KKU Res.j. 2015; 20(2) : 177-188
http://resjournal.kku.ac.th

Improvement of Eri Silkworm (*Samia ricini* D.) Tolerant to High Temperature and Low Humidity Conditions by Continuously Regime

Duanpen Wongsorn[1, 2] Sivilai Sirimungkararat[1, 2, 3] and Weerasak Saksirirat[2, 3]*

[1]Entomology Section, Department of Plant Science and Agricultural Resource, Faculty of Agriculture, Khon Kaen University, Khon Kaen 40002, Thailand
[2]Cultivation and Product Development Research Group of Wild Silkmoths and Economic Insects for Value Added Creation, Khon Kaen University, Khon Kaen 40002, Thailand
[3] Agricultural Biotechnology Research Center for Sustainable Economy and Center of Agricultural Biotechnology (AG- BIO/PERDO-CHE), Faculty of Agriculture, Khon Kaen University, Khon Kaen 40002, Thailand
** Correspondent Author: sivilai@kku.ac.th*

Abstract

Improvement of eri silkworm tolerant to high temperature condition was carried out by selection and continuous rearing (1-12 generation) under high temperature (42±1 °C) and low relative humidity (50±5%R.H.). The eri silkworm rearing at a normal temperature (25±2 °C; 80±5%R.H.) served as control treatment. The result revealed that eri silkworm was continuously tolerant to high temperature of 42±1˚C with low relative humidity (50±5%R.H.) up to 12 generations. Among treated generations with high temperature, the silkworms survived from larva stage (1st-5th instar) to adult in all generations ranking 16.67-54.17%. Between F12 and F1, survival rate of larva-adult stage of F12 was 43.33% less than F1 (54.17%), but not significantly different ($P<0.05$). For cocoon yields, fresh cocoon weight, pupa weight and cocoon shell weight of F12 were lower than F1. Of F12, shell ratio (11.84%), total cocoon shell weight (5.72g) and fresh cocoon weight/10,000 larvae (9.39 kg) were not significantly different to F1 (11.61 %, 6.10 g and 10.55 kg, respectively). It was clearly, if the egg yields were considered, egg laying/moth, total egg laying and total hatchability of F12 were higher than F1. The result indicates that the thermotolerant property of eri silkworm SaKKU1 is heritable by high temperature at least 12 generations. This study is a first report on continuously heat tolerant improvement of eri silkworm in Thailand and elsewhere.

Keywords: *eri silkworm, tolerance, high temperature, low humidity, Samia ricini*

1. Introduction

A multipurpose and polyphagous insect, eri silkworm *Samia ricini* D. has been researched in complete cycle particularly in the Northeast of Thailand (1). In addition to industrial insects such as domesticated silkworm (*Bombyx mori*), eri silkworm plays a significant role in various industries, especially the textile industry of the world. It has also been studied and utilized in various fields such as cosmetic products, processed foods, health care products, etc. (2, 3, 4). Currently, in Thailand, eri silkworm rearing is being widely promoted in government, private and public sectors. The success of sericulture industry normally depends upon several factors such as biotic and abiotic factors, which are vital importance. Among abiotic factors, the temperature plays a major role on growth and productivity in domesticated mulberry silkworm, *B. mori* (5). Similarly in eri silkworm, optimum temperature for growth of young stage is about 26-28°C, relative humidity 85-90% and later stage 24 -26°C, relative humidity 70-80% (6). Recently, eri silkworm rearing at high temperature as in summer temperatures is forced to do by global warming. At temperatures above 35°C, it often negatively affects growth of eri silkworm. As a result, eri silkworm can not grow well and eventually be infected easily by the pathogens causing dead, which makes impossible to maintain eri silkworm varieties. This may also lead to reduce yields in both quantity and quality (7). Eri silkworm was introduced to Thailand for long time ago. It has been promoted and researched in various aspects towards industrial level (8). In Thailand, rearing this silkworm in hot season especially between March-April is obstructed by high temperature. Low yields and high mortality were observed. For eri silkworm breeding to withstand high temperatures has no research except the study of Sirimungkararat et al. (9) on high temperature and high humidity (80±5%R.H.) that the silkworm was not able to survive and complete its life cycle and our study (10) on screening of tolerant ecoraces to different high temperatures and low humidity(50±5%). The ecorace SaKKU1 show high temperature tolerant property up to 42±1°C with low humidity (50±5%). Hence, improvement of heat tolerant ecorace or variety of eri silkworm is one of the promising solutions and the way to overcome lower productivity problem caused by global warming and climate change in the near future. Improvement of eri silkworm to withstand against high temperatures was conducted previously by treatment of high temperature of 42±1°C and alternation by rearing at 25°C in some generations (11). However, the improvement of eri silkworm with continuous high temperature has never been studied before. Hence, this present work aims to improve eri silkworm ecorace (SaKKU1) that can tolerate to high temperature by continuously high temperature treatment and to evaluate effect of high temperature and low relative humidity on growth and yield.

