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Zinc Stress Alters Sugar Content in Rice Plants and the Reproduction and Trehalose Metabolism in *Nilaparvata lugens*

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Abstract: Excessive zinc (Zn) is toxic to plants, but the effect of zinc-stressed plants on herbivorous insects is still unclear. Hence, we assessed the effect of zinc-stressed rice plants on its feeding pest, *Nilaparvata lugens*. The soil–rice–*N. lugens* system was treated with Zn^{2+} solution. Sugar content in rice was measured, and the reproduction and trehalose metabolism in *N. lugens* were assessed. The trehalase activity in rice significantly decreased at 100 mg·kg^{-1} Zn^{2+}, and the trehalose content increased. The glucose and starch content increased at higher Zn^{2+} concentrations. The fecundity and trehalose content of *N. lugens* decreased after feeding on zinc-stressed rice, and the glucose content in the high Zn^{2+} group was significantly higher than that in the low Zn^{2+} group. In addition, the soluble trehalase activity of *N. lugens* significantly decreased under the 125 mg·kg^{-1} treatment, while the activity of membrane-bound trehalase significantly increased under the 150 mg·kg^{-1} treatment. Quantitative RT-PCR indicated significantly lower expressions of *NlTre1-1*, *NlTre2*, and *NlTps* after Zn treatment. In conclusion, Zn^{2+} treatment significantly altered the sugar content in rice plants; it also decreased the fecundity of *N. lugens*, which may be mediated by alterations in trehalose metabolism.

Keywords: carbohydrate metabolism; fecundity; heavy metal; pest; planthopper; agriculture

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1. Introduction

Rice (*Oryza sativa* L.) is a major staple crop worldwide, supporting the diets and livelihoods of more than 3.5 billion people and other animals [1,2]. Non-essential elements, such as cadmium, lead, and arsenic, are toxic to rice plants as well as to humans [3–5]. However, the hazards of essential trace elements cannot be ignored. Zinc (Zn), an essential trace element, plays a catalytic or regulatory role as a structural cofactor of many enzymes and regulatory proteins, and it is indispensable in the growth and development of rice plants [6]. The known Zn-containing proteins in plants include carbonic anhydrase, alcohol dehydrogenase, copper/zinc superoxide dismutase, and a large numbers of zinc finger domain proteins with transcriptional regulation [7]. Although Zn deficiency in plants is common due to high pH, low redox potential, organic matter, and other factors [6], excessive Zn is found in certain areas. The sampling results of farmland in China showed that although zinc pollution is slight in China, 6.55% of the areas still exceed the standard [8]. Excessive Zn inhibits seed germination and root and aerial growth [9,10]. However, limited studies have been conducted on the accumulation characteristics of Zn and its effect on the metabolism in rice plants. Furthermore, herbivorous insects are exposed to heavy metal pollution [11], which affects their behavior, development, and reproduction [12]. The brown planthopper *Nilaparvata lugens*, a key pest in paddy fields, has a short growth period

and exhibits rapid reproduction and strong environmental adaptability [13–15]. The results of isotopic research showed Zn enrichment in *N. lugens* [13]. Therefore, it is necessary to assess the effects of zinc pollution on *N. lugens* at macro and micro levels.

Carbohydrates and their metabolism play key roles in animals and plants. Glucose, a product of photosynthesis, is a substrate of cellular respiration and acts as a signal molecule that regulates gene expression and metabolite production [16,17]. Although glucose is the primary source of energy in plants and animals, trehalose is the main player in insect hemolymph [18]. Trehalose is a soluble sugar composed of two glucose molecules and is widely found in bacteria, fungi, plants, and invertebrates [19,20]. It provides the energy necessary for flight and other physiological activities in insects [18,21,22]. Although trehalose content in plants is extremely low, it plays an important role in drought and low-temperature stress [23,24]. In addition to glucose and trehalose, starch and glycogen are important energy-storing carbohydrates in plants and animals, respectively [25]. Previous studies have shown that the content of trehalose, glucose, and starch in rice plants were significantly changed after *N. lugens* sucking [26], and the sugar content in *N. lugens* significantly decreased after feeding on the resistant varieties [27]. These results suggest that carbohydrates play an important role in plant–insect interactions. Carbohydrate metabolism, in turn, is affected by heavy metals; for example, the soluble sugar content of *Aster tripolium* significantly decreased under Cd and Pb stress [28]. It remains unclear how the sugar content of rice plants changes under heavy metal stress. Additionally, the trehalase inhibitor, validamycin, stimulates reproduction in *N. lugens*, and it increases the glucose content in rice plants [29]. Hence, these raise important questions, such as (1) whether heavy metal stress alters carbohydrate content and metabolism in rice plants, and (2) whether the fecundity of *N. lugens* changes with the carbohydrate content of the heavy-metal-contaminated rice. Therefore, in this study, we firstly explored the effects of excessive Zn^{2+} on the sugar content of rice plants; secondly, we determined effects of feeding on Zn-stressed rice plants on the fecundity and trehalose metabolism of *N. lugens*.

