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The effects of triazophos on the trehalose content, trehalase activity and their gene expression in the brown planthopper *Nilaparvata lugens* (Stål) (Hemiptera: Delphacidae)

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ABSTRACT

A previous study demonstrated that the flight capacity of *Nilaparvata lugens* adults treated with triazophos was enhanced significantly. However, the physiological and regulative mechanisms of the flight enhancement are not well understood. Trehalose is a primary blood sugar in insects, and the enzyme trehalase is involved in energy metabolism. The present study investigated the effects of triazophos on the trehalose content, trehalase activity (soluble trehalase and membrane-bound trehalase) and the mRNA transcript levels of their corresponding genes (*NlTre-1* and *NlTre-2*) in fifth instar nymphs, as well as in the brachypterous and macropterous *N. lugens* adult females. Our findings showed that the trehalose content in fifth instar nymphs as well as in the brachypterous and the macropterous adults significantly decreased following triazophos treatment. However, the glucose content, soluble trehalase activity and expression level of *NlTre-1* mRNA increased significantly compared to the controls. No significant enhancement of *NlTre-2* expression was found, indicating that regulation of energy metabolism of triazophos-induced flight capacity in *N. lugens* was not associated with *NlTre-2* expression. In addition, soluble trehalase activity and the expression level of *NlTre-1* mRNA in the macropterous females was significantly higher than that in the brachypterous females. The present findings provide valuable information on the molecular and regulative mechanisms of the increased flight capacity found in adult *N. lugens* after treatment with triazophos.

1. Introduction

The brown planthopper, *Nilaparvata lugens* (Stål) (Hemiptera: Delphacidae), is a major rice pest in many parts of Asia and a long-distance migratory pest in temperate eastern Asia. It is also a typical recurrent pest induced by pesticides [1–3]. Our previous study demonstrated that the soluble sugar content in adults treated with triazophos significantly increased [2] and the flight capacity of treated adults was also significantly enhanced [4]. However, the physiological mechanism of flight enhancement induced by triazophos is not yet understood. Trehalose is considered a blood sugar in insects [5]. Trehalase can catabolize one mole of trehalose to two moles of glucose. Catabolism of these sugars provides energy for flight and other physiological activities [6]. Thus, trehalose is the main sugar reserve in the hemolymph of larvae, pupae and adult insects [5,7–10]. It has been demonstrated that energy substances used in the *N. lugens* flying process are mainly derived from glycogen in hemolymph [11]. Therefore, the flight capacity of insects is associated with trehalose content and trehalase activity. In addition, *N. lugens* adults possess two wing forms: long wings (macropterous) for flight and short wings (brachypterous) for non-flight.

The objective of the present study is to examine changes in trehalose content and trehalase activity in adults treated with triazophos. Additionally, we measure the expression levels of two genes (*NlTre-1* and *NlTre-2*) to understand the regulative mechanisms of flight enhancement in triazophos-treated adults.

2. Materials and methods

2.1. Rice variety and culture

Rice (*Oryza sativa* L.) variety Nijing 4 (japonica rice) was used in trials. This variety of rice was selected because it is commonly planted in the Jiangsu province, China. Seeds were sown outdoors in standard rice-growing soil in cement tanks (height 60 cm, width 100 cm and length 200 cm). When seedlings reached the six-leaf stage, they were transplanted into 16-cm diameter plastic pots with four hills per pot and three plants per hill. All rice plants used in experiments reached the tillering stage.

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2.2. Insect culture and insecticide

A laboratory strain of *N. lugens* that was originally obtained from the China National Rice Research Institute (CNRRI; Hangzhou, China) was reared in a greenhouse at an ecological laboratory at Yangzhou University. *N. lugens* was kept in an ecological laboratory at 26 ± 1 °C, with 70–80% humidity and a 16-h light/8-h dark photoperiod. Technical triazophos (87% [AI]) was purchased from the Shenli Pesiticide Co., Ltd., Ningguo, Anhui, China.

2.3. Experiments

Triazophos was dissolved with acetone. Ten percent emulsifier was then added and diluted into four concentrations (10, 20, 40 and 80 ppm) based on previous results from a sublethal test [2,12]. A total of 160 third instars per hill were released onto potted rice plants. Rice plants at the tillering stage were sprayed with a series of concentrations of triazophos 24 h after insects were released using a Jacto sprayer (Maquinas Agricolas Jacto S.A., Brazil) equipped with a cone nozzle (1-mm diameter orifice, pressure 45 psi, flow rate 300 ml/min). Control plants at the same stages were sprayed with the same amount of acetone and emulsifier. Each treatment and control was replicated three times. The treated and control plants were covered with cages (screen size: 80-mesh). Nymphs on the treated and control plants were collected at the fifth instar stage following foliar sprays. Trehalose content and trehalase activity in the fifth nymphs were measured. To measure the trehalose content and trehalase activity in adults (macropterous and brachypterous), a single fifth instar nymph was placed into a glass jar (10-cm diameter, 12-cm height) and reared with untreated rice plants at 26 ± 1 °C with a 16L:8D photoperiod until adult emergence. After the adults emerged, females were separated. The trehalose content and trehalase activity were measured at 1, 2 and 3 days after emergence (1, 2 and 3 DAE). Twenty milligrams of fifth instar nymphs or adults were used for each replication in each treatment and control.

