTGTAGCATGTTGAA	
AB353856.1	qPCR-NlVg-F	TCTTCATCATCCTCCTCCTCTTC	173 bp
	qPCR-NlVg-R	TCCTGGTTGTTGTCATTGTCATT	

2.3 | Synthesis of dsRNA

Two double-stranded RNAs (dsRNAs) were synthesized for knockdown of *Nl1TOR*. The first dsRNA (ds1*NlTOR*, 550 bp) targeted between 2,764th and 3,313rd nucleotides, and the second dsRNA (ds2*NlTOR*, 492 bp) located between 1,736th and 2,227th nucleotides. T7 polymerase promoter was added at the 5′-end of gene-specific primers. These primers were used to amplify the cDNA of *NlTOR*. The resulting PCR products containing T7 polymerase promoter at both 5′-ends were used as the template for dsRNA synthesis. QIAquick™ PCR purification kits (Code No.: 28104, Qiagen, Dusseldorf, Germany) were used to purify PCR products. dsRNAs were synthesized using a T7 RiboMAX Express RNAi System (Code No.: P1700, Promega, Madison) following the manufacturer's instructions. *GFP* (GenBank accession no. U76561) dsRNA (ds*GFP*) served as a negative control. The sequences of primers used to synthesize dsRNA are ds1*NlTOR*-F/R and ds2*NlTOR*-F/R (Table 1).

2.4 | RNAi using microinjection

The third-instar nymphs (6–12 h) of BPH were collected for dsRNA injection using microinjection as described by Liu et al. (2015) using a Nanoliter 2000 injector (WPI, Sarasota). Approximately 300 ng dsRNA (ds1*NlTOR*, ds2*NlTOR*, or

ds*GFP*) was injected into each nymphal conjunction of the prothorax and mesothorax. A total of 250 nymphs were treated in each replicate with three replicates for each treatment (i.e., in total 750 nymphs were injected for each dsRNA). The treated nymphs were cultured as described by Liu et al. (2015). The survival of BPHs was recorded daily after injection. At the same time, five insects were sampled randomly for qRT-PCR analysis at 3, 6, 7, 9, 11 days after injection.

2.5 | Developmental duration and ovary observation

Each BPH nymph treated with dsRNA was observed at 6 h time intervals to examine the ecdysis of insects. Newly emerged females were coupled with wild-type males. The BPH ovaries were dissected using high-precision tweezers (WPI, Sarasota) at 1, 3, 5, and 8 days after adult emergence, in $1 \times$ phosphate-buffered saline (PBS) followed by fixation in 4% formaldehyde in $1 \times$ PBS for 20 min at room temperature. Dissected ovaries were washed three times for 10 min with 0.2% Triton-X 100 (Code No.: T9824, Santaclara, Sigma) in $1 \times$ PBS. After washing, the ovaries were photographed with an Olympus stereomicroscope (SZX16, Olympus, Tokyo, Japan) connected to a P90 Pro Digital Camera (P90, Nikon, Tokyo, Japan). Rice seedlings hosting BPH were dissected to measure the number of eggs.

2.6 | qRT-PCR

TRIzol reagent (Code No.: 15596018, Invitrogen, Carlsbad) was used to extract total RNA. *Actin1* (GenBank accession number of EU179846.1) was used as an internal control gene. SYBR Premix Ex Taq (Code No.:RR420A, Takara, Dalian, China) was used for qRT-PCR analysis on an ABI Prism 7300 instrument (Applied Biosystems) according to the manufacturer's instructions. Each pair of primers was designed to amplify approximately 100–300 bp of PCR products (Table 1), and PCR amplification efficiency were validated using the standard curve method. Only the primers with 85–110% efficiency and a 98% correlation coefficient were selected in the qRT-PCR analysis. Each RNA sample was examined by three independent reactions, and the signal intensity of the target gene was presented as the average value. Three biological replicates were set for each treatment. The relative expression level of genes was calculated according to the method of Livak and Schmittgen (2001). Sequences of primers for qRT-PCR are presented in Table 1.

