

## Article

# Characterizing the Mechanism of Serotonin Alleviates Rice Resistance to Brown Planthopper *Nilaparvata lugens* (Homoptera: Delphacidae) Nymphs

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**Abstract:** Serotonin is a well-known secondary metabolite that plays an important role in many growth and developmental processes, as well as biotic and abiotic stress responses of plants. Yet, whether serotonin biosynthesis regulates the resistance of rice to the brown planthopper (BPH) *Nilaparvata lugens* and its underlying mechanisms have not been entirely investigated. In this study, we found that expression levels of four serotonin biosynthesis gene, *OsTDC1*, *OsTDC2*, *OsTDC3*, and *OsT5H*, and the serotonin content were significantly induced by BPH nymph infestation. The rice seedlings pretreated with serotonin were more attractive to BPH nymphs and promoted their feeding behavior as indicated by increased honeydew excretion. In addition, serotonin application to artificial diets increased the survival rates of BPH nymphs in a dose-dependent manner compared to the controls. Moreover, the exogenous serotonin application to rice seedlings increased the average injury scale and functional plant loss indices caused by BPH nymph infestation. Moreover, supplemented serotonin enhanced soluble sugar and free amino acid contents, as well as the activities of SOD, POD, and PPO, but repressed the production of flavonoids. Our findings illustrated that serotonin plays a negative role in the regulation of rice resistance to BPH nymphs probably by modulating contents of soluble sugars, free amino acids, and flavonoids.

**Keywords:** rice; *Nilaparvata lugens*; serotonin; insect resistance



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## 1. Introduction

When plants are attacked by herbivorous insects, their own immune systems rapidly initiate relevant defense-related signaling networks upon recognition of herbivore-derived signals, resulting in the elicitation of biosynthesis and production of a series of defensive metabolic compounds [1–3]. Some of the metabolic compounds can serve as signal molecules in modulating herbivore-induced plant defenses [4]. Recent research advances have demonstrated that the essential aromatic amino acid tryptophan (Trp) and its derived compounds have regulatory functions in plant stress responses [5]. In *Arabidopsis thaliana*, for instance, mutants lacking Trp-derived defensive metabolites are more sensitive to the two-spotted spider mite, *Tetranychus urticae* [6]. Moreover, the indole glucosinolates derived from tryptophan are key parts of AtMYC2/AtMYC3/AtMYC4-regulated defenses against mites [6].

The monoamine serotonin, also known as 5-hydroxytryptamine, is a well-studied metabolite synthesized from tryptophan as the precursor [5]. Initially found in animals, serotonin functions as an important neurotransmitter in mammals and can directly influence insect feeding behavior, reproduction, and immunity [7]. In plants, serotonin is widely present in over 90 species and 37 plant families [8]. The serotonin biosynthesis in

plants consists of two enzymatic steps: tryptophan is first decarboxylated by tryptophan decarboxylase (TDC) to produce tryptamine, which is then hydroxylated into serotonin by tryptamine 5-hydroxylase (T5H) [8,9]. In rice *Oryza sativa* L. (Poales: Poaceae), there are three copies of TDC, named *OsTDC1*, *OsTDC2*, *OsTDC3*; furthermore, overexpression of *OsTDC1*, *OsTDC2*, or *OsTDC3* leads to enhanced serotonin levels [10,11]. An increasing number of studies demonstrate that serotonin acts as a vital signaling molecule in modulating nearly all aspects of plant growth and development, such as seed formation, root growth and development, fruit ripening, and senescence [8,9,12]. In addition, the roles of serotonin in the plant response to several abiotic stresses such as UV-B stress, cold, salinity, drought, heavy metals, and thermal stress are also well characterized [9,13–16].

Aside from the pivotal role of serotonin in the modulation of growth and developmental processes, and abiotic stress responses in plants, serotonin also has been spotted to be involved in plant responses to biotic stresses [17,18]. For example, leaves of barley *Hordeum vulgare* accumulate high levels of serotonin in response to infection with the filamentous fungus *Bipolaris sorokiniana* [19]. Infection of rice leaves with the rice brown spot fungus *Bipolaris oryzae* enhanced accumulation of high concentrations of serotonin. A rice mutant deficient in serotonin production displayed increased susceptibility to *B. oryzae* infection [20]. Similarly, serotonin was also found to serve as a scavenger of oxygen radicals to suppress oxidative damage caused by hypersensitive response in rice plants infected with the blast fungus *Magnaporthe oryzae* [21]. Likewise, several studies have unveiled the evident role of serotonin in mediating plant defense against herbivory. In *Setaria viridis*, for instance, exogenous application of serotonin to the plant medium reduced the survival of *Rhopalosiphum padi* aphid adults, as well as the body weight of the surviving aphid adults [22]. It has also been reported that serotonin content clearly increased following the feeding of rice striped stem borer *Chilo suppressalis* in rice leaves [23]. Recently, Lu et al. [24] found that rice serotonin accumulation was significantly increased with the infestation of gravid brown planthopper (BPH) *Nilaparvata lugens* female adults, and the serotonin levels of BPH-resistant rice varieties were significantly lower than that of BPH-susceptible varieties. Moreover, *CYP71A1* knockout rice mutants defected in serotonin synthesis showed enhanced resistance to BPH female adults [24]. However, it remains to be explored further whether the serotonin is involved in BPH nymphs-induced rice defense and its underlying mechanism of function, while the response of rice plants to planthopper nymphs is different from that of gravid planthopper females [25].