2. Materials and Methods

2.1. Zn^{2+} Treatment, Sampling, and Zn Detection

The rice variety tested in this experiment was *Oryza sativa* L. Taichung Native 1 (TN1). According to the references and Risk Control Standards for Soil Contamination of Agricultural Land in China, $ZnCl_2$ powder was dissolved in running water to prepare the Zn^{2+} solutions (0, 75, 100, 125, and 150 mg·kg^{-1}). Plastic basins (50 × 40 cm) were filled with 10 kg soil, irrigated with solutions with different concentrations of Zn^{2+} solution, and the 3-week-old seedlings were carefully inserted into the soil. Nine rice plants were inserted into each pot in a 3 × 3 arrangement, and each treatment was repeated three times. No insecticides or zinc-containing fertilizers were used. The soil and rice plants were collected during the stooling stage to determine the Zn content, and the rice stems were used to determine sugar content and trehalase (Tre) activity. The collected soil was air-dried in the dark, ground, and assessed for Zn content. The rice plants were rinsed with distilled water and deionized water, heated for 30 min at 105 °C to inactivate enzymes, and dried at 70 °C before the estimation of Zn content.

2.2. Insect Rearing, Sampling, and Fecundity Measurement

The *N. lugens* maintained in the laboratory were used as the experimental insect, and they were obtained from local rice fields at the China National Rice Research Institute, Hangzhou, Zhejiang, China. The rearing conditions in the artificial climate chamber were set as follows: temperature 25 ± 1 °C, relative humidity 70 ± 5%, and photoperiod 14L:10D. At the stooling stage, the three rice plants in the center row were covered with a clear plastic hard membrane with a gauze top to ensure ventilation and prevent the *N. lugens* from escaping (Figure 1). Three emerging females and three males were put in the cover, and the adults were removed after free mating for 7 d (Figure 1). The number of nymphs was recorded when the first nymphs appeared until almost no nymphs hatched. The

experiment was conducted in three biological replicates. After the hatched *N. lugens* grew to the fifth instar, thirty nymphs from each replicate were selected for assessment of sugar content and enzyme activity; five nymphs were used to determine the relative expression of trehalose metabolism-related genes.

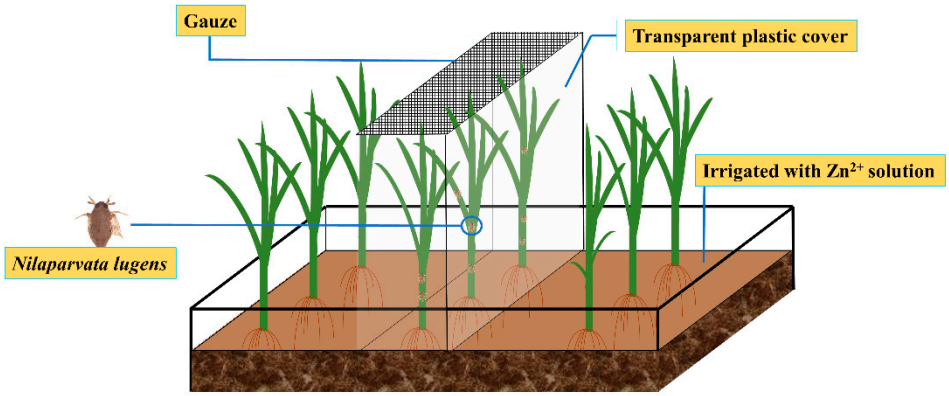

Figure 1. Zinc-treated soil–rice–*Nilaparvata lugens* pattern. Soil was irrigated with different concentrations of Zn^{2+} solution (0, 75, 100, 125, and 150 mg·kg^{-1}). Three rice plants during the stooling stage in the middle of the plastic pot were covered with a clear plastic hard membrane, and the top was covered with gauze to ensure ventilation and prevent the *N. lugens* from escaping. Three emerging females and three males were put into the cover, and the adults were removed after free mating for 7 d.

2.3. Determination of Sugar Content and Trehalase Activity in Rice Plants and N. lugens

The rice stems were ground into powder under liquid nitrogen, and 100 mg of powder was placed into 1.5 mL EP tubes containing 200 µL PBS. Similarly, 15 fifth-instar nymphs were placed into 1.5 mL EP tubes containing 200 µL PBS and ground using an electric homogenizer. The homogenates of *N. lugens* and rice plants were lysed using an Bioruptor UCD-200 (Diagenode, Seraing, Belgium) for 30 min at 320 W, 800 µL PBS was added, and the mixture was centrifuged at 1000× *g* for 20 min at 4 °C to obtain a clear supernatant [30].