2.7 | Sequence comparison and phylogenetic relationship

Phylogenetic tree were constructed based on the amino acid sequences of known homologous genes, respectively, from insects by the neighbor-joining method using the Molecular Evolutionary Genetics Analysis (MEGA) software. The robustness of each cluster was verified in 1,000 replicates.

2.8 | Data analysis

All data were analyzed with SPSS version 18. One-way ANOVA was used to analyze the differences between means, and repeated-measures ANOVA was used to analyze survival response to dsRNA. Percentage values were converted to arcsine before statistical analysis.

3 | RESULTS

3.1 | *NITOR* expression patterns during different developmental stages in BS and MS BPH

The results showed that *NITOR* was expressed in both the nymph and adult stages in BPH; it was highly and mainly expressed in the adult stage. During the nymphal stages, the *NITOR* expression level was significantly lower in BS than MS at the first instar, but there were no significant differences in *NITOR* expression level at the other nymphal stages. The *NITOR* expression levels in BS adults were significantly higher than those in MS adults at 1, 2, and 4 days after
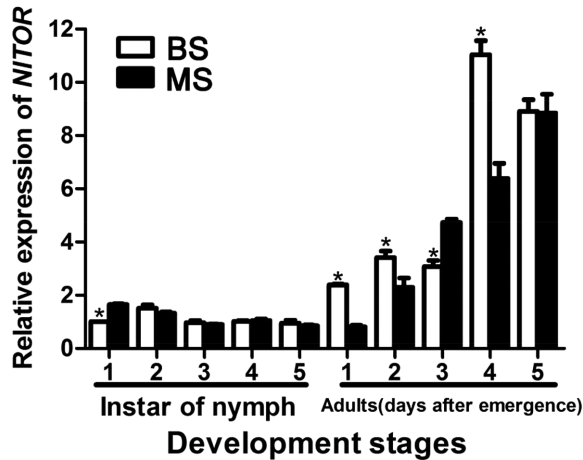
FIGURE 1 The relative expression pattern of *NITOR* at different developmental stages of MS and BS; BPH *actin1* was used as a reference control. The expression level was quantified relative to the value of the first day of BS. The average expression level was based on three biological replicates. Error bars indicate standard errors. Bars labeled with an asterisk differed significantly between the BPHs of MS and BS, as determined using one-way ANOVA ($P < 0.05$)
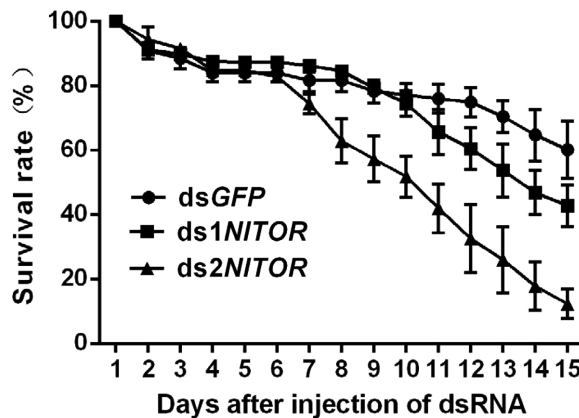

FIGURE 2 Survival rate of BS BPH in response to ds1*NITOR* and ds2*NITOR* injection. ds*GFP* injection served as a control. Each of the groups included 45 nymphs. Survival responses to ds*GFP*, ds1*NITOR*, and ds2*NITOR* were analyzed using repeated-measures ANOVA

emergence, whereas the *NITOR* expression level was lower in BS than MS 3 days after emergence (Figure 1). The *NITOR* expression level did not differ significantly at day 5 after emergence between BS and MS (Figure 1).

3.2 | Effects of knockdown of *NITOR* on the survival of BS BPH

Because the expression levels of *NITOR* in BS adults were significantly higher than those in MS adults at 1, 2, and the 4 days after emergence, ds*NITOR* was injected into nymphs of BS to investigate the role of *NITOR* in reproduction diapause in BPH.