Rice is the most important staple food source for more than 60% Chinese population. The annual total growing area of rice in China is about 30 million hectare with a total of approximately 200 million tons of rice yield [26]. BPH is one of the most devastating pests in rice, which may cause rice “hopper-burn” under severe infestation, resulting in serious yield reduction and harvest failure worldwide [26–28]. Previous studies have demonstrated that nutrient constituents, such as soluble sugars and free amino acids, are important indexes of host plant quality and are associated with BPH-induced rice defense [29,30]. In addition, the role of flavonoids, and defensive proteins including peroxidase (POD), superoxide dismutase (SOD), catalase (CAT), and polyphenol oxidase (PPO) in regulating rice resistance to BPH has also been well documented [31–34].

Given the role of serotonin played in plant defense, the objectives of this study were to investigate the function and molecular mechanism of serotonin in regulating rice resistance against BPH nymphs. To address the function of serotonin in mediating rice resistance to BPH nymphs, we analyzed the expression patterns of serotonin biosynthetic genes and serotonin contents in response to BPH nymph infestation. Furthermore, we demonstrated that serotonin promotes the feeding performance of BPH nymphs and alleviates rice tolerance to BPH by affecting the contents of soluble sugars, free amino acids, and flavonoids, as well as activities of defensive proteins.

## 2. Materials and Methods

### 2.1. Plants and Insects

The rice variety Zhonghua 11 (*Oryza sativa* L. ssp. *japonica*) (ZH11, a BPH medium-resistant variety) was selected, and seeds were soaked in water for 2 days, and germinated in an artificial greenhouse ( $28 \pm 2$  °C, 70–80% relative humidity and 16:8 h photoperiod). Ten-day-old seedlings were then hydroponically cultivated in the same artificial greenhouse with rice nutrient solution [35]. Thirty-eight days old rice seedlings were transplanted into individual black plastic cups (8 cm in diameter and 15 cm in height) for the following experiments.

The BPH population was originally obtained from the China Rice Research Institute (Hangzhou, China) and reared in the greenhouse of the Ecology Laboratory of Yangzhou University under the above conditions.

### 2.2. Transcriptome Analysis

Transcriptome data from our previous research were analyzed [36]. Detailed information including plant treatments and transcriptome analysis can be obtained from Li et al. [36]. FPKM (fragments per kilobase of transcript per million fragments mapped) values of four serotonin biosynthesis genes, *OsTDC1* (accession number Os08g0140300), *OsTDC2* (accession number Os07g0437500), *OsTDC3* (accession number Os08g0140500), and *OsT5H* (also called cytochrome P450 gene *CYP71A1*, accession number Os12g0268000) from BPH-infested and control rice plants were selected to generate the heatmap using TB tools [37].

### 2.3. Quantification of Serotonin in Rice after BPH Nymph Infestation

To verify the effect of BPH nymph infestation on levels of serotonin in rice, 30 4th-instar BPH nymphs were individually released onto each rice seedling enclosed with a transparent tube (diameter 4 cm, height 8 cm). Non-infested plants covered with an empty transparent tube were used as the control. Rice leaf sheaths were rapidly harvested and frozen in liquid nitrogen at 8 h post infestation. The serotonin levels were quantified using the method as described in Kang et al. [9], with slight modifications. In brief, the homogenized samples (0.5 g) were extracted with 8 mL of methanol and vortexed for 10 min. The samples were then centrifuged at 13,000 rpm for 5 min. The supernatant was collected and passed through a 0.22 µm filter membrane (SCAA-104, ANPEL, Shanghai, China). The filtrate was mixed with 2 mL (1:4 *v/v*) of distilled water and passed through a Waters Sep-Pak C18 Cartridge. The cartridge was then washed with 10 mL of 80% methanol. The mixture of passing and washing solutions was evaporated to dryness in vacuo at 40 °C and the residue was dissolved in 500 µL of 50% methanol. The samples were measured using reversed-phase high-performance liquid chromatography following the method described by Kang et al. [9]. Four independent replications were used for each treatment.