For the *N. lugens* samples, 350 µL supernatant was used to determine trehalose and glycogen concentration as well as protein. The remaining 350 µL supernatant was ultracentrifuged at 20,800× *g* for 1 h at 4 °C. The ultracentrifuged supernatant was used to determine the glucose and protein contents and soluble trehalase (Tre1) activity, while the ultracentrifuged precipitate was resuspended in 300 µL PBS and used to measure glucose and protein contents and membrane-bound trehalase (Tre2) activity [30]. Rice samples were treated in a similar manner as the *N. lugens* samples, except that, instead of glycogen, the starch content was determined in the 1000× *g* centrifuged supernatant.

Sugar content and enzyme activity are expressed by their ratio to the total protein. BCA Protein Assay kit (Beyotime, Haimen, China) was used to determine the total protein. Glucose content was measured using the Glucose Assay Kit (Sigma-Aldrich, St. Louis, MO, USA). Trehalose content was determined by the anthrone method [30]. The glycogen content was determined according to the instructions of Glucose Assay Kit after the 1000× supernatant (containing glycogen or starch) was treated with amylotransglucosidase (Sigma-Aldrich, St. Louis, MO, USA). In principle, trehalose is decomposed into glucose by trehalase. A glucose–standard curve was generated, the ultracentrifuged supernatants and precipitates were incubated with 40 mmol/L trehalose for 1 h (Sigma-Aldrich, St. Louis, MO, USA), and the trehalase activity was determined using the standard curve [21,30].

2.4. Gene Expression Levels Pertaining to Trehalose Metabolism in N. lugens

The total RNA from *N. lugens* nymphs was extracted with trizol and resolved by 1% agarose gel electrophoresis to determine its quality. RNA concentration and purity were determined by NanoDrop 2000 (Thermo Fisher Scientific, Waltham, MA, USA). The first

strand of cDNA was synthesized by reverse transcription with Prime Script®RT reagent Kit with gDNA Eraser (TaKaRa, Kyoto, Japan). The expression of trehalose metabolism-related genes in *N. lugens* was determined by qRT-PCR using cDNA as a template, actin gene as an internal control, and specific primers (Table 1) [30,31]. The qRT-PCR mixture was 10 µL and consisted of 10 pmol forward primer, 10 pmol reverse primer, 5 µL SYBR buffer (TaKaRa, Kyoto, Japan), 3.2 µL ddH_2O, and 1 µL template cDNA. The reaction was performed on Bio-Rad CFX96TM real-time PCR detection system (Bio-Rad, Hercules, CA, USA) using the following protocol: initial denaturation at 95 °C for 3 min, 35 cycles of denaturing at 95 °C for 5 s, annealing at 60 °C for 10 min, and extending at 72 °C for 30 s, followed by a final extension at 72 °C for 10 min. The specificity of primers was detected according to the dissolution curve, and technical experiments were repeated three times. The relative mRNA levels were calculated using the $2^{-\triangle\triangle CT}$ method [32].

Table 1. Primers for quantitative real-time polymerase chain reaction.

Primer Name	Forward Primer (5′–3′)	Reverse Primer (5′–3′)
NlTre1-1	CCTCGGCTCTATTCGTTC	ACCGCTTGACCAGTGAGA
NlTre1-2	GATCGCACGGATGTTTA	AATGGCGTTCAAGTCAA
NlTre2-2	CGTGCCAGGTGGACGGTTTA	ATGGGAGCGAGCAGAGGGAG
NlTps1	AAGACTGAGGCGAATGGT	AAGGTGGAAATGGAATGTG
NlTps2	GACAGGCGGTTGAAGAAGA	CAGTAGTCGCTGATGTGGAA
Actin	CCGAGATTTGACCGATTAC	GGTTGCCATTTCCTGTTC

2.5. Statistical Analyses

All the figures in this study are presented as mean ± SEs ($n = 3$). The relative expression of genes in *N. lugens* was analyzed by the t-test; a double asterisk indicates an extremely significant difference in mRNA levels ($p < 0.01$), and a single asterisk indicates a significant difference ($p < 0.05$). All the remaining data were analyzed using one-way analysis of variance (ANOVA) and Tukey's test. In addition, the Zn content in soil and rice plants after Zn^{2+} irrigation was analyzed with regression analysis.