2. Materials and Methods

2.1 Eri silkworm stock culture

Ecorace SaKKU1 of eri silkworm (*S. ricini*) was selected as stock to be improved for heat tolerance, based on its morphological character, growth, yields

(12), genetic characterization(9) and thermotolerant property tested preliminarily for suitable ecorace at 42±1°C, 50±5%R.H. After female adults laid eggs and newly hatched larvae (3-5 hours after hatching) developed to new adult stage, it was defined as filial generation (F). These hatched larvae were randomly selected and defined as the first filial generation (F1). They were reared at normal temperature (25±2°C, 80±5%R.H.) and fed with castor (cultivar TCO 101) leaves as food plant until 5th instar day 3. These larvae were separated into 2 groups for continuously high temperature treatment (42±1°C, 50±5%R.H.) and control treatment (25±2 °C, 80±5%R.H.).

2.2 High temperature treatments

Procedure of high temperature treatment or thermal exposure was modified from methods of Suresh Kumar et al. (13), Rao et al. (14) and Singh and Kumar (15, 16). Briefly, the larvae of 5th instar day 3 in 1st generation (F1) were randomly selected and reared at high temperature of 42±1°C, 50±5%R.H. in an incubator (Umax Scientific: Model Um-TTM004 Leec, England) for 6 hours/day (10.00-16.00). After high temperature treated of each day, they were moved to normal temperature (25±2 °C, 80±5%R.H.). This procedure was performed until the survival larvae reached to the mature stage, then they were allowed to spin cocoons at this normal temperature. Cocoon harvesting was carried out on the 7th day after spinning. Cocoon yield parameters were assessed on the subsequent days. After adult moths of each replication have random mating, the eggs were laid. The 1st instar larvae (3-5 hours after hatching) from various moths of each treatment were selected by random. They were reared at normal temperature and fed with castor leaves according to the method of Sirimungkararat et al. (17). The high temperature (42±1°C, 50±5%R.H.) exposure treatment and control treatment (25±2°C, 80±5%R.H.) were conducted continuously from 1st generation (F1) until F12. The improvement diagram of high temperature exposure and control treatment is shown in Figure 1.

2.3 Experimental design, data collection and statistic analysis

The completely randomized design (CRD) was used. Twelve generations (F1 – F12) served as treatments. There were 3 replications per treatment. Each replication contained 50 larvae. The data pertaining traits *viz*. survival {larva stage (1st-5th instar), larva stage(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/moth, percentage of hatchability, total egg laying and total hatchability) were recorded. The data were statistically analyzed according to analysis of variance (ANOVA) and the means were compared by using Duncan's multiple range test (DMRT).