### 2.4. Measurement of BPH Feeding Preference and Honeydew Production

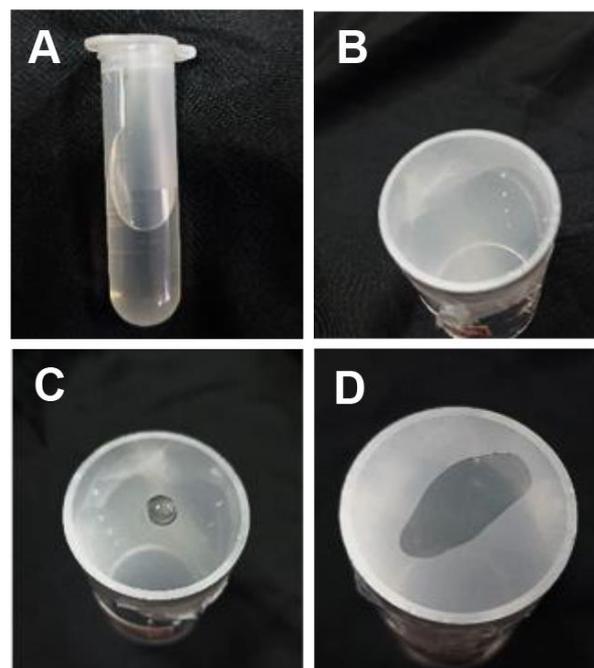
For serotonin treatment, rice seedlings were grown in nutrition solution supplemented with 300 µM serotonin for 24 h as Lu et al. [24] described. Seedlings grown in normal nutrition solution without serotonin supplementation were used as control.

For BPH feeding preference experiment, ten pairs of plants (one serotonin-treated plant and one control plant) were covered with a transparent tube (as stated above). Twenty 3rd-instar BPH nymphs were placed at the center of the paired plants. The number of BPH nymphs settled on each plant was observed at 1, 2, 4, 8, 12, 24, and 48 h after BPH release. Ten independent replications were used for each treatment.

For honeydew production measurement, the basal stem of each plant was enclosed in a pre-weighed Parafilm sachet containing one 3rd-instar BPH nymph. The honeydew excretion in each sachet was weighted 24 h after infestation. Each treatment was replicated ten times.

### 2.5. Direct Effects of Serotonin on BPH Nymphs

To determine the direct effect of serotonin on the survival rate of BPH nymphs, serotonin was added to artificial diets (Figure 1A) at concentrations of 0.1, 1, 2, or 4  $\mu\text{g}/\text{mL}$  [38]. The glass cylinder (8 cm  $\times$  4 cm) with one end covered by a well-stretched Parafilm sandwich containing a 40  $\mu\text{L}$  artificial diet was used as a feeding chamber (Figure 1B–D). Twenty 2nd-instar BPH nymphs were introduced into the feeding chamber. After BPH release, the other end and the glass cylinder were covered with tin foil paper. The BPH nymphs fed on the artificial diet without serotonin were used as control. Each treatment was replicated six times. The number of surviving BPH nymphs in each chamber were counted daily for consecutive 5 days. Survival rate of BPH nymphs was counted with the proportion of surviving BPH nymphs in each chamber in the overall twenty BPH nymphs.



**Figure 1.** Schematic of artificial feeding installation. (A) The solution of BPH artificial diet; (B) artificial feeding chamber (8 cm long and 4 cm in diameter) with one end covered by a well-stretched Paraffin membrane; (C) an artificial diet droplet on the center of Parafilm membrane; (D) the artificial diet droplet sandwiched between two layers of Parafilm membrane.

### 2.6. Determination of Rice Average Injury Scales and Functional Plant Loss Indices

Rice seedlings were individually sprayed with 10 mL of 300  $\mu\text{M}$  serotonin every 24 h for 3 days, and the control was sprayed with 10 mL of water. Seedlings were randomly assigned to BPH infestation at a density of 30 3rd-instar nymphs per plant. The average injury scales and functional plant loss indices of serotonin-treated and control plants were determined as described previously in Liu et al. [39] and Sun et al. [40]. Ten independent replications were used for each treatment.

### 2.7. Quantification of Soluble Sugar, Free Amino Acid, and Flavonoid Content

To determine the content of soluble sugars, free amino acids, and flavonoids in rice, rice seedlings were sprayed with 10 mL of serotonin solutions at concentrations of 50, 100, 200, and 500  $\mu\text{M}$  containing 0.5% Tween-20, respectively. Control plants were sprayed with an equal quantity of water containing 0.5% Tween-20. Leaf sheath samples were collected from the serotonin-treated and control plants 24 h after treatment. Each treatment was replicated three times. The concentrations of soluble sugars, free amino acids, and flavonoids were determined using commercial kits (Suzhou Keming Biotechnology

Co., Ltd., Suzhou, China, product number: KT-1-Y, LHT-1-G, AA-1-W, respectively) according to the manufacturer's instructions.

### 2.8. Measurement of the Activities of Antioxidant Enzymes

To measure the activities of antioxidant enzymes, POD, SOD, CAT, and PPO, the above-described leaf sheath samples were subjected to analyses using commercial kits (Suzhou Keming Biotechnology Co., Ltd., product number: POD-1-Y, SOD-1-Y, CAT-1-Y, PPO-1-Y, respectively) according to the manufacturer's instructions.