3. Results

3.1. Zn Concentration in Soil and Rice Plants after Zn^{2+} Irrigation

Zn content in the soil increased with the increase in Zn^{2+} irrigation concentration (Figure 2A,B). The linear regression analysis also indicated that the Zn content in soil had significant correction with Zn^{2+} concentrations (Table 2). However, the Zn content in rice stem was non-linear. Within a certain range, the Zn content in the rice stem increased with the increase in Zn^{2+} irrigation concentration. However, when Zn^{2+} concentrations were 125 and 150 mg·kg^{-1}, a slight decrease of Zn content was observed in rice plants compared with 100 mg·kg^{-1} concentration, and it may indicate that the Zn content is relatively stable in rice plant irrigated with solution containing higher Zn^{2+} concentrations (Figure 2B). The results of curve-fitting also showed that the relationship between Zn content in rice stem and Zn irrigation concentration was closer to the growth curve (Table 2).

3.2. Nymph Numbers of N. lugens

Since the first day (Day 1), *N. lugens* nymphs began hatching; the hatching number in the control (CK) group was the highest on Day 4, while those in the Zn75, Zn125, and Zn150 groups were the highest on Day 3, and that in Zn100 group was the highest on Day 5 (Figure 3A). In addition, the daily hatching number and total hatching number of *N. lugens* in the Zn treatment groups were lower than in those in the CK group, and the hatching number of *N. lugens* in the Zn75 group was the lowest (Figure 3A). However, compared with the Zn75 group, the number of nymphs increased with the increase in Zn^{2+}, and in the Zn150 group, the number of nymphs recovered to almost the same as that in the CK group (Figure 3B).

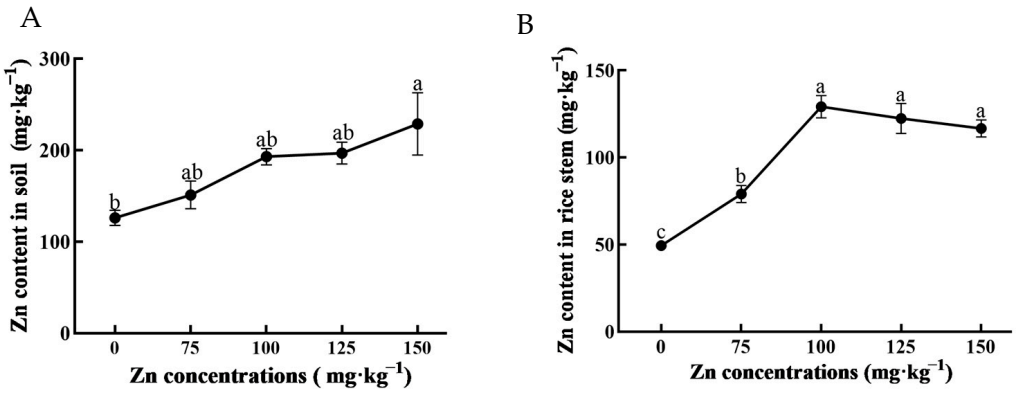

Figure 2. Zn content accumulated in soil (**A**) and rice plants (**B**) after irrigating with different concentrations of Zn^{2+} solution. The data are represented as mean ± SE (n = 3). Tukey's test was used to detect the significant differences among different groups, and different letters indicate significant differences.

Table 2. Regression analysis of Zn accumulation in soil and rice stem.

	Zn^{2+} Concentrations		Regression Type
Zn content in soil	Pearson correlation	0.957	Linear
	Sig.	0.005	
	R^2	0.917	
	N	5	
	Regression equation	$y = 0.675x + 118.48$	
Zn content in rice stem	Model	Growth curve	Curvilinear
	Sig.	0.028	
	R^2	0.842	
	Regression equation	$y = e^{(3.957+0.06)\cdot x}$	

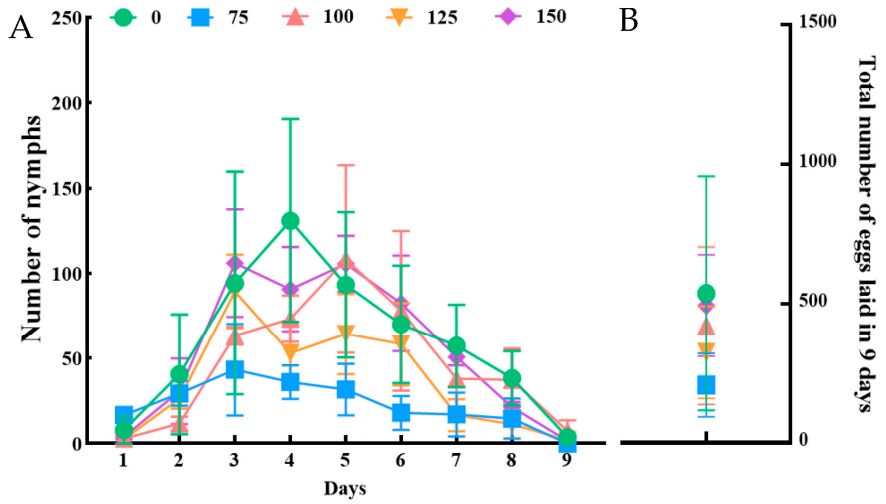

Figure 3. Effects of Zn-treated rice plants on reproduction of *N. lugens*. The figure on the left (**A**) represents the number of nymphs hatched by three females in a single day, and the figure on the right (**B**) represents the total number of nymphs hatched by three females in 9 days. The data are represented as mean ± SE (n = 3).