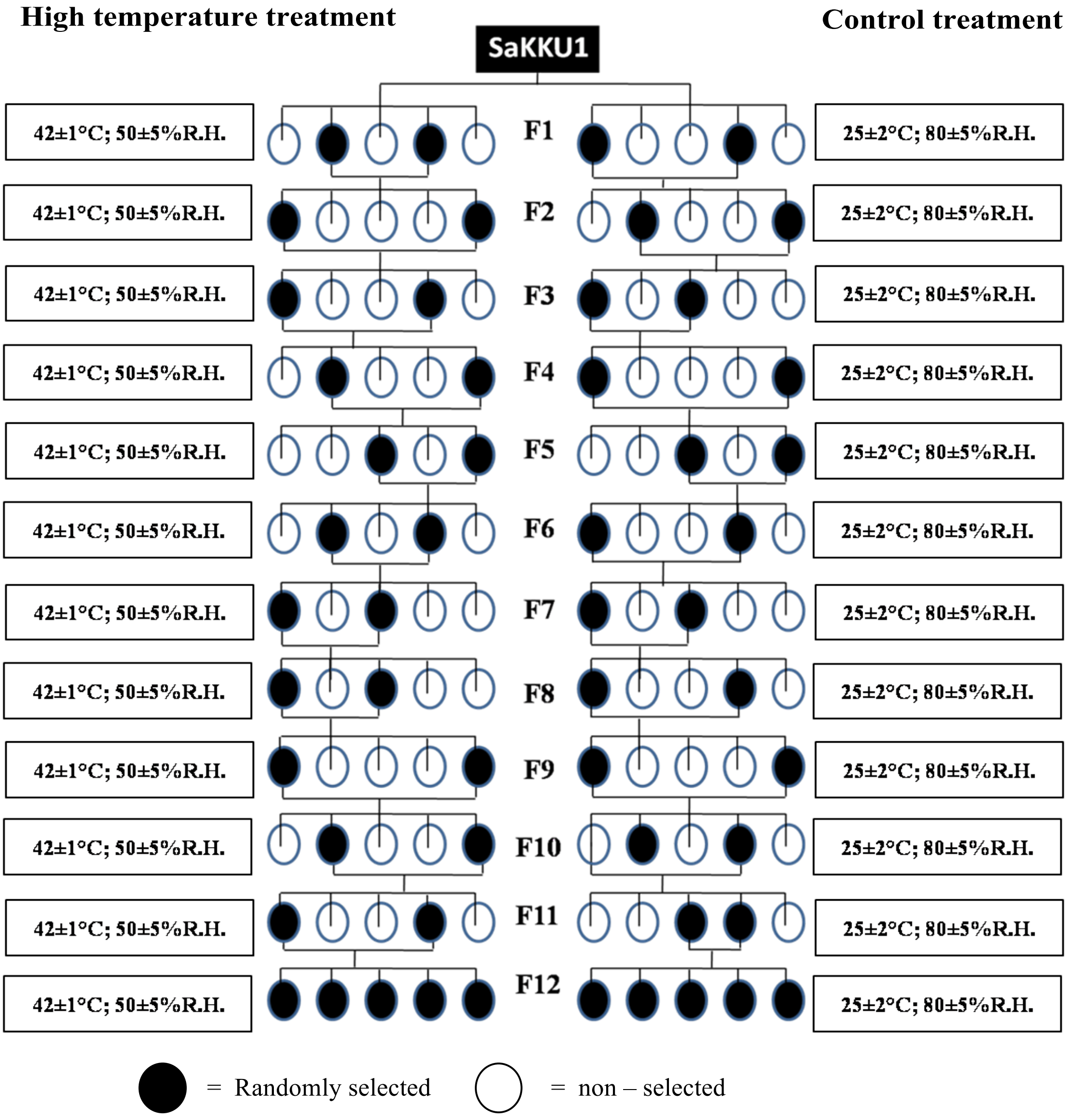

Figure 1. Improvement plan of eri silkworm (*Samia ricini* D.) ecorace SaKKU1 tolerant to 42±1°C and 50±5%R.H.

3. Results

3.1 Survival and cocooning rate of eri silkworm

Mean values of survival and cocooning rate of SaKKU1 under high temperature and low humidity (42±1°C and 50±5%R.H.) and control treatment (25±2°C; 80±5%R.H.), by continuous rearing (1-12 generation) presented in Table 1. The data revealed that the survival percentages of larva stage (1st-5th instar) and larva (1st-5th instar)-adult stage at 42±1°C and 50±5%R.H. ranges from 56.00-98.00% and 16.67-54.17%, respectively. After treatment of high temperatures and low humidity, the eri silkworm from F1 survived from generation to generation until 12th generation. Among generations, they survived beginning 56.00%, based on minimum survival rate of larva stage (1st-5th instar) in F5. However, if the survival rates of larva (1st-5th instar)-adult stage were considered, these survival rates were lower than those of larva stage. The minimum percentage was 16.67% in F2. In comparison of both parameters between last generation (F12) and first generation (F1), the survival rates in F12 (74.67 and 43.33%) were comparable and not significantly different to F1 (78.33 and 54.17%). Cocooning rate is also an indicator of thermotolerant property of eri silkworm. Likewise the survival rates, cocooning rate from F1 (34.00%) decreased temporarily to F5 (32.67%). From F6 (51.33%) onwards it increased temporarily until to F12 (49.33%), which was higher than F1 (34.00%) and significantly different (P<0.05) except F7. In control treatment, the eri silkworm was reared at normal temperature (25±2°C and 80±5%R.H.) showed principally higher values of all parameters (survival rates and cocooning rate) excluding survival rate of larva stage in F2. In comparison between F1 and F12, survival rate of larva stage (1st-5th instar) and larva (1st-5th instar)- adult stage were statistical differences of F1 (97.50, 97.50%, respectively) and F12 (100, 84.67%, respectively).

Table 1. Survival and cocooning rate of eri silkworm (*Samia ricini* D.) reared at two conditions of 42±1°C, 50±5%R.H. and 25±2°C, 80±5%R.H.

Generation	42±1°C and 50±5%R.H.[1/]			25±2°C and 80±5%R.H.[1/]		
	Survival rate (%)			Survival rate (%)		
	Larva stage (1st-5th instar)	Larva (1st-5th instar)-adult stage	Cocooning rate (%)	Larva stage (1st-5th instar)	Larva (1st-5th instar)-adult stage	Cocooning rate (%)
F1	78.33±12.58 c	**54.17±12.58 a**	34.00±7.21 c	97.50±2.50 bc	**97.50±2.50 a**	97.33±3.21 ab
F2	**98.00±2.00a**	16.67±2.31 e	21.33±4.62 d	96.67±1.15c	90.00±4.00 a-d	94.00±2.00 b
F3	94.00±5.29 ab	34.67±5.77 cd	21.33±2.31 d	99.33±1.15ab	87.33±5.03 b-d	94.00±2.00 b
F4	97.33±2.31 a	31.33±4.16 cd	15.33±3.06 d	**100.00±0.00 a**	85.33±1.15 cd	97.33±2.31 ab
F5	56.00±5.29f	40.00±2.00 b-d	32.67±1.15 c	99.33±1.15 ab	92.00±2.00 a-c	99.33±1.15 a
F6	67.33±5.03 de	30.00±4.00 d	51.33±7.57 a	**100.00±0.00 a**	90.67±3.06 a-c	**100.00±0.00 a**
F7	62.67±5.03 ef	36.67±6.11 b-d	40.67±4.16 bc	98.67±1.15 a-c	85.33±5.77 cd	98.00±2.00 a
F8	97.33±2.31 a	31.33±7.02 cd	52.67±6.43 a	**100.00±0.00 a**	80.67±8.33 d	98.00±2.00 a
F9	79.33±3.06 c	48.67±6.11 ab	**53.33±8.08 a**	**100.00±0.00 a**	95.33±3.06 ab	**100.00±0.00 a**
F10	76.67±7.57 cd	38.00±8.72 b-d	44.67±6.43 ab	97.33±2.31 bc	90.00±4.00 a-d	96.00±3.46 ab
F11	86.67±2.31 bc	34.00±8.72 cd	**53.33±3.06 a**	99.33±1.15 ab	92.00±9.17 a-c	99.33±1.15 a
F12	74.67±3.06 cd	43.33±4.16 a-c	49.33±6.43 ab	**100.00±0.00 a**	84.67±5.03 cd	96.67±3.06 ab
Mean	**80.69±4.65**	**36.57±9.63**	**39.17±5.04**	**99.01±0.09**	**89.24±4.79**	**97.50±2.07**
F-test	**	**	**	*	*	*
C.V.(%)	**7.06**	**18.13**	**14.40**	**1.25**	**5.60**	**2.11**