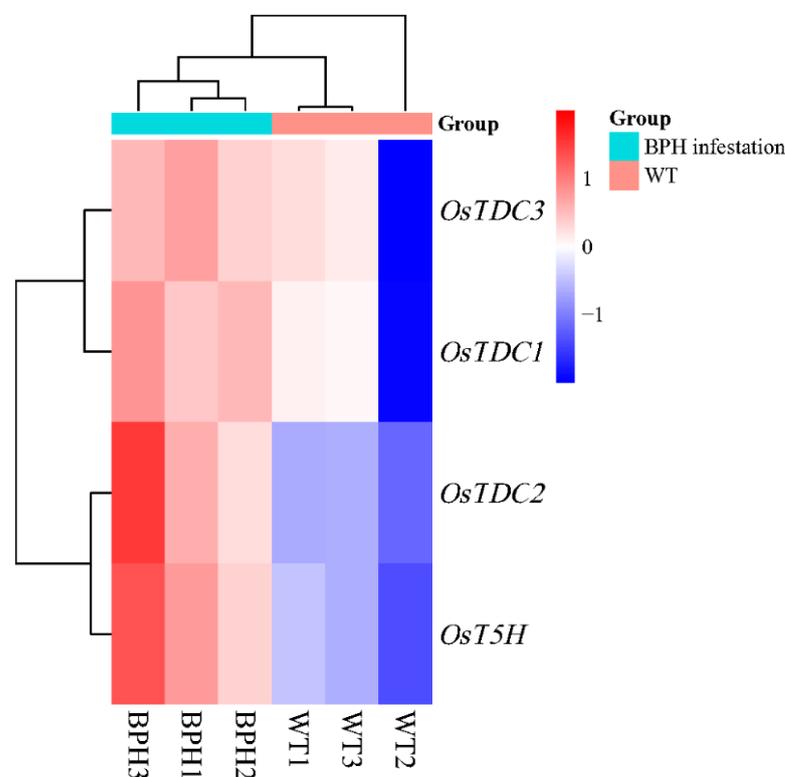
### 2.9. Statistical Analysis

SPSS v 26.0 statistical software (SPSS Inc., Chicago, IL, USA) was used to analyze all data. One-way analysis of variance (ANOVA) followed by the LSD method was used for significance analysis for multiple comparisons. The statistical differences between the two treatments were analyzed by Student's *t*-test. Data were expressed as mean + standard error (SE).

## 3. Results

### 3.1. BPH Nymph Infestation Induces the Expression of the Key Genes in Serotonin Biosynthesis

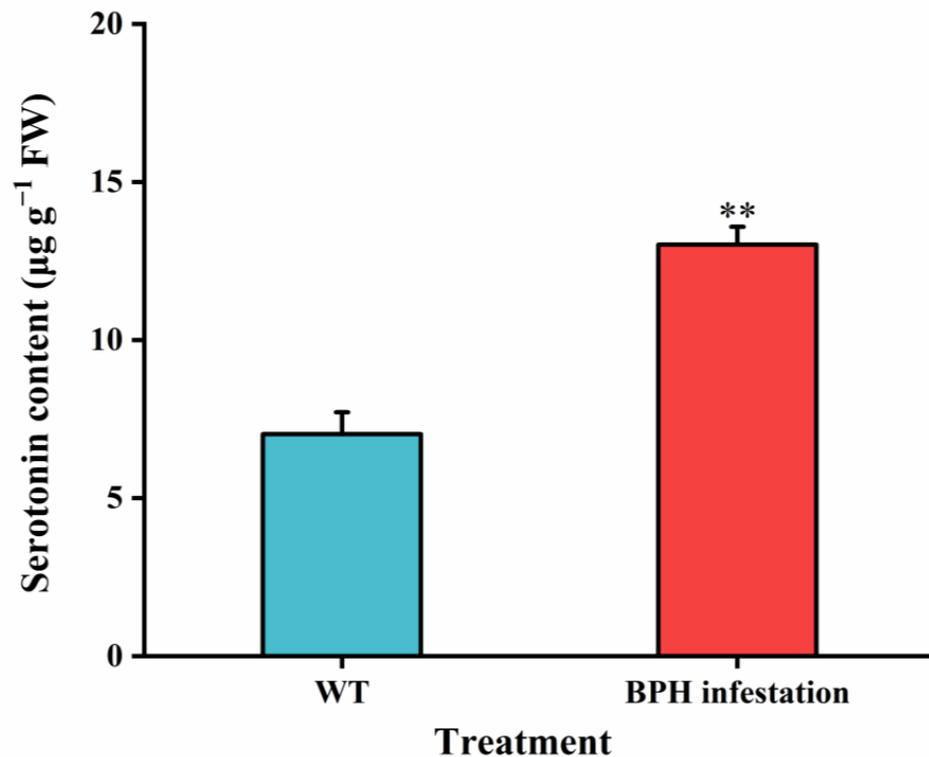
In order to examine whether BPH nymph infestation could induce biosynthesis of serotonin, we analyzed the expression levels of four key biosynthesis genes, including *OsTDC1*, *OsTDC2*, *OsTDC3*, and *OsT5H* in rice at 12 h post BPH nymph infestation by conducting a comprehensive RNA-seq analysis using our previous data [36]. The expression patterns showed that all four genes were upregulated in rice by BPH nymph infestation (Figure 2). This result indicates that serotonin might be involved in BPH nymph-induced rice defense.



