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Original Article

Improvement of eri silkworm (*Samia ricini* D.) tolerance to high temperature and low humidity conditions by discontinuous regime

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Abstract

Improvement of eri silkworm tolerance to high temperature was carried out under high temperature (42±1°C) and low relative humidity (50±5% R.H., relative humidity). Eri silkworm rearing at normal temperature (25±2°C; 80±5% R.H.) served as control treatment and was compared to rearing at high temperatures. Directional selection was undertaken with the batches reared at 42±1°C and 50±5% R.H. until the 5th generation. From 6th, 8th, 10th and 12th generations, rearing was conducted as normal rearing (25±2°C; 80±5% R.H.), while for 7th, 9th, and 11th generations directional selection was done as 1st – 5th generations. Various parameters were used as indexes for high temperature tolerance. When the silkworms were reared until to F12 (12th generation), the survival rate of larva (1st–5th instar) (95.33%) and larva (1st–5th instar) – adult (69.33%) including cocooning rate (80.67%) of F12 were the highest compared to F1 and F11. For cocoon yields among F1, F11, and F12, the highest values of fresh cocoon weight (2.7144 g), pupa weight (2.3490 g), shell weight (0.3671 g), shell ratio(13.53%), total cocoon shell weight(17.01 g), and fresh cocoon/10,000 larvae (25. 16 kg) were achieved from F12, which were significantly different ($P< 0.05$) to F1 and F11. In the same manner for egg yields, F12 provided the maximum numbers in case of eggs/moth (311.33 eggs), total eggs (6,693.33 eggs) and total hatching eggs (4,842.67 eggs) except hatching eggs (73.15%). Of these evaluations between F11 and F1, it was found that F11 was higher than F1 and statistically different ($P<0.05$) in all parameters excluding the percentage of hatching eggs, which was not significant difference. These results indicate that the property of high temperature tolerance was improved and heat tolerant property of eri silkworm (SaKKU1) is heritable. This is a first to report on heat tolerance improvement of eri silkworm. Although it is a first trial that was carried out in a laboratory, it can be applied on eri silkworm rearing in the future to cope with recent global warming trends.

Keywords: eri silkworm, tolerance, high temperature, low humidity, selection

1. Introduction

Eri silkworm (*Samia ricini* D.) is a multivoltine, polyphagous wild silkworm, and feeds on a wide range of host plants (Sirimungkararat, 2014). In addition to industrial insects such as domesticated silkworm (*Bombyx mori*), eri silkworm plays a significant role in various industries, especially the worldwide textile industry. It has also been studied and utilized in various fields such as cosmetic products, processed food supplements, health care products, etc. (Akai, 2002; Sirimungkararat *et al.*, 2010; Sirimungkararat, 2012). Currently, in Thailand, eri silkworm rearing is being widely promoted in

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government, private and public sectors. The success of sericulture industry normally depends upon several factors such as biotic and abiotic factors, which are of vital importance. Among abiotic factors, the temperature plays a major role on growth and productivity in domesticated mulberry silkworm, *B. mori* (Kumari *et al.*, 2011). Similarly, in eri silkworm the optimum temperature for growth of young stage is about 26-28°C with a relative humidity of 85-90% and at later stage 24-26°C, with 70-80% (Singh and Saratchandra, 2012). Recently, eri silkworm rearing at high temperature as in summer temperatures might be seen as a scenario due to global warming. Temperatures above 35°C often negatively affect growth of eri silkworm. As a result, eri silkworm cannot grow well and eventually be infected easily by the pathogens causing dead, which makes it impossible to maintain eri silkworm varieties. This may also lead to reduce yields in both quantity and quality (Sahu *et al.*, 2006). Eri silkworm was introduced to Thailand several decades ago. It is promoted and researched in various aspects towards industrial level (Sirimungkararat, 2013). In Thailand, the rearing of eri silkworm in hot season, especially from March to April, is hampered by high temperatures. Low yields and high mortality were observed. There has been no research about the impact of high temperature on eri silkworm breeding except Sirimungkararat *et al.* (2013a) on high temperature and high humidity (80±5% R.H.), where the eri silkworm was not able to survive and complete its life cycle, and the study of Wongsorn *et al.* (2014) on the screening of ecoraces tolerant to different high temperatures and low humidity (50±5% R.H.). Ecoraces are populations that have been isolated geographically over centuries and by this have been adapted to particular ecological niches. The ecorace SaKKU1 shows a high temperature tolerance of up to 42±1°C with low relative humidity (50±5%). Hence, improvement of a heat tolerance ecorace or a variety of eri silkworm is one promising solution and a way to adapt to global warming trends and climate changes in the future. Therefore, this present work aims to improve eri silkworm ecorace (SaKKU1) that can tolerate high temperatures and to evaluate the effect of high temperature and low relative humidity on growth and yield of eri silkworm.