After injection of dsRNA (ds1*NITOR*, ds2*NITOR*, and ds*GFP*) into third-instar nymphs, the survival rates of nymphs treated with ds*NITOR* and ds*GFP* did not differ from 1 to 7 days after injection ($F_{2,6} = 0.538$; $P = 0.609$; Figure 2). In contrast, the survival rate of the insects treated with ds*NITOR* was significantly lower than that of insects treated with ds*GFP* from 8 to 15 days after injection (BS: $F_{2,6} = 12.568$; $P = 0.007$; Figure 2). From 1 to 7 days after injection, treated
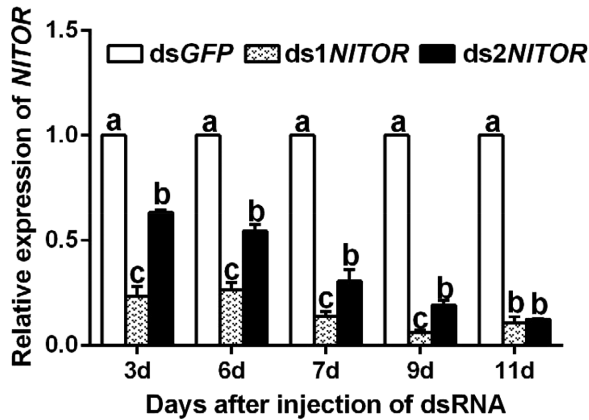
FIGURE 3 The relative expression levels of *NITOR* after injection of dsRNA into the third-instar nymphs in BS. Error bars indicate standard errors. The expression level was quantified relative to the value of the nymphs injected with ds*GFP*. Bars labeled with different letters indicated that there were significant differences among the treatments on the same day as determined using one-way ANOVA ($P < 0.05$). The average expression level was based on three biological replicates

BPHs were in the nymphal stage, while from 8 to 15 days after injection, treated BPHs were in the adult stage. This result indicated that ds*NITOR* had lethal effects on BPH adults but not on BPH nymphs.

3.3 | Effects of dsRNA injection on *NITOR* gene expression in BS

In BS, the injection of ds1*NITOR* and ds2*NITOR* into third-instar nymphs significantly suppressed the expression of endogenous *NITOR* mRNA at all five sampling points. From 3 to 11 days after the injection of ds*NITOR*, the transcript levels of *NITOR* were decreased significantly by 36.9–94.1% (Figure 3) ($P < 0.05$, one-way ANOVA) compared to ds*GFP* treatment.

3.4 | ds*NITOR* injection prolonged the duration of nymphal development in BS

The injection of ds1*NITOR* and ds2*NITOR* significantly prolonged the developmental duration of nymphs in BS for both males and females compared with ds*GFP*. After the injection of ds1*NITOR* and ds2*NITOR*, the developmental durations of BS males were 8.8 h longer and 7.8 h longer, respectively, and the developmental durations of females were 15.7 h longer and 15.2 h longer, respectively, compared to ds*GFP* injection (Figure 4A).

3.5 | Effects of ds*NITOR* injection on genes involved in ecdysone signal transduction

Previous reports demonstrated that knockdown of *NITOR* dramatically decreased the expression of juvenile hormone (JH) acid methyltransferase (*NIJHAMT*), while application of JH III on the RNAi (*NITOR*) females could partially rescue the phenotypes caused by RNAi (Lu et al., 2016). In the present study, BS BPHs treated with ds1*NITOR* and ds2*NITOR* exhibited development retardation. These results prompted us to hypothesize that *NITOR* not only influences the expression of genes involved with JH signal but also affects the ecdysone signal pathway. Thus, we measured the transcriptional levels of the two import members involving ecdysone signal pathway. *NlE74B* (GenBank accession no.KY827832, its identity was validated with phylogenetic analysis) (Figure 5A), which is a direct target of *EcR*, as an indicator of the ecdysone signaling levels (Thummel, 2002), and we also determined the transcriptional level of *NlEcR* (ecdysteroid receptor, Yu et al., 2014) in BS. The expression level of *NlE74B* decreased significantly from 3 to 6 days after ds1*NITOR* injection in BS (Figure 5B), while *NlEcR* was significantly decreased after 3 days but not at 6 days (Figure 5C) ($P < 0.05$, one-way ANOVA).
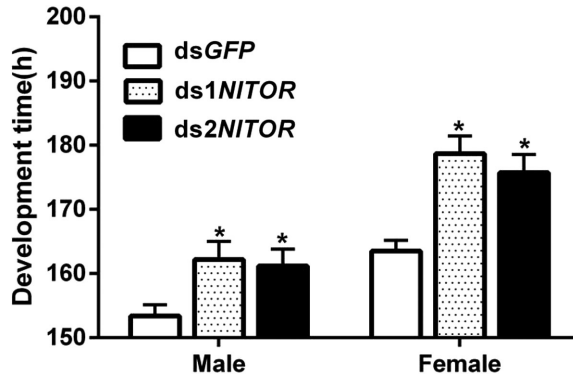
FIGURE 4 The effects of ds1*NlTOR* and ds2*NlTOR* injection on the development duration of BS BPH nymphs from third instar to adults. All error bars indicate standard errors as determined from three independent replicates. Bars labeled with different letters indicated that there were significant differences among the treatments, as determined using one-way ANOVA ($P < 0.05$)