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

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

3.2 Cocoon yields of eri silkworm

The mean values of cocoon yields: fresh cocoon weight, pupa weight, cocoon shell weight, shell ratio, total cocoon shell weight and fresh cocoon weight/10,000 larvae of eri silkworm ecorae SaKKU1 under high temperature (42±1°C) and low humidity (50±5%) condition in incubator and control treatment (25±2°C; 80±5%R.H.) are presented in Table 2. At 42±1°C and 50±5%R.H., the highest fresh cocoon weight was observed in the 2nd generation (F2)(2.5008 g) and the least at F6(1.6136 g). After continuously high temperature treatment from F1 to F12, fresh cocoon weight, pupa weight and cocoon shell weight of F12 were lower than F1. There were statistical difference ($P<0.05$) of fresh cocoon weight and pupa weight between F1 and F12. Cocoon shell weight of F1 (0.2628 g) and F12 (0.2325 g) were not significantly different. Fresh cocoon weight was slightly decreased from 2.2853 g of F1 to 1.8867 g of F12. Pupa weight was from 1.9333 g of F1 to 1.6457 g of F12. In control treatment at normal temperature, means of fresh cocoon weight, pupa weight and cocoon shell weight of all generations varied between 2.4634-3.0391 g, 2.0900 -2.6381 g and 0.3360-0.4533 g. respectively. Even though, these parameters of F12 were decreased from F1 significantly ($P<0.05$) except cocoon shell weight. For shell ratio, total cocoon shell weight and fresh cocoon weight/10,000 larvae, it was not significantly different between F1 and F12 during high temperature exposure. The average values of shell ratio, total cocoon weight and fresh cocoon weight/10,000 larvae between F1 and F12 were 11.61 and 11.84%, 6.10 and 5.72% and 10.55 and 9.39 kg, respectively. While in control treatment, shell ratio and total cocoon shell weight were not significantly different between F1 and F12. The means of 2 parameters were comparable of 13.36 and 14.11% for shell ratio, whereas of 19.79 and 18.01 g for total cocoon shell weight. However, fresh cocoon weight/10,000 larvae of F1 (29.81 kg) was significantly higher than F12 (26.36 kg).

3.3 Egg yields of eri silkworm

The mean values of importance egg yields *viz*., egg laying/moth, percentage of hatchability, total egg laying and total hatchability are presented in Table 3. At 42±1°C and 50±5%R.H., the egg laying/moth was ranged from 149.00 – 295.50 eggs with maximum of 295.50 eggs indicated in F9 and a minimum of 149.00 in F1. While, at 25±2°C and 80±5%R.H., there was 302.20 – 428.47 eggs with a maximum of 428.47 achieved from F12 and a minimum of 302.20 from F7. In comparing between F1 and F12 of high temperature regime, it was interesting that egg laying/moth (149 eggs), total egg laying (746.11 eggs) and total hatchability (444.67 eggs) of F1 were lower and increased significantly ($P<0.05$) in F12 with values of 285.00, 2,857.60 and 2,180.07 eggs, respectively. Whereas in control treatments egg laying/moth, total egg laying and total hatchability also increased significantly ($P<0.05$) between F1 and F12 and reached the maximum in F12 were 346.67 and 428.47 eggs 6,360.03 and 10,207.13 eggs and 5,577.93 and 8,874.73 eggs, respectively.

Table 2. Cocoon yields of eri silkworm (*Samia ricini* D.) reared at two conditions of 42±1°C, 50±5%R.H. and 25±2°C, 80±5%R.H.