2. Materials and Methods

2.1 Eri silkworm stock culture

The eri silkworm ecorace SaKKU1 was selected as the stock to be improved for heat tolerance, based on its morphological character, growth, yields (Wongsorn *et al.*, 2013), genetic characterization (Sirimungkararat *et al.*, 2013a), and preliminary thermotolerant property tested with 42±1°C and 50±5% R.H. The hatched larvae of selected ecorace (SaKKU1) defined as the first filial generation (F1), were reared at room temperature (25±2°C, 80±5% R.H.) until 5th instar day 2. After that the 5th instar day 3 larvae were separated into 2 groups for high temperature treatment (42±1°C, 50±5 %R.H.) and control treatment (25±2°C, 80±5% R.H.).

2.2 High temperature treatment

The method of high temperature treatments or thermal exposure was modified from methods of Suresh Kumar *et al.* (2002), Rao *et al.* (2007), and Singh and Kumar (2010a,b). Briefly, the larvae of 5th instar day 3 (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 the high temperature treatment of each day, they were moved to normal temperature condition (25±2°C, 80±5% R.H.). This procedure was performed until the larvae reached to the ripe stage; then they were allowed to spin cocoons at this normal temperature. Cocoon harvesting was carried out on the 7th day. Cocoon yield parameters were assessed on the subsequent days. Adult moths were allowed to mate and couple until laying eggs. When larvae hatched from the mixed eggs from various moths of each treatment, the 1st instar larvae (3-5 hours after hatching) were selected randomly. The completely randomized design (CRD) was used with three replications. Twelve generations (F1-F12) served as treatments. There were three replications per treatment. Each replication contained 50 larvae, which were reared at normal temperature and fed with castor (cultivar TCO 101) leaves as food plant. Rearing was done according to the method of Sirimungkararat *et al.* (2002). When the larvae developed to 5th instar day 3, they were randomly selected and exposed to high temperature as described above. The survival larvae were reared until developing to adults. The male and female moths of each replication were randomized to mate and allowed to lay eggs. After female adults laid eggs and newly hatched larvae developed until to new adult stage, it was defined as filial generation (F). The high temperature exposure experiment was conducted continuously from 1st generation (F1) until F5. For F6, F8, F10, and F12, the eri silkworms were reared at 25±2°C and 80±5% R.H., whereas F7, F9, and F11 were reared under above mentioned high temperature and low humidity (42±1°C, 50±5% R.H.). Details of the improvement plan were based on temperature regime and control treatment conducted at 25±2°C, 80±5% R.H. from F1 to F12 was followed as shown in Figure 1.

2.3 Data collection and statistical analysis

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 (eggs/moth, hatching egg percentage, total eggs and total hatching eggs) 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).