3.6 | Knockdown of *NlTOR* inhibited ovarian development

We found that female BS emerging from nymphs treated with ds1*NlTOR* laid no eggs and generated no offspring. However, females of BS injected with ds*GFP* laid many eggs and large numbers of nymphs hatched (about 100 nymphs/female). Therefore, we dissected the ovaries of females at 1, 3, 5, and 8 days after emergence to examine ovary development. The results showed that ovarian development was inhibited and oviduct development was nearly terminated by ds1*NlTOR* injection, meaning that eggs could not form in the ovarian tubules at 3, 5, and 8 days. According to grading criteria for BPH ovarian development proposed by Lu (2011), ovaries of ds*NlTOR*-treated BS female remained in the first to second stages throughout the observation period. By contrast, the ds*GFP*-treated female ovaries developed normally, and eggs were found in the ovarian tubules after 3, 5, and 8 days (Figure 6). Therefor we deduced that the ovaries of the BS females emerging from nymphs treated with ds*NlTOR* remains reproduction diapause status based on the reports of Chen et al. (1979).

3.7 | Effects of in vivo knockdown of *NlTOR* on the expression of *NlFoxA* and *NlVg* related to ovarian development

Previous studies have shown that *FoxA* and *Vg* (*Vitellogenin*) are involved in ovarian development (Dong et al., 2011). The expression levels of *NlFoxA* and *NlVg* were determined in females by qRT-PCR at 1, 3, and 5 days after emergence. At 1, 3, and 5 days after emergence, *NlFoxA* expression in females treated with ds1*NlTOR* was reduced by 95.9, 89.7, and 25.5%, respectively (Figure 7A) in comparison to the control. At 1, 3, and 5 days after emergence, *NlVg* expression in females treated with ds1*NlTOR* decreased significantly by 71.6, 60.8, and 51.2%, respectively (Figure 7B), compared to the control ($P < 0.05$, one-way ANOVA).

