A study on Performance of Iranian Commercial Silkworm Lines under Heat and Cold Stress

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Summary

One of the main objectives of the new breeding programs for silkworms is to consider the ability to withstand environmental fluctuations. In this study, the performance of the Iranian commercial silkworm lines was investigated under heat and cold stress. Seven Iranian commercial silkworm lines including 31, 32, 103, 104, 151, 153, and 154 were reared under standard conditions at Iran Silk Research Center. To induce heat and cold stress, 300 larvae on the third day of the fifth instar including three replicates of 100 larvae, were placed in an incubator at 35 °C and for 2 and 12 hours, respectively, and then returned to the standard rearing conditions at 25 °C until the end of the breeding period. The characteristics of cocoon weight (cw), cocoon shell weight (csw), and cocoon shell percentage (csp) were investigated under control and stress treatments. The generalized linear model (GLM) procedure of SAS software was used for statistical analysis. The results of the variance analysis showed that the effect of all sources of variance including genotype, stress, sex, two-way interaction effects of genotype × stress, genotype × sex, and stress × sex and three-way interaction effect of genotype × stress × sex were significant for all investigated traits. Therefore, the averages related to the three-way interaction effect of genotype × stress × sex were considered and discussed. The results of the comparison of the means showed that in general, the larvae of the control group, especially lines 154 and 153, had better performance in all the examined traits compared to the larvae under heat and cold stress groups and a significant difference was observed between them ($P < 0.001$). In the same way, the larvae of the heat stress group had a higher performance than the larvae of the cold stress group, which indicates the thermal tolerance of silkworm to higher temperatures than to low temperatures. In conclusion, the performance of Iranian commercial silkworm lines is affected by heat and cold stress conditions, and lines 154 and 153 had higher tolerance than other investigated lines and can be used and prioritized in the production of commercial hybrids in terms of resistance to temperature stress, production and economic statistics.

Key words

silkworm, thermotolerance, cocoon traits, environmental fluctuation

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Received: October 28, 2023 | Accepted: April 2, 2024 | Online first version published: May 1, 2024

Introduction

The potential responses of insects to temperature fluctuations are of increasing interest to a broad range of research studies, which is partly a consequence of the need to accurately predict the effects of climate change on their abundance and distribution, biodiversity and ecosystem function (Simpson and Douglas, 2013).

The domestic mulberry silkworm (*Bombyx mori* L.), is a sensitive organism, and domestication processes over centuries have excluded this commercial insect from the chance to acquire thermotolerance (Kumari et al., 2011). Among the different factors responsible for the insignificant performance of the silkworm strains following tropical situations (especially in bivoltine strains), the primary one is the lack of thermotolerance, as many quantitative characteristics decrease sharply in suboptimal conditions (Kumari et al., 2011).

Temperature is one of the main crucial environmental factors which directly influences the life cycle of the silkworm. It is reported that silkworm larvae spin the best cocoon at 22 °C (Ramachandra et al., 2001) and also shows that high-quality cocoons are produced in the temperature range of 22 – 27 °C, and values outside this condition could affect the quality of the cocoon (Nabizadeh and Jagdeesh Kumar, 2011). Recent studies have shown the genetic basis of thermal tolerance in silkworms (Pillai and Krishnaswami, 1980 and 1987; Kato et al., 1989). But there is limited information regarding the application of these aspects of parental stock selection for breeding programs. Some early studies selected genotypes of silkworms in terms of heat tolerance by identifying heat-resistant genotypes (Shirota, 1992; Kumar et al., 2001). However, establishing a clear and accurate understanding of the genetic basis and variation in the expression of quantitative and qualitative traits with exposure to high or low temperatures is an important step for selecting heat or cold-tolerant parental sources for breeding programs (Kumari et al., 2011).

To date, no special attention has been paid to the field of studying and improving adaptation traits in Iranian silkworms. The current research aimed to investigate different strains of Iranian silkworms' response to heat and cold treatments. The outcomes from this research will ensure sustainable silkwork production facing a changing climate.

