Journal of Thermal Biology 36 (2011) 1–6

Contents lists available at ScienceDirect

Journal of Thermal Biology

journal homepage: www.elsevier.com/locate/jtherbio

Fat body catalase activity as a biochemical index for the recognition of thermotolerant breeds of mulberry silkworm, *Bombyx mori* L.

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ARTICLE INFO

Article history:
Received 15 May 2010
Accepted 17 August 2010

Keywords:
Bivoltine
Bombyx mori L.
Catalase activity
Thermotolerance

ABSTRACT

The fifth instar larvae of the silkworm *Bombyx mori* L. were exposed to selected high temperatures (35 and 40 °C) in order to understand the changes in the level of catalase activity in the three tissues of fat body, midgut and haemolymph of the five selected bivoltine breeds and their 9 quantitative traits, namely larval weight, cocoon weight, shell weight, shell ratio, filament length, filament weight, denier, renditta and effective rearing rate (ERR), also the correlation between them under high temperature conditions were examined. Catalase activity resulting in fat body revealed a positive correlation between the control (28 ± 1 °C) and 40 ± 1 °C. The CSR_2 breed showed the most level of thermotolerance and catalase activity, compared with the CSR_4, JROP, NB_4D_2 and KA breeds. It was found that the level of catalase activity in fat body may be a reliable biochemical index to recognize thermotolerant breeds in order to develop resistant hybrids for tropical areas.

1. Introduction

The silkworm, *Bombyx mori*, is an important economic insect for production of silk. Recently, it has shown to be a good model insect for biological science due to its excellent biological characteristics, such as ease of rearing, large body and the availability of much genomic information (Mita et al., 2004; Xia et al., 2004). The silkworm strains of temperate countries like Japan, China, etc. are bivoltine, which are characterized by longer larval duration with high silk content and superior silk quality. However, they are highly susceptible to tropical climatic conditions and less tolerant to high temperatures. Temperature plays a major role in growth and productivity of silkworm, as the silkworm is poikilothermic (Benchamin and Jolly, 1986). There is ample literature showing that good quality cocoons are produced within a temperature range of 22–27 °C and levels above these makes the cocoon quality worse (Krishnaswami et al., 1973). The effect of temperatures higher than 30 °C on silkworm larvae was reported earlier by Takeuchi et al. (1964) and Ohi and Yamashita (1977). India is a tropical country where the air temperature in the summer can range between 28–35 °C at night and 35–40 °C in the day. These fluctuations in temperature have an adverse effect on the survival and pupation of silkworms, especially the bivoltine breeds, incurring heavy loss to the industry. Many silkworm characteristics are not only controlled by genes but also influenced by environmental factors such as temperature (Watanabe, 1918, 1919, 1924, 1928 and Kogure, 1933). However, in order to introduce bivoltine races in a country with a tropical climate, it is necessary to maintain stability of breeds in cocoon crop production under a high temperature environment (Suresh Kumar et al., 2002).

The environment is dynamic and hence brings about profound changes in the physical and biotic factors dominating the expression of commercial characters in silkworm (Kobayashi et al., 1986). Earlier reports suggest that environmental stresses diminish antioxidant status and cause oxidative stress in lepidoptera (Grubor-Lajsic, 1997). Oxidative stress is the result of an imbalance between pro-oxidant species and the levels of the defences resulting from the generation of reactive oxygen species (Santoro and Thiele, 1997). Living organisms need mechanisms regulating reactive oxygen species (ROS) such as hydrogen peroxide and superoxide anion. Catalase (H_2O_2:H_2O_2 oxidoreductase, EC 1.11.1.6, CAT) is one of the antioxidant enzymes and catalyzes the degradation of H_2O_2 to water and oxygen (Switala and Loewen, 2002). Protective roles of the antioxidant enzymes in high temperature have been reported for a number of plants (Almeselmani et al., 2006). In the Antarctic midge *Belgica antarctica*, oxidative stress was observed on exposure to heat stress and gene encoding catalase was also elevated in response to dehydration (Lopez-Martinez et al., 2008). ROS is harmful to living organisms because ROS tend to give oxidative damage to proteins, nucleic acids and lipids (Hermes-Lima and Zenteno-Savín, 2002). In this context, ROS has been

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doi:10.1016/j.jtherbio.2010.08.008

recognized to be related to aging and life span (Sohal et al., 1990; Orr and Sohal, 1994; Parkes et al., 1999). ROS stimulates a signal transduction and mediates various responses such as cell growth and apoptosis (Suzuki et al., 1997). On the other hand, ROS plays a helpful role in the innate immunity system of an insect (Hao et al., 2003; Kumar et al., 2003). In insects, CAT is recognized as the key enzyme to be solely responsible for the scavenging of ROS (Felton and Summers, 1995). Developmental changes in the activity of CAT were reported in the European corn borer, *Ostrinia nubilalis*, and the *Elateridae*, *Pyrearinus termitilluminans* (Barros and Bechara, 2001; Jovanović-Galović et al., 2004). However, there is little information on insect CAT, especially on its biochemical properties.