4 | DISCUSSION

TOR can respond to nutritional signaling and regulate cell, tissue, and organ development in living organisms (Oldham et al., 2000; Zhang et al., 2000). In insects, *TOR* plays key roles in reproduction system development (Lu et al., 2016; Maestro et al., 2009; Park et al., 2006). *NlTOR* has been cloned and functions in ovary and sperm development in BPH (Lu et al., 2016; Zhai et al., 2015; Zhuo et al., 2017). During migration, the ovary of BPH migratory females is in development arrest, that is, reproduction diapause status (Chen et al., 1979). Whether there are direct relations between *NlTOR* and BPH reproduction diapause remains unknown. In the present study, we found that *NlTOR* is expressed from
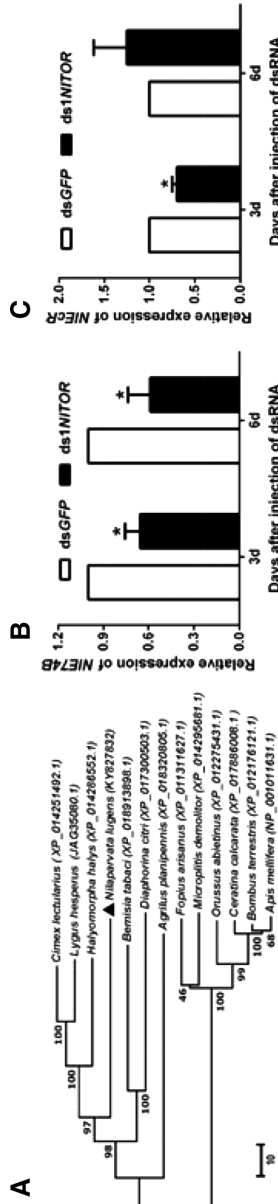
FIGURE 5 Phylogenetic tree of the *ecdysone-induced protein 74EF isoform B (E74B)* of various insect species and the expression levels of *NlE74B* and *NlEcR* after ds1*NlTOR* injection into the third-instar nymphs in BS. (A) The tree was constructed with neighbor-joining (NJ) method using MEGA software, the robustness of each cluster was verified in 1,000 replicates, and lengths are proportional to sequence divergence. The bar indicates 10 substitutions per site. The GenBank accession no. of each E74B from various insects is in the parentheses. (B and C) The expression levels of *NlE74B* and *NlEcR* after ds1*NlTOR* injection into the third-instar nymphs in BS. All error bars indicate standard errors as determined from three independent replicates. The expression level was quantified relative to the value of the insects injected with ds*GFP*. Bars labeled with an asterisk differed significantly between the treatments on the same day, as determined using one-way ANOVA ($P < 0.05$)
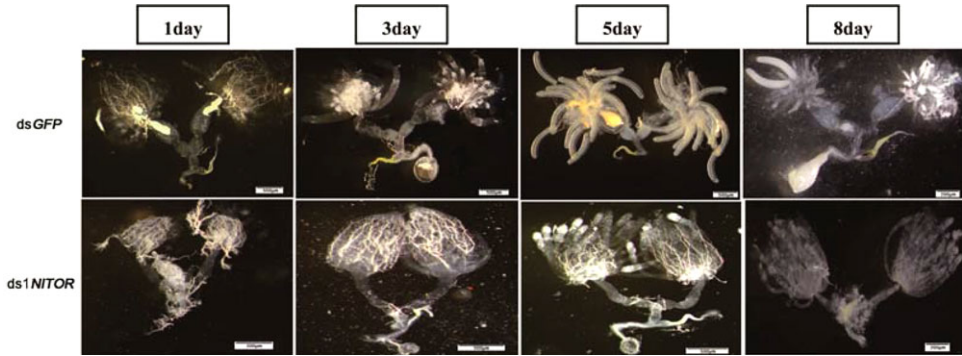
FIGURE 6 Effect of ds1*NITOR* on ovary development. ds1*NITOR*-treated females (300 ng/insect) had complete ovarian development inhibition at 1, 3, 5, and 8 days after emergence. ds*GFP* (300 ng/insect) had no suppression effects on ovarian development of females at 1, 3, 5, and 8 days after emergence
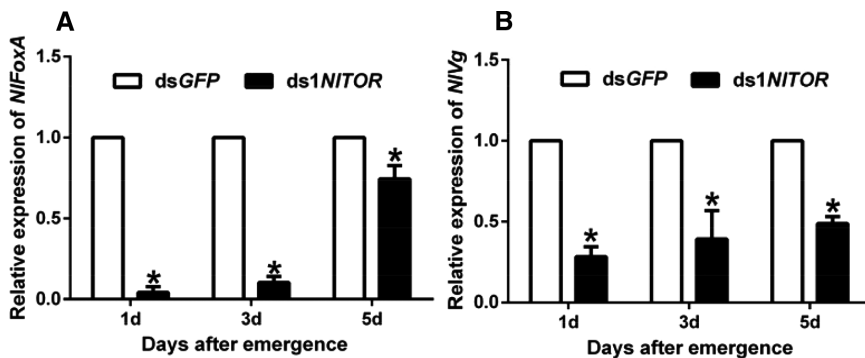

FIGURE 7 The relative expression levels of *NlFoxA* and *NlVg* after injection of dsRNA. (A) The relative expression levels of *NlFoxA* after injection of ds*1NITOR* into the nymphs at third instar. (B) The relative expression levels of *NlVg* after injection of ds1*NITOR* into the nymphs at third instar. Error bars indicate standard errors. The expression level was quantified relative to the value of the nymphs injected with ds*GFP*. Bars labeled with an asterisk differed significantly between the treatments on the same day as determined using one-way ANOVA ($P < 0.05$). The average expression level was based on three biological replicates

the nymphal to adult stages, with differing expression levels of *NITOR* between MS and BS at the first nymphal stage and at 1, 2, and 4 days posteclosion. Injection of ds*NITOR* into BS nymphs retarded ovary development in BS BPH females in a manner corresponding to the status of reproduction diapause. This finding represents first report of direct relations between *NITOR* and BPH reproductive diapause.

