



Better cold tolerance of Bt-resistant *Spodoptera exigua* strain and the corresponding cold-tolerant mechanism



Honghua Su, Jincheng Zou, Qjuxia Zhou, Qi Yu, Yong Yang, Yizhong Yang *

School of Horticulture and Plant Protection, Yangzhou University, Yangzhou 225009, Jiangsu, China

ARTICLE INFO

Article history:

Received 31 January 2017

Received in revised form 31 May 2017

Accepted 2 June 2017

Available online 7 June 2017

Keywords:

Spodoptera exigua

Bt-resistant strain

Cold tolerance

Body water content

Anti-freeze substances

ABSTRACT

Spodoptera exigua is a secondary target pest of Bt cotton commercialized in China. With the continuous adoption of Bt cotton, populations of *S. exigua* have gradually increased. However, the cold tolerance ability of Bt-resistant *S. exigua* and the effect of continuous Bt diet on anti-cold materials are unknown. In our study, it was found that Bt-resistant *S. exigua* (Bt10) developed better with shorter larval and pupal duration and higher pupation rate compared to CK at the suboptimal low temperature. The supercooling points and freezing points of the Bt-resistant *S. exigua* strain were determined, and body water content and anti-cold materials such as total sugar, trehalose and glycogen, glycerol and fat were examined to explore the effect of Bt toxin on overwintering and on population increase. The results showed that the supercooling point and the freezing point of the Bt-resistant *S. exigua* pupae were both significantly lower than that of the Bt-susceptible strain. No difference was found in the body water content of pupae and adults between the two strains. Total sugar content of the Bt-resistant strain at both the pupal and adult stages was higher than that of the susceptible strain at the corresponding stages, and glycogen content of the Bt-resistant strain at the larval stage was higher than that of the susceptible larval *S. exigua*. Fat content of the Bt-resistant larvae, pupae and adults was for each higher than that of the susceptible strain, but the difference was not significant except for that of the 3rd instar larvae. Glycerol content of the Bt-resistant strain at larval, pupal and adult stages was for each higher than that of the corresponding life stages of the susceptible strain. It can be seen that more glycerol was accumulated in Bt-resistant *S. exigua*. The results indicate that Bt-resistant *S. exigua* has better cold tolerance. The contents of the anti-freeze substances of progeny, especially glycerol, were increased after previous generations were continuously fed on Bt protein, which means that the Bt-resistant secondary target pests could more easily overcome the overwinter season and become a source of crop damage the following year.

© 2017 Published by Elsevier Inc.

1. Introduction

Cold tolerance refers to the ability of insects to maintain a supercooled condition to prevent freezing injury. Insects, especially those living in temperate and cold regions, have to overcome a period of cold weather by regulating their body temperatures via supercooling to enhance their cold hardiness, and in this way the population can be maintained [1]. The supercooling phenomenon means that when the surrounding temperature is lower than the insects' freezing point (FP), body fluids remain in the liquid state. The supercooling point (SCP) is often used as the indicator to represent cold tolerance [2,3]. In winter, the temperature is usually lower than the FP; thus, insects can avoid freezing damage by supercooling their body fluids.

There are many factors which affect insect cold tolerance. First, seasonal temperature changes have some influence on cold hardiness [4]. Second, geographical environments also play important roles in cold

hardiness [5,6]. Usually insects living in high altitudes and latitudes have better cold tolerance ability than those in low altitudes and latitudes. For example, the cold hardiness of *Aedes albapictus* eggs collected from different geographical regions was significantly different [7]. Third, growth stages also have significant influences on cold tolerance [8]. Insects in an overwintering state, hibernation or diapause can tolerate lower temperatures compared to those in developmental stages [8,9]. The cold hardiness of *Spodoptera exigua* at different developmental stages was found to be eggs < adults < larvae < pupae [10]. Low temperature acclimation could significantly enhance insect cold tolerance [11–13]. Last but not least, the food the insects feed on also has a great impact on their cold tolerance [14–16].

Insects could enhance their cold hardiness by decreasing their body water content, excreting ice nucleating agents, producing anti-freeze proteins or accumulating anti-cold substances [17–21]. Body water decreases, and hydrophilic substances are segregated, which results in supercooling of the body fluids. At present, the known small molecular anti-freeze substances include sorbitol, mannitol, glycerol, trehalose, glucose and fat [22].

* Corresponding author.

E-mail address: zyyang@yzu.edu.cn (Y. Yang).

S. exigua does not diapause. In the south of China, it can propagate all year round and cause serious damage to crops; in the north of China, however, the overwinter period is one of the most important stages in its life cycle. Its cold tolerance determines its distribution, overwinter survival and its population size next spring. In China, it was found that the SCP of pupae was the lowest compared to that of larvae, prepupae and pupae [10,23]. Pupae were thought to be the overwintering stage for *S. exigua*. Additionally, based on its cold tolerance, it was thought that the northern limit of overwinter distribution was near 38° (north latitude) [10].

Since the commercialization of Bt cotton in China, target pests like cotton bollworm have been effectively controlled, but with the decreased use of chemical insecticide spray, the populations of secondary target pests and non-target pests have increased gradually [24,25]. *S. exigua* is a secondary target pest of Bt cotton grown in China. It has caused serious damage to Bt cotton in many cotton-producing areas [24,26,27]. However, there has been no study about the influence of Bt cotton on the cold tolerance of *S. exigua* and the cold hardiness mechanism. In this study, the supercooling points and freezing points of the Bt-resistant *S. exigua* strain were determined, and the anti-cold materials were examined to explore the effect of Bt toxin on overwintering and on population increase.