3.3. Sugar Content and Tre Activity in Rice Plants under Zn Stress

The trehalose content in the rice stems of the Zn100 group significantly increased compared with that in other treatment groups (Figure 4A). Glucose content significantly increased when Zn^{2+} irrigation concentration reached 125 mg·kg^{-1} and 150 mg·kg^{-1}, but there was no significant difference between the control group and the group subjected to lower Zn concentrations (Figure 4B). Similarly, the starch content was significantly higher in the Zn125 group, while there were no significant differences among the other Zn treatment groups and the control group (Figure 4C). In agreement with the trehalose content, Tre activity was significantly lower in the Zn100 group than in the CK group (Figure 4D). However, no significant differences in trehalose content and Tre activities were observed among the other Zn treatment groups (Figure 4A,D).

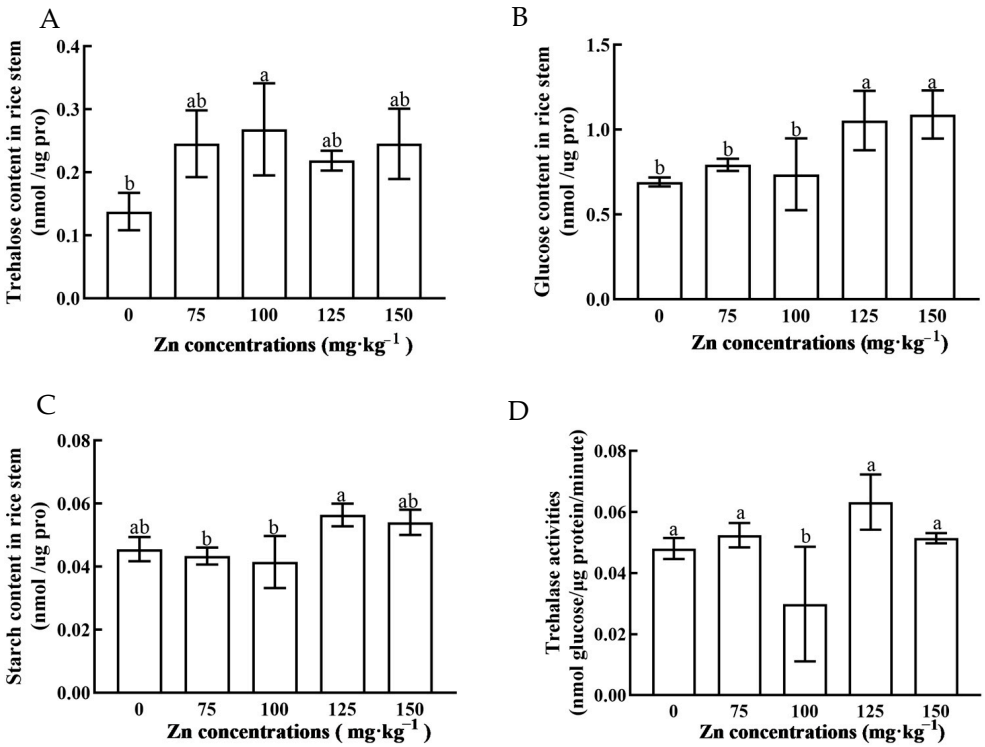

Figure 4. Effects of Zn accumulation on trehalose (**A**), glucose (**B**), and starch (**C**) contents and trehalase activity (**D**) in rice stems. The data are represented as mean ± SE (n = 3). Tukey's test was used to detect the significant differences among different groups, and different letters indicate significant differences.

3.4. Comparison of Sugar Content in N. lugens Feeding on Zn-Stressed Rice

The trehalose content of *N. lugens* decreased moderately after feeding on rice plants exposed to Zn concentrations lower than 150 mg·kg^{-1} (Figure 5A). Compared with the CK group, the glucose content of *N. lugens* in Zn-treated groups did not change significantly, but the glucose content in the Zn150 group was significantly higher than those in the Zn75 and Zn100 groups (Figure 5B). However, no differences were observed in the glycogen content among all the groups (Figure 5C).