BPH wing morph is determined by *Insulin Receptor 1* and *Insulin Receptor 2* at the nymphal stage (Xu et al., 2015), indicating that reproductive diapause of the migratory females is also predetermined before adult emergence. To investigate the molecular factors involved in reproductive diapause, genetically defined macropterous and BSs must be used for investigation. After 5 years of successive selection, a BPH strain with predominantly MF (MS, ~85% MF) and a BPH strain with pure BF (BS, 100% BF) were constructed in our lab. These strains provided the opportunity to examine the roles of *NITOR* during the nymph stage in presumptive macropters or brachypters. Three days after injection, the expression of *NITOR* in treated nymphs already decreased by 73.7%. Therefore, newly eclosed BS females that emerged from dsRNA-treated nymphs exhibited significantly lower expression levels of *NITOR*. This condition allowed us to investigate the functions of *NITOR* in reproduction diapause as early as wing morph determination.

The dose of ds*NITOR* used in this study was 300 ng, which is substantially higher than that used in Xu, Xue, Lu, Zhang, Zhuo, and Zhang (2015). Liu et al (2015) used a 300 ng dose of ds*NlapA*; however, ds*NlapA* had no lethal effects on BPH. Therefore, we believe that the mortality is caused not by the specific dose but by ds*NITOR* itself. In fact, ds*NITOR* had

lethal effects on only BPH adults, not on BPH nymphs. Whether this result was due to the fact that *NlTOR* plays a more important role in adults than in nymphs or due to the higher repression efficiency of *NlTOR* in adults than in nymphs needs to be studied further.

Previous reports demonstrated that *NlTOR* knockdown dramatically decreased the expression of JH acid methyltransferase (*NlJHAMT*), and the application of JH III on the RNAi (*NlTOR*) females could partially rescue the phenotypes caused by RNAi, indicating that the TOR pathway induces JH biosynthesis to regulate AAs-mediated Vg synthesis in *N. lugens* (Lu et al., 2016). Our results suggested that *NlTOR* also influences the ecdysone signal pathway. *NlTOR* knockdown also significantly decreased the expression levels of *NlE74B* and *NlEcR*. The relationship among the TOR pathway, JH biosynthesis, and ecdysone signal pathway needs to be studied further.

In the current study, *NlTOR* downregulated the expression level of *NlFoxA* and *NlVg*. These results were in accordance with the results of Zhai et al. (2015). We can infer that TOR signaling functions in BPH reproduction system development, playing important roles in reproductive diapause in migratory females by regulating the expression of *NlFoxA* and *NlVg*.

LITERATURE CITED

Chen, R., Cheng, X., Yang, L., & Yin, X. (1979). Brown planthopper ovarian development and its relationship with the journey. *Acta Entomologica Sinica*, *22*, 280–288. (In Chinese).

Dong, X., Zhai, Y., Zhang, J., Sun, Z., Chen, J., Chen, J., & Zhang, W. (2011). Fork head transcription factor is required for ovarian mature in the brown planthopper, *Nilaparvata lugens* (Stål). *BMC Molecular Biology*, *12*, 53.

Johno, S. (1963). Analysis of the density effect as a determining factor of the wing-form in the brown planthopper, Nilaparvata lugens. *Japanese Journal of Applied Entomology and Zoology*, *7.1*, 45–48.

Kisimoto, R. (1956). Effect of crowding during the larval period on the determination of the wing-form of an adult plant-hopper. *Nature*, *178*(4534), 641–642.