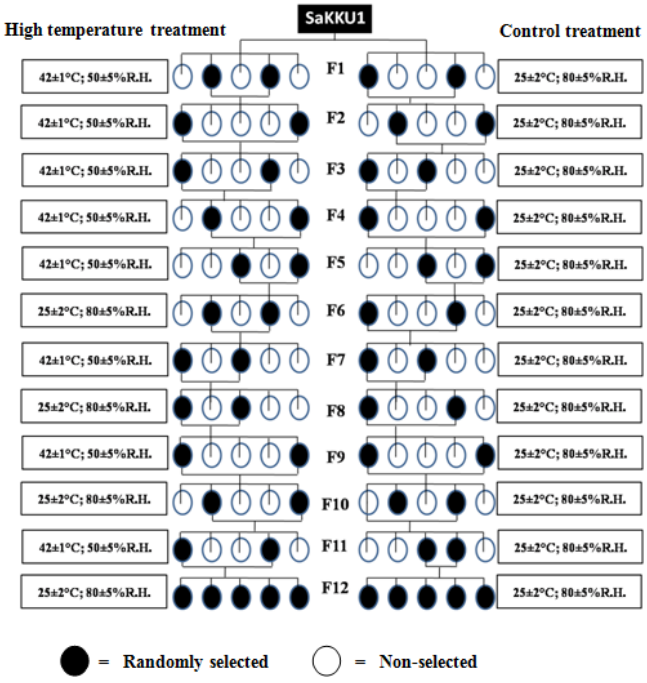

Figure 1. Improvement plan of eri silkworm (*Samia ricini* D.) ecorace SaKKU1 tolerant to 42±1°C and 50±5% R.H. F1-F5, F7, F9, and F11 = 5th instar larvae, day 3 were randomly selected and exposed to high temperature (42±1°C and 50±5% R.H.) for 6 hours/day otherwise were reared at normal temperature until reached to the ripe stage. F6, F8, F10, and F12 = silkworms were reared at normal temperature (25±1°C and 80±5% R.H.).

3. Results

3.1 Survival and cocooning rate of eri silkworm

Rearing eri silkworm at high temperature condition of 42±1°C, 50±5% R.H. in F1-F5 and F7, F9, and F11, while F6, F8, F10, and F12 at 25±2°C, 80±5% R.H. (Table 1) revealed the average values of survival and cocooning rates: survival rate from larva stage (1st-5th instar), the F1 survival rate was 78.33% whereas F5 was 56.00%, which was significantly different ($P<0.05$). When the silkworms were reared until to F11, the survival rate increased practically and F11 had a higher value (85.33%) than F1 (78.33%), which was not significantly different. The result of survival from larva (1st-5th instar) – adult stage showed that F1 (54.17%) was not significantly different from F11 (45.33%). For cocooning rate of F1-F5 treated with high temperature, the rate decreased temporarily. Thereafter, the cocooning rate of high temperature exposed treatments increased continuously until F11 with a maximum of 52.67% and it was significantly different ($P<0.05$) from F1 (34.00%). Moreover, in comparison of all generations treated and non-treated with high temperature, F12 of all checked parameters had higher values and was significant difference ($P<0.05$) from both F11 and F1. In control treatments, means of survival rates and cocooning rate among F1-F12 were similar and comparable. Survival rate at larva stage (1st-5th instar) from F1 to F12 was 97.50 to 100.00%, respectively. While for larva (1st-5th instar) – adult stage from F1 onwards to F12 was from 97.50 to 84.67%, respectively. There were significantly statistical differences ($P<0.05$) between F1 and F12 on both survival rates, but no difference on cocooning rate.