**Figure 2.** Heatmap indicates the expression profiles of four key genes involved in serotonin biosynthesis induced by BPH nymph infestation for 12 h. The colors illustrate the expression levels of each gene based on values of  $\log_2$ FPKM within groups of BPH-infested and non-infested (WT) rice plants. Red, upregulation; blue, downregulation.

### 3.2. BPH Nymph Infestation Induces Accumulation of Serotonin in Rice

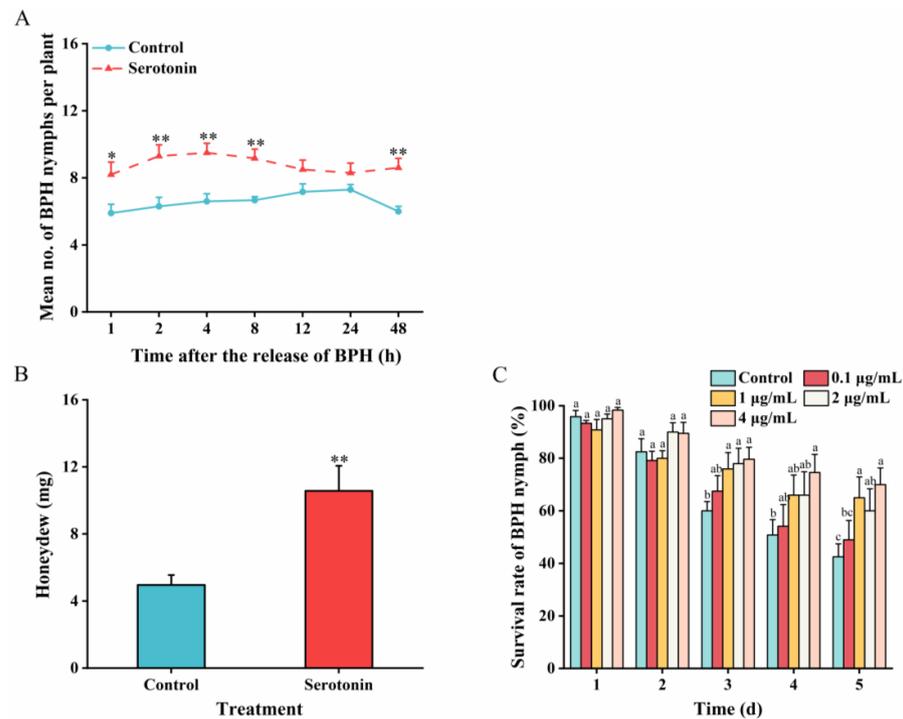
As reported in previous results, the levels of rice serotonin could be induced by gravid BPH female infestation [24]. In this study, to test whether BPH nymph infestation influenced the accumulation of serotonin, we analyzed serotonin levels in rice plants after infestation with BPH nymphs. Consistent with the increased expression of key genes involved in serotonin synthesis, the infestation of BPH nymphs significantly enhanced the accumulation of serotonin in rice 8 h post infestation. The serotonin level in rice plants infested by BPH nymphs was 1.85-fold greater than that in non-infested plant (Figure 3).



**Figure 3.** Influence of BPH nymph infestation on the accumulation of serotonin in rice plants. The data are means (+SE) of serotonin content in rice plants at 8 h post infestation without (WT) or with BPH nymphs. FW, fresh weight. Asterisks indicate significant differences in BPH-infested plants compared with non-infested plants (WT) (\*\*,  $p < 0.01$ ; Student's  $t$ -test).

### 3.3. Exogenous Serotonin Affects BPH Performance

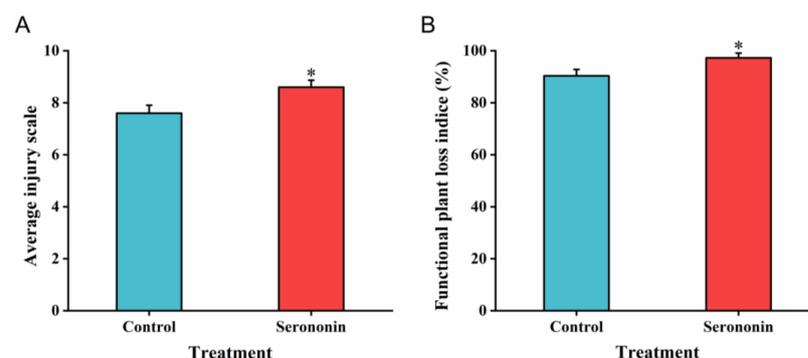
To observe the effect of exogenous serotonin on the BPH nymph performance, we tested the host choice selectivity for BPH nymphs and the amount of honeydew excretion within serotonin-treated or control plants. The BPH nymphs preferred to feed on serotonin-treated rice rather than on control plants (Figure 4A). The honeydew excretion indicates the host suitability, hence the increased (2.13-fold) honeydew excreted by BPH nymphs supports the finding that BPH nymphs prefer to feed on serotonin-treated rice plants (Figure 4B). When BPH nymphs fed on the artificial diet containing varied concentrations of serotonin, their survival rate increased at 3 d post addition of serotonin, and this increase continued until 5 d post addition of serotonin (Figure 4C). These results indicated that exogenously serotonin application promoted BPH nymph performance on rice plants.