2.4. Measurement of trehalose content

Trehalose content was estimated by the method designed by Steele et al. [13] and Feng [14]. Twenty milligrams of whole insect bodies was homogenized at 0 °C after adding 2 ml of 20 mM phosphate buffer (PBS, pH 5.8) and then vibrated on a supersonic cell pulverizer (Ninbo Xin Yi Science Instrument Ltd., Co., Ninbo, Zhejiang) for 30 min. The sample solution was centrifuged at 1000 rpm for 10 min at 4 °C, the cuticle debris was removed and then centrifuged at 12,000 rpm for 10 min at 4 °C. Two hundred microliters of the supernatant was put into a 5 ml tube, 200 μl of 1% H_2SO_4 was added, the tube was bathed in 90 °C boiling water for 10 min, and 200 μl of 30% NaOH was added after it cooled on ice for 3 min. The supernatant was bathed in 90 °C boiling water for 10 min again and then bathed in 3 ml of developer with 0.02 g anthrone (Sigma, USA). Then, 10 ml of 80% H_2SO_4 was added after it cooled on ice for 3 min. The absorbance at 630 nm was determined in the UV755B spectrometer (Shanghai Precision Instrument Co., Ltd., Shanghai, China). Trehalose content was calculated based on a standard curve.

2.5. Measurement of glucose content

Glucose levels were determined using the glucose oxidase method (Sigma, USA) [15]. Two milliliters of 20 mM phosphate buffer (PBS, pH 5.8) was added into 20 mg of whole insect bodies and then homogenized at 0 °C. The sample solution was centrifuged at 10,000 rpm for 10 min at 4 °C. Fifty microliters of 0.6 mol L^{-1} perchloric acid was added into 50 μl of the supernatant to remove the protein in solution, centrifuged at 3000 rpm for 5 min at 4 °C, and 450 μl of 0.2 mol L^{-1} sodium phosphate buffer (pH 7.4) was then added into 50 μl of the supernatant. Two hundred microliters of chromogen reagent (Covance, New Jersey, USA) and 150 μl of glucose oxidase were added to 50 μl of the sample solution, which was incubated at 37 °C for 5 min. The absorbance at 625 nm was determined with a UV755B spectrometer (Shanghai Precision Instrument Co., Ltd., Shanghai, China). The glucose content in the sample solution was calculated based on a standard curve.

2.6. Measurement of soluble trehalase and membrane-bound trehalase activity

The separation of trehalase proteins was carried out as described by Tatun et al. [16] and Gu et al. [17]. Two milliliters of 20 mM phosphate buffer (PBS, pH 5.8) was added into 20 mg of whole insect bodies, homogenized at 0 °C, and then vibrated on a supersonic cell pulverizer (Ninbo Xin Yi Science Instrument Ltd., Co., Ninbo, Zhejiang) for 30 min. The sample solution was centrifuged at 1000 rpm for 10 min at 4 °C, and the cuticle debris was removed and centrifuged at 105,000 rpm for 10 min at 4 °C. The supernatant was directly used for measuring the activity of soluble trehalase. The residual was resuspended with 100 μl PBS for the measurement of membrane-bound trehalase. We followed the procedure described by Li et al. [18] to measure protein content using Coomassie Brilliant Blue G-250 (Shanghai Chemical Agent Co., Ltd., Shanghai, China). A standard curve was established based on a standard protein (bovine serum albumin, Shanghai Biochemistry Research Institute, Shanghai, China). The absorbance at 595 nm was determined in the UV755 B spectrometer (Shanghai Precision Instrument Co., Ltd., Shanghai, China). Fifty microliters of 40 mM trehalose (Sigma, St. Louis, MO, USA) and 110 μl of PBS were added into 40 μl of the supernatant (soluble trehalase), and 40 μl of the suspended solution (membrane-bound trehalase) was added into 50 μl of 40 mM trehalose (Sigma, St. Louis, MO, USA) and 110 μl PBS. The mixture solution was incubated at 37 °C for 30 min, bathed in 95 °C boiling water for 5 min, centrifuged at 12,000 rpm at 4 °C for 10 min after cooling on ice for 10 min. Then, the coagulated protein was removed. Trehalase activities (soluble and membrane-bound trehalase) were detected by measuring the quantity of released glucose after adding hexokinase and glucose-6-phosphate dehydrogenase [19]. Fifty units of hexokinase, 100 U of glucose-6-phosphate dehydrogenase, 2 mM NADP and 2.8 mM ATP (Roche Diagnostics GmbH, Mannheim, Germany) were added in the reaction solution and incubated at 37 °C for 30 min. Trehalase activity was determined based on a calibration curve established by a glucose standard (Sigma, St. Louis, MO, USA). The enzyme activities were expressed as μmol. mg^{-1} Protein.min^{-1}.

2.7. Total RNA isolation and cDNA preparation

Total RNA was isolated from 10 adult females using an SV Total Isolation System Kit (Promega Corporation, Madison, WI, USA). Synthesis of first-strand cDNA was carried out according to the PrimeScript™ RT reagent Kit (TaKaRa Biotechnology Dalian Co., Ltd). The first-strand cDNA synthesis was performed in a 10 μl total reaction volume containing 0.5 μg total RNA, 0.5 μl PrimeScript™ RT Enzyme mix I, 0.5 μl Oligo dT Primer (50 μM), 2 μl random hexamers (100 μM), 2 μl 5 × PrimeScript™ Buffer (for Real Time), *X* μl total RNA, and the addition of RNase-free dH_2O up to 10 μl. The cDNA reverse transcriptase polymerase chain reaction was done with the following cycling regime: 37 °C for 15 min, 85 °C for 5 s and 4 °C for 5 min.