3.2 Cocoon yields of eri silkworm

For cocoon yields, mean values of fresh cocoon weight, pupa weight, shell weight, shell ratio, total cocoon shell weight, and fresh cocoon/10,000 larvae were evaluated under alternatively treated with high temperature condition (F6-F12) (Table 2). The fresh cocoon weight of F1-F5, continuously exposed to 42±1°C, 50±5% R.H., trended to decrease temporarily. Fresh cocoon weight of F5 was 2.1370 g, after that the weight decreased slightly. The value of F11 was 2.0861 g, but not significantly different to F1 (2.2853 g). Pupa weight of F1 was 1.9333 g, however, not significant different to F11 (1.8836 g). For shell weight, the value of F1 (0.2628 g) was higher than of F11 (0.1944 g). Interestingly, when the experiment was continued to F12 all of these checked values of F12 were the highest except when compared to F10 and F8. Shell weight of F11 (0.1944 g) was less than that obtained from F1 (0.2628 g) and significantly different ($P<0.05$). In control treatments, from F1 to F12 a slight decrease was observed. The values of all parameters varied depending on different generations. Fresh cocoon weight of F1 and F12 was from 3.0411 to 2.7270 g, pupa weight of F1 and F12 was from 2.6236 to 2.3322 g and shell weight of F1 and F12 from 0.4037 to 0.3829 g. F1 and F12 were statistically different ($P<0.05$) on fresh cocoon weight and pupa weight, however not significant different on shell weight. In consideration on means of fresh cocoon weight, pupa weight and shell weight of F12 in high temperature treatment, the values were similar and comparable to those obtained from F1-F12 in control treatments. The shell ratio from F1 (11.61%) decreased until F11 (9.65%), and it is significantly different with F11. Statistically, there is no change between F2 (9.73%) and F11 (9.65%). In the case of total cocoon shell weight and fresh cocoon/10,000 larvae, F1 was 6.10 g and 10.55 kg, respectively. Between F2 and F11 of both parameters increased from 1.76 to 3.55 g (total cocoon shell weight) and from 3.68 to 7.42 kg (fresh cocoon/10,000 larvae). Besides, these values are above mean values, with F12 gave a value close to F10, which is close to the highest (F10) and significantly different ($P<0.05$). For the control treatments from F1 to F12 there was a slight increase observed in the shell ratio, but there was a larger decrease in fresh cocoon/10,000 larvae (shell ratio F1 to F12 from 13.36 to 14.11% and fresh cocoon/10,000 larvae F1 to F12, from 29.81 to 26.36 kg). Only the values on fresh cocoon weight/10,000 larvae between F1 and F12 were statistically different ($P<0.05$). Shell ratio, total cocoon shell weight and fresh cocoon/10,000 larvae derived from F12 (after high

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/,2/]			25±2 °C and 80±5% R.H. [1/,2/]		
	Survival rate (%)			Survival rate (%)		
	Larva stage (1st - 5th)	Larva (1st - 5th) – adult stage	Cocooning rate (%)	Larva stage (1st - 5th)	Larva (1st - 5th) – adult stage	Cocooning rate (%)
F1	78.33±12.58 c	**54.17±12.58 d**	34.00±7.21 e	97.50±2.50 bc	**97.50±2.50 a**	97.33±3.21 ab
F2	**98.00±2.00 a**	16.67±2.31 g	21.33±4.62 f	96.67±1.15 c	90.00±4.00 a-d	94.00±2.00 b
F3	94.00±5.29 ab	34.67±5.77 f	21.33±2.31 f	99.33±1.15 ab	87.33±5.03 b-d	94.00±2.00 b
F4	97.33±2.31 a	31.33±4.16 f	15.33±3.06 f	**100.00±0.00 a**	85.33±1.15 cd	97.33±2.31 ab
F5	56.00±4.00 d	40.00±2.00 ef	32.67±1.15 e	99.33±1.15 ab	92.00±2.00 a-c	99.33±1.15 a
F6	(98.67±1.15 a)	(90.00±2.00 a)	(98.67±1.15 a)	**100.00±0.00 a**	90.67±3.06 a-c	**100.00±0.00 a**
F7	53.33±5.77 d	36.00±2.00 f	41.33±5.03 de	98.67±1.15 a-c	85.33±5.77 cd	98.00±2.00 a
F8	(99.33±1.15 a)	(79.33±3.06 b)	(98.67±2.31 a)	**100.00±0.00 a**	80.67±8.33 d	98.00±2.00 a
F9	58.67±13.32 d	48.67±6.11 de	46.67±8.33 cd	**100.00±0.00 a**	95.33±3.06 ab	**100.00±0.00 a**
F10	(98.00±2.00 a)	(86.00±5.29 ab)	(94.67±2.31 a)	97.33±2.31 bc	90.00±4.00 a-d	96.00±3.46 ab
F11	85.33±2.31 bc	45.33±3.06 de	**52.67±3.06 c**	99.33±1.15 ab	92.00±9.17 a-c	99.33±1.15 a
F12	(95.33±6.43 ab)	(69.33±1.15 c)	(80.67±11.72 b)	**100.00±0.00 a**	84.67±5.03 cd	96.67±3.06 ab
Mean[3/]	77.63±19.08	38.35±11.63	33.17±13.25	99.01±0.09	89.24±4.79	97.50±2.07
F-test	**	**	**	*	*	*
C.V.(%)	7.56	9.68	10.05	1.25	5.60	2.11

