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Effect of Temperatures on Growth, Yields and Heat Shock Protein Expression of Eri Silkworm (*Samia ricini* D.)

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Abstract

The effect of high temperatures on growth, yields and heat shock protein (HSP) production of eri silkworm (*Samia ricini* D.) was carried out by exposure the 5th instar day 3 larvae to different temperatures, 36±1, 40±1, 42±1, 45±1 and 48±1°C for 3 hours, compared to control treatment by rearing eri silkworm continuously at normal temperature (25±2°C, 80±5%R.H.). After treated with high temperature condition, the eri silkworm larvae were reared at 25±2°C, 80±5%R.H. until cocooning, pupation, adult stage, coupling and laying eggs. The result exhibited that survival rates, cocooning rate and almost yields varied inversely to temperatures especially between 42±1-48±1°C. At the highest temperature, survivals and yields were the lowest, while those values were the maximum in almost control treatments. At 48±1°C treatment, the means of all parameters were the lowest; larva survival (50.00%), survival of larva-adult (38.33%) and cocooning rate (41.67%), which were significantly different to other treatments ($P<0.05$). Other yields affected by 48±1°C were also the lowest; fresh cocoon weight (2.5078 g), pupa weight (2.1508 g), shell weight (0.3429 g), total cocoon shell weight (2.88 g), fresh cocoon weight/10,000 larvae (10.47 kg), egg laying/moth (287.56 eggs), hatchability (72.67%), total egg laying (1,121.33 eggs) and total hatchability (800.11 eggs). Detection of HSP of 5th instar larvae day 3 of eri silkworm treated with 5 different temperatures (36±1, 40±1, 42±1, 45±1 and 48±1°C) was performed using sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS–PAGE), compared to control treatment (25±2°C; 80±5%R.H.). The result was clearly that the eri silkworm treated with all high temperatures expressed HSP bands of approximate 50 kDa. Whereas HSP band was not detectable in control treatment. The HSP is applicable in the program for thermotolerant variety improvement of eri silkworm.

Keywords: *eri silkworm, high temperature, yield, heat shock protein, effect*

1. Introduction

Among wild silkmoths researched recently in Thailand, the eri silkworm (*Samia ricini* Donovan) is a high potential silkworm for commercial production and export. Its products are various type such as textiles, safety food and cosmetics. Both governmental and private sector in Thailand including international levels are interested in this silkworm and products. Normally optimal rearing temperature and relative humidity (R.H.) of eri silkworm are 26-28 °C and 85-90%R.H. for young larvae and 24 - 26°C, 70 - 80%R.H. for late instar larvae (1). Higher temperature than 35°C causes adversely on growth. In some cases give rise to dead larvae and devastating growth and quality of yields, e.g. cocooning, survival of larva and pupa including of adult development (2). In general, the organisms produce defense protein so called heat shock protein (HSP) under unfavorable stress such as oxidative stress, nutritional deficiency, ultraviolet radiation, chemicals, harmful microorganisms and ischemia reperfusion injury (3). This HSP plays an important role in the stabilizing of biochemical process in cells and other protein protection (4). There are reports on the HSP expression in mulberry silkworm, which molecular weight around 90, 70 and 20 kDa. The HSP expression and survival of silkworm depend on temperature, variety, growth stage and age (5), (6). However, it has still no report on the HSP expression in eri silkworm. Therefore, the present study was focused on the objectives that to evaluate on the effect of high temperatures on growth, yields and to detect the HSP in eri silkworm for further application as protein marker of thermotolerant property.