3.5. Tre Activity of N. lugens Feeding on Zn-Stressed Rice

Tre1 activities in the Zn100 and Zn150 groups of *N. lugens* were significantly higher than those in Zn75 groups, but there was no significant difference compared with the CK group (Figure 6A). In addition, Tre1 activity in the Zn125 group of *N. lugens* was significantly more reduced than those in the Zn100 and Zn150 groups (Figure 6A). Compared with the

CK group, Tre2 activity significantly increased in the Zn150 group, which corroborated the change in trehalose content (Figure 6B).

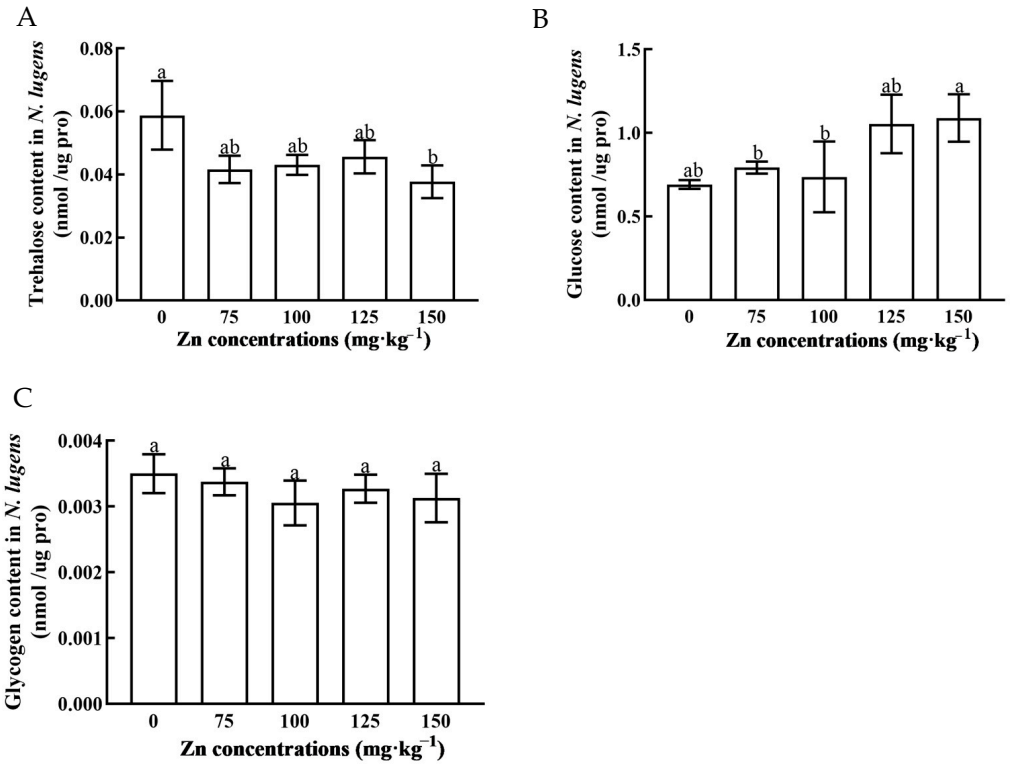

Figure 5. Effects of Zn accumulation in rice plants on trehalose (**A**), glucose (**B**), and starch (**C**) contents of *N. lugens*. The data are represented as mean ± SE (n = 3). Tukey's test was used to detect the significant differences among different groups, and different letters indicate significant differences.

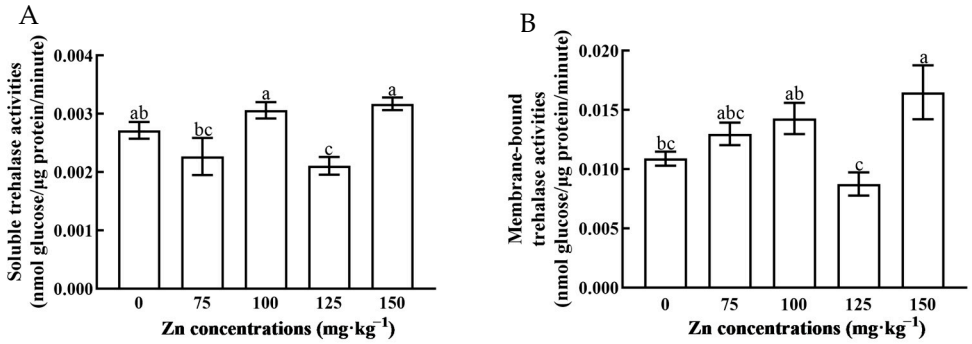

Figure 6. Effects of Zn accumulation in rice plants on the soluble (**A**) and membrane-bound (**B**) trehalase activities of *N. lugens*. The data are represented as mean ± SE (n = 3). Tukey's test was used to detect the significant differences among different groups, and different letters indicate significant differences.