[1/] means followed by the same letter within a column are not significantly different (DMRT, P >0.05). [2/] numbers in parentheses are the values of a generation reared in room temperature 25±2°C humidity 80±5% (F6, F8, F10, and F12). [3/] the values of an average of a generation tested at high temperature (F1-F5, F7, F9 and F11) except in control treatment (25±2°C; 80±5%R.H.).
*, ** = Significantly different at 95 and 99% level, respectively.

temperature exposed treatment, F11) were comparable to the values from F1-F12 in control treatments. These results indicate an improvement in the high temperature tolerance of eri silkworm SaKKU1 in this study.

3.3 Egg yields of eri silkworm

The mean values of egg yields were presented in Table 3. Under the treatment with high temperature eggs/moth, hatching egg percentage, total eggs and total hatching eggs increased from F1 to F12 (149.00 to 311.33, 77.00 to 73.15, 746.11 to 6,693.33 and 444.67 to 4,842.67, respectively) and were significantly different (P<0.05), except hatching eggs. In control treatments, all parameters of F1 and F11 were 346.67 and 357.69, 6,360.03, and 7,524.69, 5,577.93, and 6,631.30 eggs, and 87.78 and 88.37%, for eggs/moth, total eggs, total hatching eggs and hatching eggs, respectively. Of these were not significant differences, although F11 had higher values than F1. In F12 after F11 (high temperature exposure), the average of eggs/moth, total eggs and total hatching eggs increased significantly (P<0.05) from F11. Interestingly, eggs/moth, total eggs and total hatching eggs of F12 in control treatments increased in the same trend as the high temperature treatment regime.

4. Conclusions and Discussion

It is well established in plants and animals that the widespread utilization of hybrids can achieve sustainability, quality and quantity oriented production increasing. Silkworm is the best animal where hybrids are used compulsorily for commercial silk product since the study of Toyama (1906). The goal of breeding is to bring together the desirable genes in appropriate combinations and allow them to recombine so as to improve the genetic performance of maximizing the yield and productivity per unit population (Kumar and Reddy, 1994). The presented study in eri silkworm was carried out to develop suitable high temperature tolerant ecorace for a target environment with high temperature (42±1°C) and low humidity (50±5%) exposed to the larvae from 3rd days of 5th instar until ripe stage. After exposure to high temperature, eri silkworm could grow continuously until a final generation (F12). Survival of larva stage, survival rate larva – adult stage and cocooning rate increased and were significantly different (P<0.05) between F1 and F12, with 78.33 to 95.33%, 54.17 to 69.33% and 34.00 to 80.67%, respectively. These results suggest that the nature of the heat tolerance is heritable and accumulated to the 12th generation. These three parameters of F12 were the highest and most significantly different