**Figure 4.** Effects of exogenous serotonin on the performance of BPH nymphs. (A) Host choice selectivity of BPH on serotonin-treated or control plants; (B) honeydew excretion of BPH on serotonin-treated or control plants; (C) survival rate of BPH nymphs fed on artificial diet containing varied concentrations of serotonin. Values are means + SE. Asterisks indicate significant differences between treatments (\*,  $p < 0.05$ ; \*\*,  $p < 0.01$ ; Student's *t*-test). Different letters represent significant differences among treatments (One-way ANOVA followed by LSD test).

### 3.4. Exogenous Serotonin Alleviates Rice Tolerance to BPH Nymphs

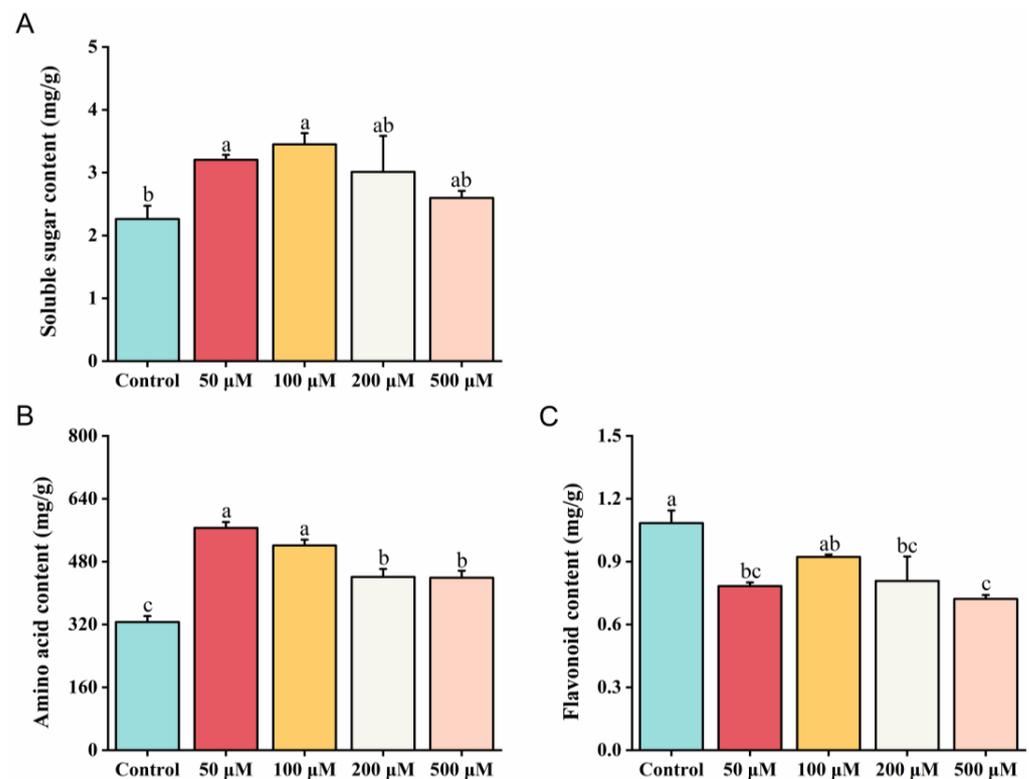
To determine the effect of exogenous serotonin on rice tolerance to BPH nymph infestation, parameters of the average injury scale and functional plant loss indices were investigated in rice plants pre-treated with or without (control) serotonin post infestation of BPH nymphs. The average damage level was significantly higher by approximately 13.16% in serotonin-treated plants relative to control plants (Figure 5A). Furthermore, the functional plant loss index in plants pre-treated with serotonin was significantly higher than in control plants post BPH nymph infestation (Figure 5B). Collectively, rice plants are more vulnerable facing BPH feeding post exogenous serotonin application.



**Figure 5.** Effects of exogenous serotonin on rice tolerance to BPH infestation. Average injury scale (A) and functional plant loss indices (B) were examined in rice plants post BPH infestation with or without (control) pre-treatment of serotonin application. Data are means + SE. Asterisks indicate significant difference between treatments (\*,  $p < 0.05$ ; Student's *t*-test).