High temperature affects nearly all biological processes, including the rates of biochemical and physiological reactions (Hazel, 1995; Willmer et al., 2004), and it eventually can affect the quality or quantity of cocoon crop in the silkworm. Several reports (Ueda and Lizuka, 1962; Shirota, 1992; Tazima and Ohnuma, 1995) demonstrated that silkworms were more sensitive to high temperature during the 4th and 5th stages; for the selection of thermotolerant silkworm breeds, the 5th instar proved to be the best stage. For the purpose of screening and evaluation of robust and thermotolerant bivoltine breeds of silkworm *B. mori* L. in the present investigation, the main objective was to understand the level of changes in the nine quantitative characters, namely larval weight, cocoon weight, shell weight, shell ratio, filament length, filament weight, denier, renditta and effective rearing rate (ERR) percentage, along with the level of changes in the catalyse activity and the correlation between them using five popular bivoltine breeds under natural temperature (28 ± 1 °C) and imposed thermal stress (35 ± 1 and 40 ± 1 °C) conditions during 5th instar larvae.

2. Materials and methods

Five bivoltine silkworm genotypes having higher cocoon productivity traits (Datta et al., 2000a, b) and known for higher temperature and disease tolerance (Mano, 1994) were drawn from the germplasm bank of the Department of Sericulture Science, University of Mysore, Mysore, India during 2009, these genotypes were JROP, KA, NB_4D_2, CSR_2 and CSR_4. The rearing was conducted in the monsoon season following the method suggested by Krishnaswami (1978). The larvae were fed with the M_5 variety of mulberry (*Morus alba*) leaves. The breeds were considered with three replications at each temperature and each replicate included 250 larvae. They were divided into experimental trays at a rearing house, based on a completely random design.

2.1. Temperature treatments

The ambient temperature (28 ± 1 °C) was the control treatment and was based on tropical countries where the air temperature in summer is 35–40 °C in the daytime. Two imposed temperatures of 35 and 40 ± 1 °C were tested using a Biological Oxygen Demand (BOD) incubator. The temperature stressed larvae were exposed to 2×2 h periods at a high temperature (35 or 40 ± 1 °C) separated by a 4 h "rest" period at 28 ± 1 °C. Control animals were maintained at a constant 28 ± 1 °C. The exposing duration to thermal stress was started from the first to fifth day of 5th instar. Appropriate plastic boxes ($25\times18\times7$ cm in size) with a net lid were made and used to transfer larvae from the rearing house to the BOD. After the heat treatment, the tested larvae were transferred to the standard rearing condition at 28 ± 1 °C. All experimental silkworms in both control and treated batches were not fed during incubation and both were fed fresh mulberry leaves 15 min after the conclusion of BOD incubation, twice per day. The humidity in the BOD was adjusted to the same rearing house humidity of $75\pm2\%$ using wet pads.

2.2. Tissue preparations

The tissue preparations were made on the 5th day of 5th instar in both control and high temperature treated batches using the method of Jagadeesh Kumar (2005). To prepare the tissues for catalase activity estimation, haemolymph samples were collected from 6 to 7 larvae by random selection. One of the thoracic legs of the larvae was amputated and placed in the prechilled centrifuge tube. The larvae were dissected in 0.9% saline at pH 6.5 on a chilled dissection tray. The fat body and midgut were collected and stored at −20 °C. From each larva 0.5 ml of haemolymph was extracted; to avoid the activity of prophenol oxidase followed by the melanization of haemolymph, 1 mg phenylthiourea was added to the haemolymph samples immediately after extraction. Then they were centrifuged for 10 min at 4000 rpm at −4 °C. The supernatant was transferred to new prechilled tubes and kept in −20 °C until the beginning of the chemical experiments.

2.3. Measurements of catalase activity

To produce the test solution, 0.1 ml of stored haemolymph, 100 mg fat body and 100 mg midgut were homogenized in 10 ml distilled water at −4 °C. Total CAT activity was spectrophotometrically measured by the method of Aebi (1984). The decrease in absorbance at 240 nm was monitored at 30 °C. To examine the distribution of the activity, the test solution was prepared by the following method, frozen tissues were homogenized in 70 mM potassium phosphate buffer (pH 6.5) containing 0.1% Triton X-100, and the insoluble substance were centrifuged. Tissue protein concentrations were measured according to Lowry's method (Lowry et al. 1951). Each measurement was considered with 6 replicates.

2.4. Measurement of quantitative traits

The performance of all genotypes was studied by analyzing nine economic characters, namely larval weight, cocoon weight, shell weight, shell ratio, filament length, filament weight, denier, renditta and ERR% using the following general methods found in sericulture science:

Larval weight: the average weights of 10 larvae were taken after the last feeding, just before beginning spinning cocoon.
Cocoon weight: the average weights of 10 cocoons were taken after removing the useless floss on the cocoons (deflossing).
Shell weight: the average weights of 10 cocoon shells were taken separately after removing the pupa.
Shell percentage: (weight of shell/weight of cocoon) × 100.
Filament length: the total length of the silk filament from a single cocoon was reeled using an epprouvette. (The mean value of 10 observations was considered).
Filament weight: the total weight in grams of the silk filament of a single cocoon was estimated. (The mean value of 10 observations was considered).
Denier: (filament Weight/filament Length) × 9000.
Renditta: weight of cocoon/weight of silk reeled from the same cocoon.
Effective rearing rate (ERR) percentage: (total number of cocoon harvested/total number of larvae retained after 4th moult) × 100.