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/, 2/]			25±2 °C and 80±5% R.H. [1/, 2/]		
	Fresh cocoon weight (g)	Pupa weight (g)	Shell weight (g)	Fresh cocoon weight (g)	Pupa weight (g)	Shell weight (g)
F1	2.2853±0.31cde	1.9333±0.16 de	**0.2628±0.03 d**	3.0411±0.03 ab	2.6236±0.02 ab	0.4037±0.01 b-d
F2	**2.5008±0.11 bc**	**2.3139±0.10 ab**	0.2404±0.01 de	2.8929±0.06 bc	2.4617±0.06 c	0.4158±0.03 bc
F3	1.9712±0.14 f	1.7999±0.14 def	0.1634±0.01 gh	**3.0901±0.20 a**	**2.6381±0.19 a**	**0.4533±0.04 a**
F4	1.9812±0.02 f	1.8213±0.04 def	0.1477±0.02 h	2.8927±0.08 bc	2.4753±0.06 bc	0.4202±0.03 b
F5	2.1370±0.12 def	1.8860±0.08 def	0.2465±0.04 de	2.7639±0.10 c	2.3939±0.08 c	0.3740±0.03 d-f
F6	(2.3397±0.07 cd)	(2.0062±0.08 cd)	(0.3337±0.04 c)	2.4731±0.11 d	2.1383±0.06 d	0.3360±0.01 g
F7	1.9367±0.13 f	1.7102±0.14 f	0.2207±0.04 ef	2.4751±0.03 d	2.1375±0.25 d	0.3526±0.02 fg
F8	(2.6030±0.02 b)	(2.1964±0.02 bc)	(0.3937±0.01 ab)	2.5482±0.05 d	2.1343±0.04 d	0.3999±0.01 b-d
F9	1.9046±0.11 f	1.7428±0.11 ef	0.1523±0.01 h	2.8526±0.18 c	2.4105±0.13 c	0.3953±0.01 b-e
F10	(2.8753±0.15 a)	(2.4364±0.15 a)	(0.4067±0.02 a)	2.7935±0.09 c	2.3768±0.07 c	0.3950±0.10 b-e
F11	2.0861±0.16 ef	1.8836±0.18 def	0.1944±0.02 fg	2.4634±0.07 d	2.0900±0.06 d	0.3614±0.03 e-g
F12	(2.7144±0.05 ab)	(2.3490±0.05 ab)	(0.3671±0.01 bc)	2.7270±0.09 c	2.3322±0.07 c	0.3829±0.02 c-f
Mean [3/]	2.1004±0.20	1.8864±0.19	0.2035±0.05	2.7511±0.22	2.3510±0.19	0.3908±0.03
F-test	**	**	**	**	**	**
C.V.(%)	6.10	5.71	8.10	3.74	3.81	4.96

Generation	42±1 °C and 50±5% R.H. [1/, 2/]			25±2 °C and 80±5% R.H. [1/, 2/]		
	Shell ratio (%)	Total cocoon shell weight (g)	Fresh cocoon / 10,000 larvae (kg)	Shell ratio (%)	Total cocoon shell weight (g)	Fresh cocoon / 10,000 larvae (kg)
F1	**11.61±1.01 b**	**6.10±0.68 c**	**10.55±0.21 d**	13.36±0.24 d	19.79±0.87 a-c	**29.81±0.84 a**
F2	9.73±0.86 cd	1.76±0.09 f	3.68±0.46 g	14.46±1.02 b-d	19.40±1.18 a-c	27.00±0.36 cd
F3	8.25±1.12 de	1.75±0.26 f	4.18±0.14 g	14.90±0.76 ab	**21.30±0.63 a**	29.25±1.99 ab
F4	7.37±1.22 e	1.13±0.28 f	3.04±0.39 g	14.66±0.59 bc	20.50±1.66 ab	28.14±1.34 a-d
F5	11.44±1.44 bc	3.27±1.64 e	6.65±0.87 f	13.65±0.58 cd	18.58±1.57 b-e	27.46±1.14 b-d
F6	(14.39±0.60 a)	(16.46±0.04 b)	(23.09±1.00 c)	13.68±0.17 cd	16.80±0.71 e	24.73±1.09 f
F7	11.43±1.32 bc	4.51±0.67 d	7.99±0.91 ef	14.29±0.80 b-d	17.28±1.03 de	24.42±0.33 f
F8	(15.14±0.79 a)	(19.42±0.77 a)	(25.68±0.43 b)	**15.83±0.28 a**	19.59±0.64 a-c	24.97±0.35 ef
F9	8.03±0.26 de	3.59±0.14 de	8.87±0.36 e	13.94±0.86 b-d	19.76±0.33 a-c	28.53±1.81 a-c
F10	(14.15±0.45 a)	(19.25±1.04 a)	(27.23±1.88 a)	14.28±0.28 b-d	18.96±0.59 b-d	26.80±0.23 c-e
F11	9.65±1.54 cd	3.55±0.69 de	7.42±0.29 f	14.81±0.77 a-c	17.95±1.39 c-e	24.47±0.72 f
F12	(13.53±0.40 a)	(17.01±1.05 b)	(25.16±1.30 b)	14.11±0.39 b-d	18.01±1.47 c-e	26.36±1.27 d-f
Mean [3/]	9.69±1.69	3.21±1.64	6.55±2.68	14.33±0.67	18.99±1.33	26.83±1.89
F-test	**	**	**	**	**	**
C.V.(%)	8.94	7.15	6.35	4.35	5.74	4.13