### 3.5. Exogenous Serotonin Affects Levels of Rice Soluble Sugars, Free Amino Acids and Flavonoids

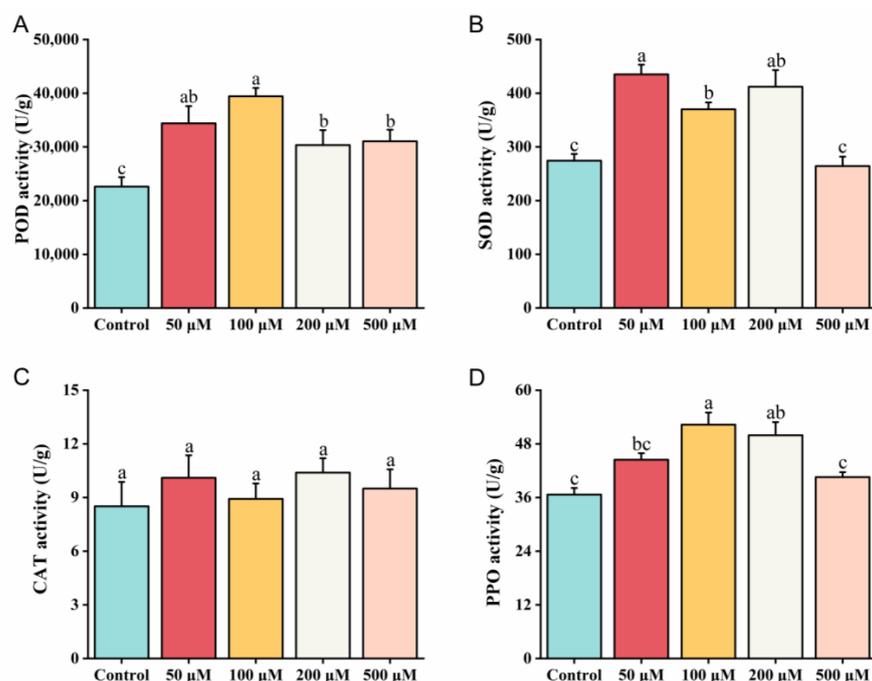
The contents of soluble sugars, free amino acids, and flavonoids were quantified to evaluate the influence of serotonin on rice physiological indexes. The respective content of soluble sugars was significantly higher in plants treated with 50 and 100  $\mu\text{M}$  serotonin compared to control plants (Figure 6A). The levels of free amino acid were significantly increased in treatments with different serotonin concentrations, reaching a peak at a concentration of 50  $\mu\text{M}$  (Figure 6B). The peak was reached at concentrations 50 and 100  $\mu\text{M}$ , as there is no statistical difference between them. Conversely, flavonoid synthesis was suppressed by the exogenous application of serotonin: the flavonoid contents were significantly lower in plants treated with 50, 200, and 500  $\mu\text{M}$  serotonin, with the lowest at the concentration of 500  $\mu\text{M}$  serotonin, compared to that of control (Figure 6C).



**Figure 6.** Effects of exogenous application of various concentrations of serotonin on the contents of soluble sugars (A), free amino acids (B) and flavonoids (C). Data are means + SE. Different letters represent significant differences among treatments (one-way ANOVA followed by LSD test).

### 3.6. Exogenous Serotonin Affects the Activities of Antioxidant Enzymes in Rice

Previous studies have shown that antioxidant enzymes, such as POD, SOD, CAT, and PPO, are involved in plant defense [31,32], therefore, we tested the activities of POD, SOD, CAT, and PPO after exogenous application of various concentrations of serotonin. The POD activities were significantly higher in serotonin-treated rice plants, reaching highest activities at concentrations of 50 and 100  $\mu\text{M}$ , compared to control plants (Figure 7A). Except for the 500  $\mu\text{M}$  serotonin concentration, the SOD activities in the other treatments were remarkably increased and reached a maximum at 50  $\mu\text{M}$  serotonin (Figure 7B). However, exogenous serotonin had no significant effect on the activities of CAT (Figure 7C). Although the PPO activities increased after serotonin addition, differences between serotonin-treated and control plants were observed at concentrations of 100 and 200  $\mu\text{M}$  (Figure 7D).



**Figure 7.** Effects of exogenous serotonin application on activities of defense proteins, including POD (A), SOD (B), CAT (C), and PPO (D) 12 h post application in rice. Data are means + SE. Different letters represent significant differences among treatments (one-way ANOVA followed by LSD test).

#### 4. Discussion

Serotonin, which exists widely throughout the plant kingdom, has been found to be involved in plant responses to abiotic and biotic stresses, besides its important roles in plant growth and development [8,18]. In this study, we demonstrated the key role of serotonin in mediating rice responses to BPH nymphs. We found that BPH nymph infestation induced the expression levels of four serotonin-biosynthesis genes, hence enhanced the accumulation of serotonin in rice (Figures 2 and 3). Additionally, the exogenous application of serotonin promoted BPH nymph feeding, which in turn decreased rice tolerance to BPH nymphs (Figures 4 and 5). Furthermore, rice plants exposed to serotonin application increased the production of soluble sugars and amino acids, as well as activities of defense-related proteins, but repressed the flavonoid production (Figures 6 and 7). These findings suggested that serotonin has a negative effect on rice resistance against BPH nymphs.