Generation	**42±1°C and 50±5%R.H.** [1/]			**25±2°C and 80±5%R.H.** [1/]		
	Fresh cocoon weight (g)	**Pupa weight (g)**	**Cocoon Shell weight (g)**	**Fresh cocoon weight (g)**	**Pupa weight (g)**	**Cocoon Shell weight (g)**
F1	2.2853±0.31 ab	1.9333±0.16 b-d	**0.2628±0.03 a**	3.0411±0.03 ab	2.6236±0.02 ab	0.4037±0.01 b-d
F2	**2.5008±0.11 a**	**2.3138±0.10 a**	0.2404±0.01 a-c	2.8929±0.06 bc	2.4617±0.06 c	0.4158±0.03 bc
F3	1.9712±0.14 cd	1.7999±0.14 d-g	0.1634±0.01 de	**3.0901±0.20 a**	**2.6381±0.19 a**	**0.4533±0.04 a**
F4	1.9812±0.02 cd	1.8213±0.04 d-f	0.1477±0.02 e	2.8927±0.08 bc	2.4753±0.06 bc	0.4202±0.03 b
F5	2.1370±0.12 bc	1.8860±0.08 c-e	0.2465±0.04 a-c	2.7639±0.10 c	2.3939±0.08 c	0.3740±0.03 d-f
F6	1.6136±0.03 e	1.4080±0.02 h	0.1993±0.01 b-e	2.4731±0.11 d	2.1383±0.06 d	0.3360±0.01 g
F7	1.8789±0.02 d	1.6225±0.12 g	0.2517±0.02 ab	2.4751±0.03 d	2.1375±0.25 d	0.3526±0.02 fg
F8	2.2744±0.04 ab	2.1076±0.06 b	0.2116±0.08 a-d	2.5482±0.05 d	2.1343±0.04 d	0.3999±0.01 b-d
F9	2.0282±0.06 cd	1.8077±0.02 d-g	0.1888±0.02 c-e	2.8526±0.18 c	2.4105±0.13 c	0.3953±0.01 b-e
F10	2.2955±0.17 ab	2.0577±0.09 bc	0.2325±0.02 a-c	2.7935±0.09 c	2.3768±0.07 c	0.3950±0.10 b-e
F11	1.9405±0.07 cd	1.6975±0.04 e-g	0.2183±0.02 a-d	2.4634±0.07 d	2.0900±0.06 d	0.3614±0.03 e-g
F12	1.8867±0.19 cd	1.6457±0.20 fg	0.2325±0.01 a-c	2.7270±0.09 c	2.3322±0.07 c	0.3829±0.02 c-f
Mean	**2.0661±0.24**	**1.8418±0.24**	**0.2163±0.02**	**2.7511±0.22**	**2.3510±0.19**	**0.3908±0.03**
F-test	**	**	**	**	**	**
C.V.(%)	**6.52**	**5.55**	14.40	**3.74**	**3.81**	**4.96**

Generation	**42±1°C and 50±5%R.H.** [1/]			**25±2°C and 80±5%R.H.** [1/]		
	Shell ratio (%)	**Total cocoon shell weight (g)**	**Fresh cocoon weight /10,000 larvae (kg)**	**Shell ratio (%)**	**Total cocoon shell weight (g)**	**Fresh cocoon weight /10,000 larvae (kg)**
F1	11.61±1.01 a-c	**6.10±0.68 a**	10.55±0.21 ab	13.36±0.24 d	19.79±0.87 a-c	**29.81±0.84 a**
F2	9.73±0.86 c-e	1.76±0.09 d	3.68±0.46 e	14.46±1.02 b-d	19.40±1.18 a-c	27.00±0.36 cd
F3	8.25±1.12 ef	1.75±0.26 d	4.18±0.14 e	14.90±0.76 ab	**21.30±0.63 a**	29.25±1.99 ab
F4	7.37±1.22 f	1.13±0.28 d	3.04±0.39 e	14.66±0.59 bc	20.50±1.66 ab	28.14±1.34 a-d
F5	11.44±1.44 a-c	3.27±1.64 c	6.65±0.87d	13.65±0.58 cd	18.58±1.57 b-e	27.46±1.14 b-d
F6	12.45±0.46 ab	5.13±1.03 ab	8.29±1.31 cd	13.68±0.17 cd	16.80±0.71 e	24.73±1.09 f
F7	**13.33±0.93 a**	5.10±0.35 ab	7.52±0.69 cd	14.29±0.80 b-d	17.28±1.03 de	24.42±0.33 f
F8	8.22±1.92 ef	5.17±1.91 ab	**11.97±1.32 a**	**15.83±0.28 a**	19.59±0.64 a-c	24.97±0.35 ef
F9	9.28±0.54 d-f	4.90±1.01 ab	10.53±1.61 ab	13.94±0.86 b-d	19.76±0.33 a-c	28.53±1.81 a-c
F10	12.88±0.97 ab	4.08±0.83 bc	6.72±1.12 d	14.28±0.28 b-d	18.96±0.59 b-d	26.80±0.23 c-e
F11	11.21±0.91 d-d	5.73±0.31 a	10.22±0.76 ab	14.81±0.77 a-c	17.95±1.39 c-e	24.47±0.72 f
F12	11.84±0.99 ab	5.72±0.59 a	9.39±2.07 bc	14.11±0.39 b-d	18.01±1.47 c-e	26.36±1.27 d-f
Mean	**10.63±1.99**	**4.17±2.27**	**7.73±0.91**	**14.33±0.67**	**18.99±1.33**	**26.83±1.89**
F-test	**	**	**	**	**	**
C.V.(%)	10.29	**19.26**	**8.07**	**4.35**	**5.74**	**4.13**

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

** = Significantly different at 99% level

Table 3. Egg yields of eri silkworm (*Samia ricini* D.) reared at two conditions of 42±1°C, 50±5%R.H. and 25±2°C, 80±5%R.H.