[1/] means followed by the same letter within a column are not significantly different (DMRT, P >0.05). [2/] numbers in parentheses are the values of a generation reared in room temperature 25±2°C humidity 80±5% (F6, F8, F10, and F12). [3/] the values of an average of a generation tested at high temperature (F1-F5, F7, F9 and F11) except in control treatment (25±2°C; 80±5%R.H.). ** = Significantly different at 99% level.

among F1, F11, and F12. In domesticated mulberry silkworm (*B. mori*), the breeder of all the sericultural countries have experienced the influence of environment during the process of breeding. The effect of higher temperature of more than 30°C on mulberry silkworm larvae was reported earlier (He and Oshiki, 1984). Attempts to create higher temperature resistant silkworm races were carried out and showed that the genetically heritable nature of thermotolerance is possible to be

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/, 2/]				25±2°C and 80±5% R.H. [1/, 2/]			
	Eggs/moth (eggs)	Hatching eggs (%)	Total eggs (eggs)	Total hatching eggs (eggs)	Eggs/moth (eggs)	Hatching eggs (%)	Total eggs (eggs)	Total hatching egg (egg)
F1	149.00±33.83 d	77.00±12.82 a-d	746.11±474.96 h	444.67±209.71 h	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 d	**86.38±5.22 a**	982.00±244.52 gh	845.52±259.34 gh	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 cd	69.50±6.40 d	1,774.89±274.76 f	1,227.11±237.52 fg	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 bc	69.04±5.63 d	1,656.56±429.47 fg	1,106.78±227.60 f-h	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 bc	75.59±2.38 a-d	3,272.89±576.70 de	2,581.44±629.90 cd	405.13±40.66 ab	88.70±7.17	9,086.73±928.11 ab	8,049.73±694.85 ab
F6	(285.00±14.27 ab)	(82.91±2.12 a-c)	(5,896.40±557.06 b)	(4,844.80±565.85 a)	392.20±23.31 a-c	86.60±1.15	7,816.67±730.95 bc	7,493.20±203.08 a-c
F7	239.80±19.71 bc	81.08±3.35 a-c	2,075.60±101.29 f	1,692.27±198.80 ef	302.20±15.38 e	87.67±4.01	6,947.07±809.88 c-e	6,096.40±749.43 c-e
F8	(308.59±24.82 a)	(84.82±3.21 ab)	(5,127.50±399.62 c)	(4,364.78±517.59 a)	329.48±17.73 de	87.89±1.41	5,485.71±357.82 e	4,795.14±212.30 e
F9	**279.87±16.96 ab**	77.07±6.22 a-d	2,802.27±339.67 e	2,164.10±361.90 de	335.40±18.34 de	90.75±3.45	8,834.60±761.70 ab	8,036.87±430.12 ab
F10	(288.00±29.21 ab)	(74.17±1.78 b-d)	(4,984.27±406.67 c)	(3,702.00±223.77 b)	420.73±51.30 a	87.93±2.15	8,896.80±199.04 ab	7,824.80±155.27 ab
F11	251.75±25.01 abc	83.45±5.49 a-c	**3,601.25±427.82 d**	**3,011.92±397.53 c**	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	(311.33±23.80 a)	(73.15±3.22 cd)	(6,693.33±562.30 a)	(4, 842.67±474.51 a)	**428.47±41.59 a**	86.95±1.06	**10,207.13±963.63 a**	**8,874.73±845.48 a**
Mean [3/]	219.08±45.70	77.39±6.17	2,113.92±1,035.54	1,635.35±890.13	368.99±41.98	87.55±2.84	7,903.04±800.29	6,903.83±735.38
F-test	**	**	**	**	**	ns	**	**
C.V.(%)	13.38	7.21	12.79	15.11	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). [2/] numbers in parentheses are the values of a generation reared in room temperature 25±2°C humidity 80±5% (F6, F8, F10, and F12). [3/] the values of an average of a generation tested at high temperature (F1-F5, F7, F9 and F11) except in control treatment (25±2°C; 80±5%R.H.). ns = non significantly different at 95% level. ** = Significantly different at 99% level.