Materials and Methods

Seven Iranian commercial silkworm lines including 31, 32, 103, 104, 151, 153, and 154 were reared under standard conditions at Iran Silk Research Center (Guilan province, Rasht, Iran). To induce heat and cold stress, 300 larvae on the third day of the fifth instar (three replicates of 100 larvae) were placed in an incubator at 35 °C and 0 °C for 2 and 12 hours, respectively, and then returned to the standard rearing conditions at 25 °C until the end of the breeding period (Chavadi et al., 2006; Ashraf and Qamar, 2023; Kang et al., 2016). These extreme temperatures cause adverse effects on life cycle, general biology, average growth and development and also affect the cocoon characters such as cocoon weight (Ashraf and Qamar, 2023). Also, the mechanism of protecting the cell from thermostress involving the expression of a polypeptide family known as heat shock proteins and anti-frozen proteins, is induced by these extreme temperatures (Chavadi et al., 2006; Kang et al., 2016). The control group included larvae that were not exposed to stress and were raised at a standard temperature.

To investigate production traits in each of the lines, the three most important economic traits including cocoon weight (CW), cocoon shell weight (CSW), and cocoon shell percentage (CSP) (Mirhoseini et al., 2005), were recorded under control and stress (heat and cold) groups for subsequent statistical analysis.

For statistical analysis, the generalized linear models (GLM) procedure was used in SAS software version 9.4 (SAS Institute Inc, 2013). For comparing the means, Tukey's HSD statistical test was used at a significance level of $P < 0.05$. The statistical model was as follows:

$$Y_{ijkl} = \mu + A_i + B_j + C_k + AB_{ij} + AC_{ik} + BC_{jk} + ABC_{ijk} + e_{ijkl}$$

where Y_{ijkl} is the phenotypic value of the production traits; μ is overall mean; A_i is the effects of i_{th} genotype/line; B_j is the effects of j_{th} stress group; C_k is the effects of k_{th} sex; AB_{ij} is the two-way interaction effect of the i_{th} genotype and j_{th} stress; AC_{ik} is the two-way interaction effect of the ith genotype and k_{th} sex; BC_{ik} is the two-way interaction effect of the j_{th} stress group and k_{th} sex; ABC_{ijk} is the three-way interaction effect of the i_{th} genotype, j_{th} stress group, and k_{th} sex; and e_{ijk} is the residual effects.

Results and Discussion

The results of the variance analysis of the studied traits are presented in Table 1. The results showed that the effects of all sources of variance, including genotype, stress, sex, and the interaction effects of genotype × stress, genotype × sex, stress × sex, and genotype × stress × sex were significant for all investigated traits. The values of the coefficient of determination (R^2) also indicate the appropriateness of the statistical model for the analysis of the experimental data.

Table 1. Analysis of variance of the studied traits

Source of Variance	Traits (F value)		
	Cocoon Weight	Cocoon Shell Weight	Cocoon Shell Percentage
Model	6423.52***	835.08***	313.31***
Genotype	32505.6***	2289.93***	127.16***
Stress	14422.5***	2289.93***	4817.28***
Sex	26998.7***	62.72***	994.07***
Genotype×Stress	490.81***	103.53***	35.07***
Genotype×Sex	365.85***	71.75***	96.47***
Stress×Sex	25.34***	56.78***	119.19***
Genotype×Stress×Sex	362.63***	20.40***	18.01***
R^2	0.99	0.99	0.99
CV%	0.272	1.161	1.132

Note: *** Significant at $P < 0.001$

The results of comparing the average effect of genotype (line), the effect of cold stress, and the effect of sex for the studied traits are presented in Tables 2 to 4, respectively. According to the results of the analysis of variance (Table 1) and the significance of the three-way interaction effect of genotype × stress × sex, it is not possible to compare the levels of the main effect of genotype and the main effect of stress or sex separately. Therefore, the independent comparisons between genotypes (Table 2) and between the levels of stress and sex groups (Tables 3 and 4) were omitted and only the averages related to the three-way interaction effect of genotype × stress × sex were reported on and discussed further (Table 5). The sorted averages of the genotypes for each trait are also given in Figs. 1 to 3.

Table 2. The main effect of the genotype (line) on the studied traits

Genotype	Traits		
	Cocoon Weight (g)	Cocoon Shell Weight (g)	Cocoon Shell Percentage (%)
31	1.345	0.266	19.757
32	1.283	0.257	20.076
103	1.217	0.229	19.141
104	1.203	0.253	20.702
151	1.429	0.277	19.360
153	1.501	0.309	20.480
154	1.638	0.336	20.488
SEM	0.00088	0.00075	0.0533

Table 3. The main effect of the stress on the studied traits

Stress	Traits		
	Cocoon Weight (g)	Cocoon Shell Weight (g)	Cocoon Shell Percentage (%)
Control	1.441	0.322	22.365
Heat	1.377	0.277	20.118
Cold	1.303	0.227	17.519
SEM	0.00057	0.00049	0.0349