3.6. Expression of Genes Related to Trehalose Metabolism in N. lugens

The relative expression levels of *NlTre1-1* and *NlTre2* were significantly lower in the Zn125 group than the CK group, and there was no significant difference among the other Zn-treated groups and the CK group (Figure 7A). However, there were no significant differences in the expression of *NlTre1-2* among any of the groups (Figure 7A). When compared with the CK group, *NlTps1* was significantly downregulated in the Zn75 and Zn100 groups, but not in the Zn125 and Zn150 groups (Figure 7B). When compared with

the CK group, *NlTps2* was significantly downregulated in the Zn75, Zn100, and Zn125 groups, but not in the Zn150 group (Figure 7B).

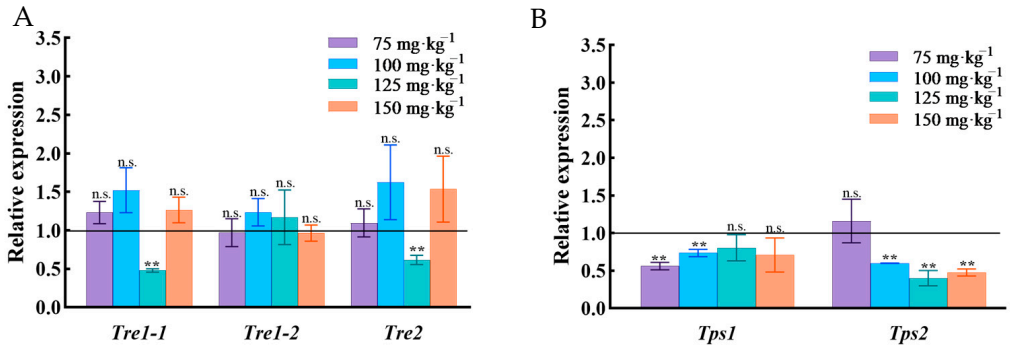

Figure 7. Effects of Zn accumulation in rice plants on the expression of *Tre* (**A**) and *Tps* (**B**) in *N. lugens*. The qRT-PCR was used to determine the relative expression of genes, and actin was used as the internal control. The horizontal line in the figure represents the gene expression level in the control group. Data are represented as mean ± SE (n = 3), and the significance of differences in gene expression between the treatment and control groups was analyzed with t-tests. * $p < 0.05$; ** $p < 0.01$.

4. Discussion

Cd, As, and Zn are known to exhibit high mobility from soil to rice roots, indicating their high bioavailability [33]. Our study also indicated that the Zn content in soil significantly increased as Zn^{2+} concentration increased in the irrigation water. Correspondingly, we found that the Zn accumulated in the rice plants treated with irrigation water with 100 mg·kg^{-1} Zn^{2+} concentration. However, unlike the hyperaccumulator plants, such as *Sedum plumbizincicola* [10,34], the rice plant has a limited uptake of Zn. According to the regression analysis, we have speculated that when the concentration of Zn^{2+} in irrigation water reaches a certain value, the accumulated Zn content in rice stems tends to be saturated, but it will not decrease significantly. Metal transporters, such as OsZIP1, promote the outflow of Zn, Cu, and Cd metal ions in rice [35]. This might be the reason why the Zn accumulation in rice did not increase at higher Zn concentrations. However, the range of zinc concentration gradient set in this study is limited, so more accurate conclusions need to be further explored.

Previous studies have shown that the fecundity of female gypsy moth larvae decreased after feeding on poplar trees planted in Zn^{2+}-contaminated soil (500 mg·kg^{-1}) [36], and heavy metal exposure reduced the hatching success of *Acartia pacifica* resting eggs in the sediment [37]. In our study, lower hatching was observed in *N. lugens* nymphs feeding on Zn-stressed rice than that in the control, likely due to the accumulation of Zn in the *N. lugens*. However, it was noteworthy that among the Zn-stressed groups, increase in Zn^{2+} concentration led to increase in hatching of *N. lugens* nymphs; it recovered to that of the control group when the Zn^{2+} concentration was 150 mg·kg^{-1}. Similar results were obtained in a Zn stress study using *Harmonia axyridis*, wherein low Zn levels prevented egg-laying, but high Zn levels increased the number of eggs [38]. Thus, insects may increase reproduction after heavy metal exposure to cope with the impact of stress on the population. However, previous studies focused mostly on the effects of heavy metals on insect reproduction by incorporating heavy metals in artificial diets, while this study adopted the plant-mediated method. Plant metabolism also has a significant effect on the brown planthopper [39]. Therefore, the decreased fecundity of the *N. lugens* may be attributed to physiological changes of the Zn-stressed plant. Considering this reason, we also tested the change of sugar content in rice plants after zinc treatment.