accumulated by selection using the pupation rate of the silkworm as an index of the thermotolerance on the larvae reared under higher temperature conditions during the 5th instar (Shirota, 1992; Tazima and Ohnuma, 1995). In addition, an increase in the temperature beyond 35°C causes less spinning, mortality of larvae and pupae, poor moth emergence and sterility at adult stage of eri silkworm (Sahu *et al.*, 2006). In this present study, cocoon yields of eri silkworm were obtained regularly from early generation until 12th generation. This indicates that the performance of eri silkworm ecorace with high temperature tolerance improved by directional selection at 42±1°C and 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 (Sirimungkararat *et al.*, 2013b). 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 effects on biochemical processes, including the physiology of the worm (Willmer *et al.*, 2004). Rahmathulla (2012) indicated in *B. mori* that relative humidity affects both directly and indirectly the physiological functions of the silkworm and rapidly dry mulberry leaves. Besides, the study of Hussian *et al.* (2011) demonstrated in *B. mori* that rearing silkworm at temperatures, 25±1, 30±1, and 35±1°C and low humidity of 55% R.H. resulted in lowest cocoon yield in almost all tested varieties. Suresh Kumar *et al.* (2001) 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 decrease of fresh cocoon weight and cocoon shell weight of high temperature improvement was observed in our study between F1 and F11 of eri silkworm compared to control group. Survival rates of eri silkworm obtained from high temperature treatment increased by directional selection towards F12 in our study. Besides, when all parameters were considered, it was clear that survivals, cocoon yields, and total hatching eggs of F12 were higher than F1 and significantly different ($P<0.05$). This suggests the adaptation of eri silkworm SaKKU1 against higher temperature suppression. There is also evidence that F11, the last generation of high temperature exposure, showed survival rates, which were comparable and not statistically different to the beginning generation (F1). In Thailand, our studies exhibited the thermotolerant property of eri silkworm. Ecorace SaKKU1 can tolerate up to 42±1°C with low humidity (50±5% R.H.) compared to other ecoraces (Sirimungkararat *et al.*, 2013a; Wongsorn *et al.*, 2014). The present study indicates that thermotolerant property of eri silkworm ecorace SaKKU1 is heritable to the progeny. This study is a pioneer work of the improvement of eri silkworm tolerance to high temperature. The derived thermotolerant eri silkworm is used for rearing tests by farmers, which then will be assessed so that new culture methods can be established to cope with recent global warming trend.

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