Previous studies showed that the levels of serotonin biosynthesis gene transcripts and serotonin could be induced by pathogen infection or herbivore attacks [12]. For examples, *AeVTDC1* and *AeVTDC2* in *Aegilops variabilis No.1* [41], 4 putative *BdTDC* genes and serotonin content in *Brachypodium distachyon* [42], were found to be highly induced by pathogen infection. In rice, the TDC activities and serotonin accumulation were clearly upregulated in rice leaves infected by *B. oryzae* [20]. Similarly, *C. suppressalis* feeding and gravid BPH female infestation led to enhanced serotonin contents in rice [23,24]. In this study, we demonstrated that BPH nymph infestation could also elicit the expression of four serotonin biosynthesis genes, including *OsTDC1*, *OsTDC2*, *OsTDC3*, and *OsT5H*, and the accumulation of serotonin (Figures 2 and 3). Our findings further confirmed the involvement of serotonin in the defense responses of rice against herbivores.

Serotonin has been reported to directly affect the performance of insect pests. The addition of serotonin to artificial diets facilitated the growth and development of the cotton bollworm, *Helicoverpa armigera*, leading to earlier pupation, decreased developmental period, and increased pupal weight of *H. armigera*. However, exogenous serotonin at extremely high concentrations decreased the survival rate of *H. armigera* larvae [43]. In rice, serotonin supplementation in artificial diet increased the survival rate of gravid BPH

females, as well as the larval weight of *C. suppressalis* [24]. Consistent with these results, we found that BPH nymphs fed on artificial diets supplemented with serotonin exhibited higher survival rates than those fed on non-supplemented diets, with the survival rates increased as the serotonin concentrations increased (Figure 4C). Moreover, our results supported the speculation that serotonin affects insect pest performance in a dose-dependent manner at low concentrations.

A recent study demonstrated that a rice knockout mutant of *CYP71A1*, which encodes a tryptamine 5-hydroxylase, is defective in serotonin production and exhibited enhanced resistance to gravid BPH females, suggesting an evident role of serotonin in modulating plant defense against insect herbivores [24]. Similarly, our results showed that the exogenous application of serotonin on rice plants improved the performance of BPH nymphs, including enhanced feeding preference and amounts (Figure 4A,B), which in turn reduced rice tolerance to BPH nymphs (Figure 5), indicating that serotonin plays a negative role in regulating rice resistance to BPH nymphs. Additionally, the enhanced resistance in *CYP71A1* knockout mutant was due to high levels of salicylic acid, which is known to positively regulate rice resistance to gravid BPH females [24,44]. However, it is reported that infestation by BPH nymph had no effect on the production of jasmonic acid, jasmonoyl-isoleucine, salicylic acid, ethylene, and H<sub>2</sub>O<sub>2</sub>, all of which play important roles in the resistance of rice to herbivores [45]. Here, to better gain insight into the underlying mechanisms of serotonin-mediated rice defense, the effect of serotonin on physiological and biochemical indexes of rice was also determined. Levels of soluble sugars and free amino acids have been observed to be negatively correlated with rice resistance to BPH [46]. It was found that the contents of soluble sugars and amino acids in the BPH-resistant varieties were lower than those in the insect-susceptible varieties [30,46]. In addition, BPH nymphs fed on the resistant plants were short of nutrients (e.g., soluble sugars and free amino acids) compared with those fed on susceptible plants [47]. Here, we found that serotonin significantly increased the levels of soluble sugars and amino acids (Figure 6A,B), which may then promote BPH nymph feeding. In a previous study, rice plants treated with flavonoids showed greater resistance to BPH, meanwhile, the flavonoid contents in different rice varieties were found to be positively correlated with the resistance of rice to BPH [33]. In the present study, we observed that serotonin treatment suppressed flavonoid contents (Figure 6C). This finding suggests that enhanced BPH nymph feeding behavior on serotonin-treated plants may also result from decreased flavonoid contents.

In addition to soluble sugars, free amino acids and flavonoids, defense-related enzymes, including POD, SOD, CAT, and PPO were reported to be implicated in BPH-induced rice defense [48,49]. We found that serotonin treatments boosted the activities of POD, SOD and PPO, but did not influence the activities of CAT (Figure 7). However, whether enhanced activities of POD, SOD, and PPO contributed to serotonin-mediated resistance of rice to BPH nymphs should be further investigated.

## 5. Conclusions

Our findings indicated that BPH nymph infestation activates serotonin biosynthesis, which in turn negatively regulates the resistance of rice to BPH nymphs by the modulation of defense-related substances in rice, including enhanced soluble sugar and total amino acid contents, and decreased flavonoid content. Thus, we provide valuable information for molecular design breeding of new BPH-resistant rice cultivars by modifying the serotonin-biosynthesis pathway.

**Author Contributions:** L.C., L.F. and J.L. (Jinglan Liu) designed the research; L.F., X.L., J.L. (Jitong Li), G.L., W.F., L.Z. and K.F. conducted the experimental works; L.C., L.F., S.W. and J.L. (Jinglan Liu) analyzed the data and wrote the manuscript; L.C. and J.L. (Jinglan Liu) acquired the funding. All authors have read and agreed to the published version of the manuscript.

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**Data Availability Statement:** Not applicable.

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**Conflicts of Interest:** The authors declare no conflict of interest.

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