Generation	42±1°C and 50±5%R.H.[1/]				25±2°C and 80±5%R.H.[1/]			
	Egg laying/moth (eggs)	**Hatchability (%)**	**Total egg laying (eggs)**	**Total hatchability(eggs)**	**Egg laying/moth (eggs)**	**Hatchability (%)**	**Total egg laying (eggs)**	**Total hatchability (eggs)**
F1	149.00±33.83 f	77.00±12.82	746.11±474.96 g	444.67±209.71 e	346.67±19.72 c-e	87.78±0.41	6,360.03±687.85 c-e	5,577.93±594.53 de
F2	160.00±79.02 ef	**86.38±5.22**	982.00±244.52 fg	854.52±259.34 de	322.16±56.96 de	**92.64±1.66**	5,903.45±922.19 de	5,480.38±927.36 de
F3	199.11±41.13 d-f	69.50±6.40	1,774.89±274.76 ef	1,227.11±237.52 d	386.17±10.45 a-c	81.96±9.45	8,756.06±234.87 ab	7,263.22±565.16 a-c
F4	234.08±27.53 a-d	69.04±5.63	1,656.56±429.47 ef	1,106.78±227.60 de	401.53±24.13 ab	83.35±2.44	9,017.33±874.28 ab	6,722.67±597.18 b-d
F5	239.11±39.70 a-d	75.59±2.38	3,272.89±576.70 bc	2,581.44±629.90 ab	405.13±40.66 ab	88.70±7.17	9,086.73±928.11 ab	8,049.73±694.85 ab
F6	253.24±12.70 a-d	78.11±8.73	1,854.44±138.77 ef	1,457.44±225.03 cd	392.20±23.31 a-c	86.60±1.15	7,816.67±730.95 bc	7,493.20±203.08 a-c
F7	220.33±20.94 b-e	77.03±1.84	2,114.47±379.62 de	1,624.73±157.09 cd	302.20±15.38 e	87.67±4.01	6,947.07±809.88 c-e	6,096.40±749.43 c-e
F8	210.00±58.28 c-f	80.14±15.30	2,062.53±587.82 de	1,661.49±638.24 cd	329.48±17.73 de	87.89±1.41	5,485.71±357.82 e	4,795.14±212.30 e
F9	**295.50±13.51 a**	79.70±4.52	**4,236.97±479.06 a**	**3,366.55±224.70 a**	335.40±18.34 de	90.75±3.45	8,834.60±761.70 ab	8,036.87±430.12 ab
F10	270.64±11.87 a-c	77.32±2.11	3,781.94±145.55 ab	2,865.44±170.38 ab	420.73±51.30 a	87.93±2.15	8,896.80±199.04 ab	7,824.80±155.27 ab
F11	286.00±50.59 ab	79.50±3.35	3,873.33±621.65 ab	3,092.92±924.17 a	357.69±12.38 b-d	88.37±1.90	7,524.69±925.94 b-d	6,631.30±741.53 b-d
F12	285.00±11.82 ab	74.26±4.32	2,857.60±397.96 cd	2,180.07±441.59 bc	**428.47±41.59 a**	86.95±1.06	**10,207.13±963.63 a**	**8,874.73±845.48 a**
Mean	**233.50±48.15**	**75.56±4.69**	**2,434.46±415.40**	**1,871.97±937.19**	**368.99±41.98**	**87.55±2.84**	**7,903.04±800.29**	**6,903.83±735.38**
F-test	**	ns	**	**	**	ns	**	**
C.V.(%)	**16.02**	9.48	20.57	23.24	**7.31**	4.30	**11.56**	12.37

[1/] 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 99% level.