2. Materials and Methods

2.1 Evaluation of different high temperatures affecting growth and yields of eri silkworm

The early hatched 3-5 hours larvae of eri silkworm ecorace SaKKU1 were reared in laboratory at 25±2°C; 80±5%R.H. until developed to 5th instar day 3. Twenty of these larvae per replication were put in screened nylon bag and hung in a 2 liter beaker. The beaker was place in a water bath setting temperatures at 36±1, 40±1, 42±1, 45±1 and 48±1 °C for 3 hours (which served as treatments). After high temperature exposure, the larvae were transferred to raise in the same condition as control treatment at 25±2°C 80±5%R.H. The rearing process was followed according to Sirimungkararat et al. (7). High temperature levels, heat treatment and data recording were modified from Malik and Reddy (6) and Howrelia et al. (8). The completely randomized design (CRD) was used, which contained 6 treatments and 3 replications. The treatments are as followings:

Treatment 1: Temperature 25±2°C, 80±5%R.H. (control)
Treatment 2: 36±1°C
Treatment 3: 40±1°C
Treatment 4: 42±1°C
Treatment 5: 45±1°C
Treatment 6: 48±1°C

Data collection : Parameters of these were recorded: life cycle, survival rate {larva stage (1st-5th instar), larva (1st-5th instar)-adult stage}, cocooning rate, cocoon yields(fresh cocoon weight, pupa weight,

shell weight, shell ratio, total cocoon shell weight and fresh cocoon weight/10,000 larvae), and egg yields (egg laying, hatchability, total egg laying and total hatchability). The data were statistically analyzed according to analysis of variance (ANOVA) and the means were compared by using Duncan's multiple range test (DMRT).

2.2 Detection of heat shock protein

2.2.1 Hemolymph collection

Ten 5th instar larvae treated by different temperatures as described previously were surface sterilized by using cotton plug soaked with 70% ethanol rubbing the abdomen and proleg of larvae. Five male and 5 female larvae of each treatment were punched on proleg by small sterile needle. The hemolymph of each larva was sucked by syrynx 100 µl/larva and kept in a micro tube filled with 0.1% phenylthiourea for protection of blood clotting. Protein content in hemolymph was quantified by the method of Bradford (9), using bovine serum albumin as protein standard.

2.2.2 Detection of heat shock protein by SDS - PAGE

Protein samples from hemolymph were suspended in sample buffer 1:1 (v/v) heated at 90-100 °C for 10 min. The denatured proteins 20 µl/sample were separated on sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) using 10% stacking gel and 12% separating gel, based on Laemlli (10). The gel was stained and protein bands were photographed by gel document BIO RAD (Chimi Doc™ MP, Bio-Rad Laboratories, Inc) and analyzed by it software.

3. Results

3.1 Effect of temperature on growth and yields of eri silkworm

3.1.1 Growth

The eri silkworms treated at different temperatures, 25±2; 80±5%R.H., 36±1, 40±1, 42±1, 45±1 and 48±1 °C for 3 hour of each temperature, had the life cycle durations approximate to 50-63 days. The most longest duration for complete life cycle was 63 days at 48±1°C (Table 1).

The survival rates were displayed in Table 2. The average survival rates varied inversely with temperatures, namely, at high temperature the rates especially 40±1-48±1°C were decreased in both larva stage (1st - 5th instar) and larva (1st-5th instar) to adult stage including cocooning rates. Survival rates of larva stage (1st-5th instar) at 25±2, 36±1 and 40±1°C were 100%, which not significantly different to the rate at 42±1°C (98.33%). At 48±1°C, it was the minimum rate (50.00%), which significantly different ($P<0.05$) than others. In the same as survival of larva stage (1st-5th instar), the survival rate of larva (1st-5th instar) - adult stage were similar rates and not significantly different. They were 96.67, 95.00, 91.67 and 83.33% for treatment of 25±2, 36±1, 40±1°C and 42±1°C, respectively. At 48±1°C, the lowest rate was 38.33%. Cocooning rates at temperatures 25±2, 36±1, 40±1°C and 42±1°C were not significantly different ranking 100, 100, 98.33 and 95.00%, respectively. The least cocooning rate was 41.67% obtained from treatment of 48±1°C.

Table 1. Life cycle durations of eri silkworm (*Samia ricini* D.) at different temperatures.