2. Materials and methods

2.1. Artificial diet preparation

Preparation of the artificial diet for susceptible *S. exigua* refers to Su et al. [24]. The endotoxin protein used to develop the Bt-resistant strain was Cry1Ac contained in MVPII (a commercial bioinsecticide formulation supplied by Mycogen, San Diego, CA). The purity of the freeze-dried, powdered MVPII was 20%. MVPII powder was dissolved in water and added to the artificial diet to obtain the Bt-treated artificial diet with a final concentration of 10 µg Cry1Ac/g diet which was used to develop the Bt-tolerant/-resistant strain (Bt10 strain).

2.2. Insect rearing

The susceptible *S. exigua* strain was provided by the Plant Protection Institute of the Chinese Agricultural Academy of Sciences. This strain had been reared on an artificial diet in the laboratory for over 10 years without any pesticide selection.

The above susceptible *S. exigua* strain was reared on a Bt-treated artificial diet (10 µg/g) for more than 40 generations. Resistance to Cry1Ac was up to 10. Then we obtained the Bt10 *S. exigua* strain.

The rearing conditions were 27 ± 1 °C, 70 ± 7% RH with a 14:10 photoperiod. The rearing room was sterilized for 0.5 h periodically.

2.3. Supercooling point and freezing point determination

The supercooling point of *S. exigua* at each growing stage was determined using supercooling point determination equipment. The heat-sensitive detector was inserted into a 1.5 mL tube and fixed with cotton around it. Thirty 3rd instar larvae were chosen and numbered. After being weighed, each larva was put on the detector which was again fixed with cotton, and the tube was put into a refrigerator. The tested larval temperature decreased with the environmental temperature. When the larval fluid started to freeze, the temperature at that moment was called the supercooling point. Because heat of crystallization was released when the insect's body fluid iced up, the larval temperature increased quickly and then dropped down gradually. There was an obvious peak in the temperature change curve; the peak value was the freezing point. Determination of supercooling points and freezing points of 5th instar larvae, pupae and adults was the same as for the 3rd instar larvae.

2.4. Determination of body water content

After determination of the supercooling points and the freezing points, the insects were dried for 24 h at 60 °C and then weighed. The body water content was calculated according to the following formula:

$$\text{Body water content (\%)} = [(\text{fresh weight} - \text{dry weight}) / \text{fresh weight}] \times 100\%$$

2.5. Determination of total sugar, trehalose and glycogen content

Zero, 0.2, 0.4, 0.6, 0.8 and 1.0 mL of D-glucose solution (0.1 mg/mL) was separately added into 6 microcentrifuge tubes, and 10% trichloroacetic acid was added to each tube to reach the final volume (1.0 mL). Four milliliters of 0.2% anthrone reagent was added into each tube which was ice-bathed in ice water. Then all the tubes were boiled in a metal bath for 10 min and cooled with distilled water and kept at room temperature for 20 min. Absorbance was measured at 620 nm using an ultraviolet spectrophotometer. Then the standard curve was drawn with the light absorption value as abscissa and the glucose content as ordinate.

Total sugar determination refers to Wu et al. [28]. The 3rd and 5th instar larvae, pupae and adults of the two strains were washed with distilled water, and then the water on the body surface was removed with filter paper. The individuals were weighed and homogenated thoroughly in 10% trichloroacetic acid, and then centrifuged at 50000 r/min for 5 min. The supernatant was collected. Then 1 mL of trichloroacetic acid was added to the precipitate and mixed thoroughly to centrifugate. All of the supernatant was collected. This was the total sugar sample. Two milliliters of 95% ethanol were added into the collected supernatant; then the solution was settled for 24 h at 4 °C. One milliliter of the solution was centrifuged at 10000 r/min for 15 min, then 1 mL of H₂SO₄ was added into the supernatant to conduct the hydrolysis for 15 min in the metal bath. Then after the solution was cooled to room temperature, 1 mL of 30% KOH was gradually added to the solution and mixed thoroughly. The solution was heated in a metal bath for 15 min, then cooled to room temperature, and then the trehalose samples were obtained. After this, the above precipitate was completely dissolved with distilled water which was used as the glycogen sample. The light absorption value was measured at 620 nm using an ultraviolet spectrophotometer. Based on the light absorbance value of each sample, the sugar content was found on the standard curve. Then contents of the total sugar, trehalose and glycogen in the insect body were calculated according to this formula:

$$\text{Sugar content (}\mu\text{g/mg)} = [\text{sugar content obtained from standard curve (}\mu\text{g/mL)} \times \text{dilution volume (mL)}] / \text{body weight (mg)}$$

2.6. Determination of glycerol content

Glycerol standard solution (0.1 mg/mL) was added to 24 2-mL centrifugal tubes, and distilled water was added to those tubes to make the glycerol concentration gradient. Oxidant was added into the glycerol solution and mixed thoroughly, and then visualization reagent was added into the mixture. The 24 tubes were put into the metal bath for 15 min. The solutions were cooled to room temperature after the color development reaction developed fully. The light absorbance value was measured at 420 nm using an ultraviolet spectrophotometer. Then the standard curve was drawn.

Glycerol determination refers to Wu et al. [28]. The 3rd and 5th instar larvae, pupae and adults of the two strains were washed with distilled water, and then the water on the body surface was removed with filter paper. The individuals were weighed, homogenated thoroughly in distilled water, and then centrifuged at 10000 r/min for 10 min. The supernatant was collected. Then some distilled water was added to the precipitate and mixed thoroughly to centrifugate, into

Download English Version:

<https://daneshyari.com/en/article/5514925>

Download Persian Version:

<https://daneshyari.com/article/5514925>

[Daneshyari.com](https://daneshyari.com)