4. Conclusions and Discussion

Eri silkworm is being promoted to produce in various parts of Thailand. Comparison to mulberry silkworm, the hybrid variety or breeding were utilized to achieve high yield and quality of silk including conservation. One of limiting factors affecting the eri silkworm rearing is the low survival and yield during high temperature especially in hot climate as in summer. Silkworm is the best animal where hybrids are used compulsorily for commercial silk product since the study of Toyama (18). This study in eri silkworm was carried out to develop suitable high temperature tolerant ecorace to the target environment with continuously high temperature (42±1°C) and low humidity (50±5%). After exposure with this high temperature condition, eri silkworm could grow continuously until a final generation (F12). Comparison between F1 and F12, F12 had higher values in shell ratio, egg laying/moth and total hatchability except shell ratio were significant difference ($P<0.05$). Although others parameters (survival rates, total cocoon shell weight) of F1 were higher than F12 but not significantly different. All of these mention parameters suggest that the nature of the heat tolerance is heritable and accumulated to the 12th generation. The similar result was reviewed from the former work under the same temperature condition (42±1°C, 50±5%R.H.) but with alternative treatment (11). In domesticated mulberry silkworm (*B. mori*), the breeders of all the sericultural countries have experienced on the influence of environment during the process of breeding. Effect of high temperature more than 30°C on mulberry silkworm larvae was reported earlier (20). Attempts to create high temperature resistant silkworm races were carried out and showed that the genetically heritable nature of thermotolerance is possible to be accumulated by selection using pupation rate of silkworm as index of thermotolerance on the larvae reared under high temperature conditions during 5th instar (21, 22). In addition, increasing in temperature beyond 35°C causes less spinning, mortality of larvae and pupae, poor moth emergence and sterility at adult stage of eri silkworm (7). In this present study, cocoon yields of eri silkworm particularly total cocoon shell weight and fresh cocoon weight/10,000 larvae were obtained regularly from early generation until at least 12 generations. This indicates the performance of eri silkworm ecorace of high temperature tolerance improved by directional selection at 42±1°C of low humidity (50±5%R.H.). However, at the same temperature but high humidity (80±5%R.H.) the eri silkworm could not survive and could not complete its life cycle (23). This emphasizes that the temperature and relative humidity are the major effective factors on growth and yield of eri silkworm. In the case of domesticated mulberry silkworm, high temperature caused adverse effect on biochemical process including physiology of the worm (24). Rahmathulla (25) indicated in *B. mori* that relative humidity affects both directly and indirectly on physiological function of silkworm and rapidly dry mulberry leaves. Besides, the result study of Hussian et al. (26) demonstrated in *B. mori* that rearing silkworm at temperatures, 25±1, 30±1 and 35±1°C and low humidity of 55%R.H., result in lowest cocoon yield in almost all tested varieties. Suresh Kumar et al. (27) showed that fresh cocoon and cocoon shell weight of *B. mori* decreased due to high temperature effect and suggested that thermotolerant property was heritable. Similar tendency for the decreasing of fresh cocoon weight and cocoon shell weight of high temperature improvement was observed in our study between F1 and F12 of eri silkworm. Survival rates of eri silkworm obtained from high temperature treatment increased temporarily by directional selection towards F12 and between F1 and F12 were not significant difference ($P<0.05$) in our study. Besides, when all parameters were considered, it was showed that survivals, total cocoon shell weight and fresh cocoon weight/ 10,000 larvae of F12 were lower but not significantly different to F1. Only total hatchability of F12 was higher and significantly different ($P<0.05$). This suggests the adaptation of eri silkworm SaKKU1 against high temperature suppression. There is also evidently that F12, the last generation of high temperature exposure showed the survival rates, which were comparable and not statistically different to the beginning generation (F1). Our studies indicated the thermotolerant property of eri silkworm exposed continuously with high temperature. Ecorace SaKKU1 can tolerate to 42±1°C with low humidity (50±5%) than other ecoraces (9, 10). The study indicates that thermotolerant property of eri silkworm ecorace SaKKU1 is heritable to the progeny. This work is a first report of the improvement of eri silkworm tolerant to high temperature by continuously regime. The discontinuous temperature regime for improving thermotolerant ecorace of eri silkworm was previously reported (11). Both works provide the thermotolerant eri silkworm, which is a high potential to be produced under a climate change condition. Consequently, the further research is ongoing in outdoor condition and farmer scale.

5. Acknowledgements

This research is supported by Plant Genetic Conservation Project under the Royal Initiation of Her Royal Highness Princes Maha Charkri Sirindhorn, KKU-Integrate Multidisciplinary Research Cluster, General Research Fund Fiscal Year 2011-2013 from Khon Kaen University (KKU), Cultivation and Product Development Research Group of Wild Silkmoths and Economic Insects for Value Added Creation, Agricultural Biotechnology Research Center for Sustainable Economy and Center of Agricultural Biotechnology KKU and AG-BIO/PERDO-CHE.

6. References

(1) Sirimungkararat S. Eri silkworm rearing in Thailand. International Journal of Wild Silkmoth & Silk. 2014;18: 1-16.
(2) Akai H. Development and contribution of international society for wild silkmoths. International Journal of Wild Silkmoth & Silk. 2002;7: 1-10.
(3) Sirimungkararat S, Nuchadomrong S, Saksirirat W, Nopparat T. By-product of eri silkworm rearing as preliminary cosmetic product development. 6th International Conference on Wild Silkmoths; 2010 Sep 21-23, Tokyo University of Agriculture, Tokyo, Japan. 2010.

(4) Sirimungkararat S. Eri silkworm products and current status of wild silkmoths in Thailand. The 7th International Conference on Wild Silkmoth and Silk “Southeast Asian Silk”; 2012 Nov 22-24, Mahasarakham University, Mahasarakham, Thailand. 2012.

(5) Kumari SS, Subbrarao SV, Misra S, Murty US. Screening strains of the mulberry silkworm, *Bombyx mori*, for thermotolerance. Journal of Insect Science. 2011;11(116): 1-14.

(6) Singh NS, Saratchandra B. Eri culture. A P H Publishing C orporation: New Delhi; 2012.

(7) Sahu M, Bhuyan N, Das PK. Eri silkworm, *Samia ricini* (Lepidoptera: Saturniidae) Donovan, seed production during summer in Assam. Proceeding of Regional Seminar on Prospects and Problems of Sericulture as an Economic Enterprise in North West India; 2006 Nov 11-12; RSRS, Dehradune, India. 2006.