Development Stage (days)	Temperature (°C)[1/]					
	25±2	**36±1**	**40±1**	**42±1**	**45±1**	**48±1**
Egg	**9-11**	**9-11**	**9-11**	**9-11**	**9-11**	**9-11**
Larva	**19-20**	**19-20**	**19-20**	**20-21**	**20-21**	**20-21**
1st instar	4	4	4	4	4	4
2nd instar	3	3	3	3	3	3
3rd instar	4	4	4	4	4	4
4th instar	3	3	3	3	3	3
5th instar	5-6	5-6	5-6	6-7	6-7	6-7
Pupa	**17-19**	**17-20**	**17-20**	**17-19**	**17-19**	**17-20**
Adult	**5-11**	**7-11**	**5-11**	**5-10**	**5-11**	**5-11**
Female	5-10	7-10	5-11	5-10	5-10	5-11
Male	5-11	7-11	5-10	8-10	5-11	5-11
Egg - Adult	**50-61**	**52-62**	**50-62**	**51-61**	**51-62**	**51-63**

[1/] Temperature 25±2°C, 80±5%R.H. (control treatment) = normal temperature throughout experiment, high temperatures, 36±1, 40±1, 42±1, 45±1 and 48±1 °C. (the larvae were treated for 3 h.)

Table 2. Survival rates and cocooning rate of eri silkworm (*Samia ricini* D.) treated with different temperatures.

Temperature (°C)[1/]	Survival (%)		Cocooning (%)
	Larva stage (1st - 5th instar)	**Larva stage (1st - 5th instar) - Adult stage**	
25±2	**100.00±0.00 a**	**96.67±2.89 a**	**100.00±0.00 a**
36±1	**100.00±0.00 a**	95.00±5.00 a	**100.00±0.00 a**
40±1	**100.00±0.00 a**	91.67±10.41 a	98.33±2.89 a
42±1	98.33±2.89 a	83.33±10.41 a	95.00±5.00 a
45±1	68.33±5.77 b	55.00±15.00 b	65.00±5.00 b
48±1	50.00±5.00 c	38.33±5.77 c	41.67±7.64 c
F-test	**	**	**
C.V. (%)	**4.82**	**12.01**	**4.40**

Means followed by the same letter within a column are not significantly different (DMRT, P >0.05).

** = Significantly different at 99% level.

[1/] Temperature 25±2°C, 80±5%R.H. (control treatment) = normal temperature throughout experiment, high temperatures, 36±1, 40±1, 42±1, 45±1 and 48±1 °C. (the larvae were treated for 3 h.)

3.1.2 Yields

For cocoon yields, it was summarized in Table 3. The maximum fresh cocoon weight, pupa weight and fresh cocoon weight/10,000 larvae were detected from control treatment (25±2°C), 2.8378 g, 2.4193 g, and 28.38 kg, respectively. These values were not significantly different to that obtain from 36±1 and 40±1°C. However, shell weight, shell ratio and total cocoon shell weight were the maximum derived from 36±1°C of 0.4000 g, 14.62% and 8.00 g, respectively. While at 48±1°C , the means of almost of all parameters were the minimum, fresh cocoon weight (2.5078 g), pupa weight (2.1508 g), cocoon shell weight(0.3429 g), total cocoon shell weight (2.88 g) and fresh cocoon weight/ 10,000 larvae (10.47 kg) which significantly different ($P<0.05$) to other treatments except shell ratio (13.69%).

Table 3. Cocoon yields of eri silkworm (*Samia ricini* D.) treated with different temperatures.