Our study showed that Zn accumulation (Zn100) in rice led to a decrease in Tre activity and an increase in trehalose content. High concentrations of exogenous trehalose decreased the absorption of lead by *Lemna Gibba* [40] and significantly mitigated the toxic effects of excessive Cu on photosynthesis and plant-growth-related parameters [41]. Thus, trehalose

may play an important role in heavy metal stress response. Previous studies have shown that the total amount of carbohydrates in plant leaves significantly increased under Pb stress [42], and glucose increased under Cd and As stress [43] Our study also showed that the glucose and starch contents in rice were significantly higher in high Zn-stress than in low Zn-stress. On the one hand, it may be due to the accumulation of heavy metals in rice tissues, which affects physiological processes, such as transpiration and production of reactive oxygen species (ROS) [42,44], resulting in a lower utilization of sugars in rice [45]. On the other hand, starch and glucose accumulation may be a physiological adaptation of rice to heavy metal stress because carbohydrate accumulation in leaves helps plants to maintain osmotic pressure and eliminate reactive oxygen species under abiotic stress conditions [42,43,46]. Previous research reported that trehalase inhibitor validamycin stimulated the reproduction of *N. lugens* by increasing the glucose content of rice plants [29]. Similarly, in our study, a moderate recovery in the fecundity of *N. lugens* was observed with the increase of glucose content in rice plant, as mentioned, indicating that Zn-mediated sugar changes in plants affected the reproduction of *N. lugens*.

N. lugens suck the juice from phloem of rice plants, which is rich in carbohydrates, amino acids, and other nutrients and has a great influence on the physiological metabolism of *N. lugens*. Results showed that the trehalose content in rice stem increased under Zn stress, but the trehalose content of *N. lugens* significantly decreased in the Zn150 group, which was contrary to *Aedes albopictus*, in which the trehalose content significantly increased under Cd stress [47]. The trehalose metabolism is closely related to reproduction. For example, as a synergistic substance in artificial diets, trehalose significantly improved the reproduction of *H. axyridis* [48], and hypertrehalosemic hormone and adipokines increased the egg production by increasing trehalose [49,50]. Hence, the falling of *N.lugens* nymphs may be caused by decreased trehalose content in *N.lugens*. As for the inconformity of trehalose content between *N.lugens* and rice stem, it may be attributed to trehalase. Trehalase in insects include the soluble trehalase (Tre1) and membrane-bound trehalase (Tre2) [18,21,51]. Tre1 acts on endogenous trehalose found in circulatory and digestive systems, while Tre2 acts on exogenous trehalose expressed in fat body, midgut, and Malpighian tubes [18]. Our results demonstrated differences in the enzyme activities of Tre1 and Tre2 in *N. lugens* fed on zinc-stressed rice plant. A lower Tre1 activity was observed in the Zn125 group, while higher Tre2 activity was observed in the Zn150 group when compared with the control group. These results suggested that Tre2 of *N. lugens* likely degraded an excess of exogenous trehalose from rice plant to cope with Zn stress. Meanwhile, Zn accumulation had minimal effect on glycogen in *N. lugens*, but the glucose content in high-Zn group was significantly higher than that in the low-Zn group, which was conductive to restoration of fecundity [52]. Obviously, this is consistent with the change of glucose content in rice plant. Therefore, the increased glucose in rice plant under high-Zn treatment may have led to the increased absorption of glucose by *N. lugens*. Certainly, it was also possible that Zn directly acts on *N.lugens*, such as regulating the transcription level of glucose-regulated protein [53]. The change of sugar content changed the trehalose metabolism gene expression. Soluble trehalase of *N.lugens* has two coding genes, namely *Tre1-1* and *Tre1-2* [54]. The expressions of *NlTre1-1* and *NlTre2* were significantly decreased when the Zn^{2+} concentration was 125 $mg \cdot kg^{-1}$, which relieved the rate of trehalose degradation to maintain the balance of trehalose concentration. In addition, the genes responsible for trehalose biosynthesis, *Tps1* and *Tps2* [55], were also downregulated after feeding on Zn-stressed rice plants, indicating that the trehalose metabolic rate of *N. lugens* decreased.

5. Conclusions

Zinc effectively accumulated in rice plants, and high concentration of Zn increased the content of trehalose and glucose in rice plants. Meanwhile, the Zn-stressed rice plants altered the trehalose metabolism and reduced fecundity in *N. lugens*. Interestingly, the reproduction of *N. lugens* exhibited recovery with an increase in Zn concentration. However, in this study, the plant-mediated method was used to explore the effects of Zn pollution

on *N. lugens*, so it is not clear whether the inhibitory effect of Zn on the reproduction of *N. lugens* is direct or indirect. In conclusion, this study explored the effects of Zn treatment on the soil–rice–*N. lugens* system at the molecular level, and it provides a theoretical basis for assessment of ecological risk from heavy metal pollution in paddy ecosystems.

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