(8) Sirimungkararat S. Innovation on eri silk products for the community and economics in Thailand. International Seminar Cum Business Matching on Mekong Silk Road; 2013 Nov 27–28; Rachawadee Resort Hotel, Khon Kaen, Thailand. 2013.

(9) Sirimungkararat S, Maskhao S, Wongsorn D, Saksirirat W. Effects of high temperature and relative humidity on growths and yields of eri silkworm (*Samia ricini* D.). National Conference on Sericulture; 2013 June 25-27; Amari Don Muang Airport, Bangkok, Thailand.; 2013a. Thai.

(10) Wongsorn D, Sirimungkararat S, Urairong H, Saksirirat W, Choo-prayoon S. Selection of eri silkworm (*Samia ricini* D.) tolerant ecorace to high temperature. The Conference of the Queen Sirikit Department of Sericulture, Ministry of Agriculture and Cooperatives; 2014 April 2-4, Asia Airport Hotel, Pathum Thani, Thailand. 2014. Thai.

(11) Wongsorn D, Sirimungkararat S, Saksirirat W. Improvement of eri silkworm (*Samia ricini* D.) tolerant to high temperature and low humidity conditions by discontinuous regime. Songklanakarin Journal of Science and Technology. 2015; 4 Jul.-Aug.

(12) Wongsorn D, Sirimungkararat S, Saksirirat W. Screening of eri silkworm ecoraces towards variety improvement. Khon Kaen Agriculture Journal. 2013;41(Special Issue): 192-198. Thai.

(13) Suresh Kumar N, Basavaraja HK, Kumar CMK, Reddy NM, Datta RK. On the breeding of CSR18x CSR19-a robust bivoltine hybrid of silkworm, *Bombyx mori* L. for the tropics. International Journal of Industrial Entomology. 2002;5(2): 153-162.

(14) Rao PS, Nayaka ARN, Mamatha MT, Sowmyashree S, Bashai I, Ilahi I. Development of new robust bivoltine silkworm hybrid SR2x-SR5 for rearing throughout the year. International Journal of Industrial Entomology. 2007;14(2): 93-97.

(15) Singh H, Kumar NS. On the breeding of bivoltine breeds of the silkworm, *Bombyx mori* L. (Lepidoptera : Bombycidae), tolerant to high temperature and high humidity conditions of the tropics. Psyche. 2010a;(892452): 1-15.

(16) Singh H, Kumar NS. On the breeding of bivoltine double hybrid of the silkworm, *Bombyx mori* L. (Lepidoptera : Bombycidae), tolerant to high temperature and low humidity conditions of the tropics. African Journal of Basic & Applied Sciences. 2010b;2(3-4): 71-80.

(17) Sirimungkararat S, Atthathom T, Saksirirat W. Development of eri-silkworm rearing technique using cassava leaf as food plant and its textile production. Proceedings of the XIXth Congress of the International Sericultural Commission; 2002 Sep 21-25, Queen Sirikit National Convention Center, Bangkok, Thailand. 2002.

(18) Toyama K. Studies on hybridology of insects. I. On some silkworm crosses, with special reference to Mendel's law of heredity. Bull. Tokyo Imp. Univ. College of Agriculture. 1906;7: 259-293.

(19) Kumar NS, Reddy SG. Evaluation and selection of potential parents for silkworm breeding. In: Reddy, SG, editor. Silkworm breeding. Proceedings of the National Workshop held; 1994 March 18 -19; 1994, P. 63-78.

(20) He Y, Oshiki T. Study on cross breeding of a robust silkworm race in low latitude are in China. J. Seri. Sci. Jpn. 1984;54: 320-324.

(21) Shirota T. Selection of healthy silkworm strain through high temperature rearing of fifth instar larvae. Reports of the Silk Science Research Institute. 1992;40: 33-40.

(22) Tazima Y, Ohnuma A. Preliminary experiments on the breeding procedure for synthesizing a high temperature resistant commercial strain of the silkworm, *Bombyx mori* L. Silk Science Research Institute Japan. 1995;43: 1-16.

(23) Sirmungkararat S, Urairong H, Wongsorn D, Saksirirat W. Searching of microsatellite primers for diversity study of eri silkworm. The Conference of the Queen Sirikit Department of Sericulture, Ministry of Agriculture and Cooperatives; 2013 June 25-27, Amari Don Muang Airport, Bangkok, Thailand. 2013b. Thai.

(24) Willmer CW, Stone G, Johnston I. Environmental physiology of animal. Blackwell Science: Oxford; 2004.

(25) Rahmathulla VK. Management of climatic factors for successful silkworm (*Bombyx mori* L.) crop and higher silk production: a review. Psyche. 2012;121234: 1-12.

(26) Hussian M, Khan SA, Naeem M, Aqil T, Khursheed R, Mohsin AU. Evaluation of silkworm lines against variations in temperature and R.H. for various parameters of commercial cocoon production. Psyche. 2011;145640: 1-11.

(27) Suresh Kumar N, Yamamota T, Basavaraja HK, Datta RK. Studies on the effect of high temperature on F1 hybrids between polyvoltine and bivoltine silkworm races of *Bombyx mori* L. International Journal of Entomology. 2001;2: 123-127.