Kisimoto, R. (1965). Studies on polymorphism and its role playing in the population growth of brown planthopper, *Nilaparvata lugens* (Stal). *Bulletin of the Shikoku National Agricultural Experiment Station*, *13*, 101–106.

Liu, F., Li, K., Li, J., Hu, D., Zhao, J., He, Y., … Hua, H. (2015). *Apterous A* modulates wing size, bristle formation and patterning in *Nilaparvata lugens. Scientific Reports*, *5*, 10526.

Livak, J. K., & Schmittgen, T. D. (2001). Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta Ct}$ method, *Methods*, *25*, p402–p408.

Lu, F. (2011). The processes of morphological change and grading criteria for ovarian development in the brown planthopper. *Chinese Journal of Applied Entomology*, *48*, 1394–1400.

Lu, K., Chen, X., Liu, W., & Zhou, Q. (2016). TOR pathway-mediated juvenile hormone synthesis regulates nutrient-dependent female reproduction in *Nilaparvata lugens* (Stål). *International Journal of Molecular Sciences*, *17*(4), 438.

Madurangi, S. A. P., Ratnasekera, D., Hemachandra, P. V., & Senanayake, S. G. J. N. (2012). Evaluation of brown planthopper *Nilaparvata lugens* (Stal) resistance in *Oryza nivara* wild rice accessions found in Sri Lanka. *Proceedings of International Forestry & Environment Symposium*, *15*, 172–175.

Maestro, J. L., Cobo, J., & Belles, X. (2009). *Target of rapamycin* (TOR) mediates the transduction of nutritional signals into juvenile hormone production. *Journal of Biological Chemistry*, *284*, 5506–5513.

Morooka, S., & Tojo, S. (1992). Maintenance and selection of strains exhibiting specific wing form and body colour under high density conditions in the brown plant-hopper, *Nilaparvata lugens* (Homoptera: Delphacidae). *Applied Entomology and Zoology*, *27*, 445–445.

Oldham, S., Montagne, J., Radimerski, T., Thomas, G., & Hafen, E. (2000). Genetic and biochemical characterization of dTOR, the *Drosophila* homolog of the *target of rapamycin. Genes & Development*, *14*, 2689–2694.

Park, J. H., Attardo, G. M., Hansen, I. A., & Raikhel, A. S. (2006). GATA factor translation is the final downstream step in the amino acid/target-of-rapamycin-mediated vitellogenin gene expression in the anautogenous mosquito Aedes aegypti. *Journal of Biological Chemistry*, *281*, 11167–11176.

Syobu, S., Mikuriya, H., Yamaguchi, J., Matsuzaki, M., & Matsumura, M. (2002). Fluctuations and factors affecting the wing-form ratio of the brown planthopper, *Nilaparvata lugens* Stal in rice fields. *Japanese Journal of Applied Entomology and Zoology*, *46*(3), 135–143.

Thummel, C. S. (2002). Ecdysone-regulated puff genes 2000. *Insect Biochemistry and Molecular Biology*, *32*(2), 113–120.

Xu, H., Xue, J., Lu, B., Zhang, X., Zhuo, J., He, S., … Zhang, C. (2015). Two insulin receptors determine alternative wing morphs in planthoppers. *Nature*, *519*, 464–467.

Yu, R., Xu, X., Liang, Y., Tian, H., Pan, Z., Jin, S., … Zhang, W. (2014). The insect ecdysone receptor is a good potential target for RNAi-based pest control. *International Journal of Biological Sciences*, *10*(10), 1171–1180.

Zhai, Y., Sun, Z., Zhang, J., Kang, K., Chen, J., & Zhang, W. (2015). Activation of the TOR signalling pathway by glutamine regulates insect fecundity. *Scientific Reports*, *5*, 10694.

Zhang, H., Stallock, J. P., Ng, J. C., Reinhard, C., & Neufeld, T. P. (2000). Regulation of cellular growth by the *Drosophila* target of rapamycin dTOR. *Genes & Development*, *14*, 2712–2724.

Zhuo, J., Xue, J., Lu, B., Huang, H., Xu, H., & Zhang, C. (2017). Effect of RNAi-mediated knockdown of *NlTOR* gene on fertility of male Nilaparvata lugens. *Journal of Insect Physiology*, *98*, 149–159.