Temperature (°C)[1/]	Average yields					
	Fresh cocoon weight (g)	Pupa weight (g)	Cocoon Shell weight (g)	Shell ratio (%)	Total cocoon shell weight (g)	Fresh cocoon /10,000 larvae (kg)
25±1	**2.8378±0.07 a**	**2.4193±0.06 a**	0.3899±0.01 a	13.82±0.13	7.80±0.25 a	**28.38±0.72 a**
36±1	2.7555±0.17 ab	2.3407±0.16 ab	**0.4000±0.02 a**	**14.62±0.61**	**8.00±0.48 a**	27.56±1.73 ab
40±1	2.7357±0.03 ab	2.3287±0.04 ab	0.3763±0.02 ab	13.87±0.46	7.41±0.55 ab	26.91±1.10 ab
42±1	2.5991±0.07 bc	2.2462±0.06 ab	0.3463±0.05 b	13.33±0.17	6.58±0.42 b	24.76±1.94 b
45±1	2.5166±0.12 c	2.1538±0.08 bc	0.3475±0.03 b	13.74±0.69	4.52±0.56 c	16.36±1.55 c
48±1	2.5078±0.04 c	2.1508±0.03 c	0.3429±0.03 b	13.69±0.99	2.88±0.74 d	10.47±2.10 d
F-test	**	*	*	ns	**	**
C.V. (%)	**3.63**	**3.69**	**5.84**	**4.26**	**8.42**	**7.12**

Means followed by the same letter within a column are not significantly different (DMRT, $P>0.05$).

ns = non significantly different at 95% level

*, ** = Significantly different at 95 and 99% level, respectively.

[1/] Temperature 25±2°C, 80±5%R.H. (control treatment) = normal temperature throughout experiment, high temperatures, 36±1, 40±1, 42±1, 45±1 and 48±1 °C. (the larvae were treated for 3 h.)

The egg yields were presented in Table 4. Egg laying/moth derived from all treatments were not significantly different. Percentage of hatchability from 36±1°C was the highest and not significant difference to others except 48±1°C. But total egg laying from control treatment (25±2°C) were the maximum of 3,039.33 eggs, which not statistically different to obtain from 36±1 and 40±1°C. The most hatchability of 2,675.13 eggs were achieved from treatment of 36±1°C and not significantly different to the derived from 25±2 and 40±1°C as 2,637.47 and

2,558.67 eggs, respectively. Besides, at 48±1°C, these yields were the minimum, egg laying /moth (287.56 eggs), hatchability (72.67%), total egg laying (1,121.33 eggs) and total hatchability (800.11 eggs).

Table 4. Egg yields of eri silkworm (*Samia ricini* D.) treated with different temperatures.

Temperature (°C)[1/]	Average yields			
	Egg laying (eggs)	Hatchability (%)	Total egg laying (eggs)	Total hatchability (eggs)
25±2	316.40±21.34	86.63±1.40 a	**3,039.33±541.67 a**	2,637.47±511.66 a
36±1	330.53±61.79	**90.69±3.23 a**	2,939.60±358.67 a	**2,675.13±406.59 a**
40±1	311.40±46.95	85.51±4.42 a	3,002.33±490.60 a	2,558.67±434.36 a
42±1	290.73±42.97	81.89±6.39 ab	2,176.73±316.26 b	1,802.73±255.20 b
45±1	307.98±24.94	81.92±1.53 ab	1,641.33±161.05 bc	1,342.58±114.38 bc
48±1	287.56±47.65	72.67±9.32 b	1,121.33±136.88 c	800.11±188.09 c
F-test	ns	*	**	**
C.V. (%)	**12.85**	**6.39**	**15.81**	**18.09**

Means followed by the same letter within a column are not significantly different (DMRT, P >0.05).
ns = non significantly different at 95% level
*, ** = Significantly different at 95 and 99% level, respectively.
[1/] Temperature 25±2°C, 80±5%R.H. (control treatment) = normal temperature throughout experiment, high temperatures, 36±1, 40±1, 42±1, 45±1 and 48±1°C. (The larvae were treated for 3 h.)

3.2 Heat shock protein detection

The protein bands appeared in the polyacrylamide gel exhibited the proteins from hemolymph of eri silkworms exposed by different high temperatures, compared to normal temperatures (25±2°C). Protein bands distributed closely pattern from high molecular weight 216 kDa to lower than 18.4 kDa. In comparison among temperatures treated samples, there was only a protein band with molecular weight of approximate 50 kDa detected from samples obtained from treated larvae at temperatures of 36±1, 40±1, 42±1, 45±1 and 48±1 °C. However, this protein band was not detectable in control treatment (lane 2, Figure 1). The bands were found and expressed only in the high temperature treatments. These were the proteins occurred by heat shock treatment.

4. Conclusions and Discussion

Survival rates and yields of eri silkworm depend on temperature. The result of this present study showed particularly at 42±1 onwards the negative relation between temperature and those survival rates and yields excluding cocoon shell weight, shell ratio, egg laying/moth, percentage of hatchability. At high temperatures, survival rates and yields were low. In the case of optimum temperature (25±2°C, 50±5%R.H.), all parameters contained the maximum values. These values decreased

principally, when temperature had obviously increased. The minimum values of all parameters were recorded at the maximum temperature treated (48°C) except cocoon shell percentage. This phenomenon was similar to the report of Hussian et al. (11) that high temperature affected yields of mulberry silkworm and also eri silkworm (12). In our present study, the heat shock protein with molecular weight approximately 50 kDa was evidently occurred when larvae were treated with temperature 36°C upwards to 48°C. There was no such protein band found from control treatment (25±2°C). The HSP was produced to defense any invasion to the cells. This protein plays an important role in cell protection and stress (13) and viable of cell (14). Normally, the HSP was detected from different tissues of silkworm and depended on growth and temperature (15). The molecular weight of HSP from this study is different from that of the mulberry silkworm, which expressed the molecular weight of 70 and 40 kDa (16). There is no report of eri silkworm HSP before in Thailand and others, so that this present work is a first report on the HSP of eri silkworm. The HSP is available to be applied as a protein marker to assist the eri silkworm thermotolerant variety improvement for future study.

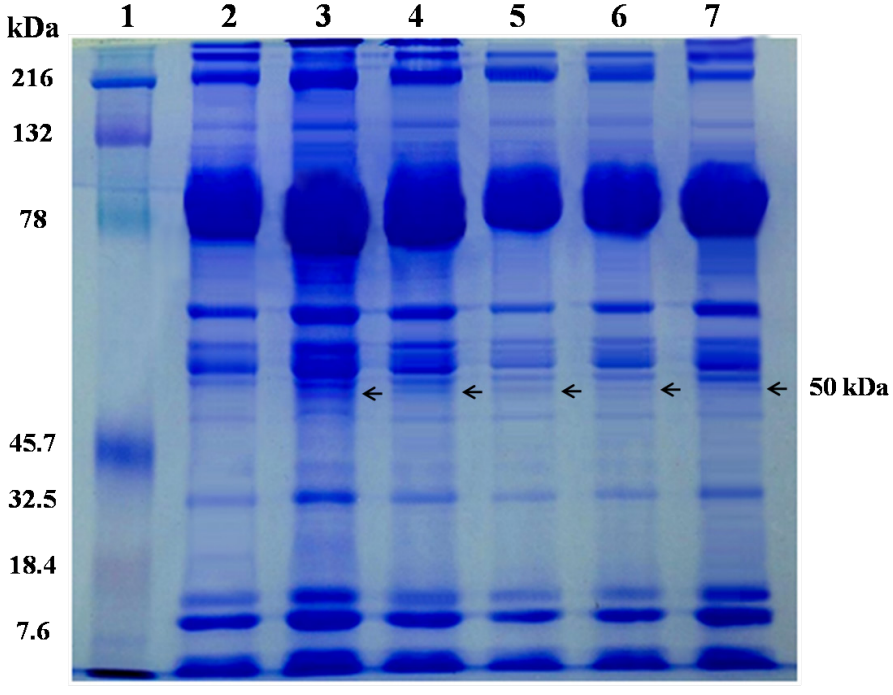

Figure 1. SDS-PAGE analysis of heat shock protein profile derived from eri silkworm (*Samia ricini* D.) treated with different temperatures.

Lane 1: Protein Molecular Weight Marker
Lane 2: Control treatment (25±2º; 80±5%R.H.)
Lane 3: 36±1°C
Lane 4: 40±1°C
Lane 5: 42±1°C
Lane 6: 45±1°C
Lane 7: 48±1°C

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