Table 4. The main effect of the sex on the studied traits

Sex	Traits		
	Cocoon Weight (g)	Cocoon Shell Weight (g)	Cocoon Shell Percentage (%)
Female	1.428	0.278	20.636
Male	1.319	0.273	19.364
SEM	0.00047	0.00040	0.0285

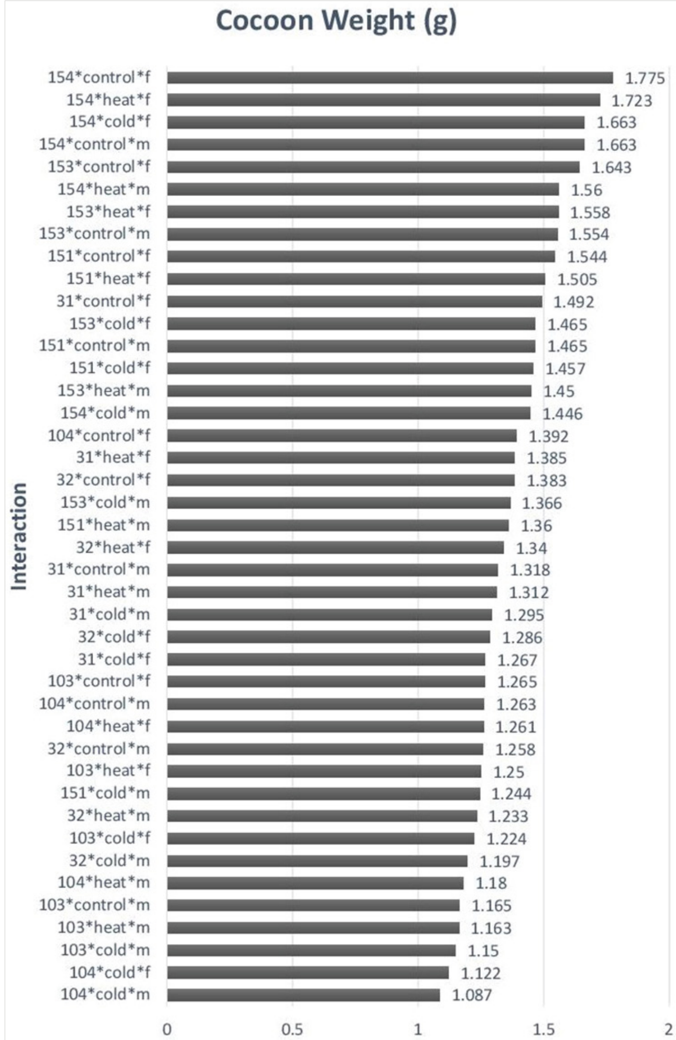

Figure 1. Sorted averages of the lines for the cocoon weight (g) under control and stress groups and sex status

In general, the highest and lowest values of traits among studied lines and under applied stress conditions were not the same, but some lines had higher performance for all three traits. Moreover, traits related to cw, csw, and csp are the most important traits in breeding purposes that have high economic value and are used to improve cocoon performance. The overall average obtained from cw in all investigated lines was 1.374 g (Table 5). Line 154 from the control group of the female sex (154*control*f) showed the highest performance with 1.775 g and line 104 from the cold stress group of the male sex (104*cold*m) had the lowest average with 1.087 g (Table 5 and Fig. 1).

For csw, the overall average was 0.275 g and line 154 from the control group of both male and female sexes (154*control*m and 154*control*f) showed the highest values with 0.398 and 0.389 g, respectively, and line 103 from the cold stress group of the female sex (103*cold*f) had the lowest average with 0.194 g (Table 5 and Fig. 2). For csp, overall average of all investigated lines was 19.87 %, and in general, the control group showed higher performance than the heat stress and cold stress groups (Fig. 3). Line 154 from the control group of the male sex (154*control*m) showed the highest performance with 23.93 % and line 151 from the cold stress group of the female sex (151*cold*f) had the lowest average with 14.90 % (Table 5 and Fig. 3).

Table 5. The least-squares means of genotype × stress × sex interaction effect for the studied traits

Treat (Genotype × Stress × Sex)	Traits		
	Cocoon Weight (g)	Cocoon Shell Weight (g)	Cocoon Shell Percentage (%)
31*control*f	1.492^{g}	0.322^{fg}	21.320^{e}
31*control*m	1.318^{m}	0.308^{h}	23.386^{abc}
31*heat*f	1.385^{j}	0.267^{lm}	19.316^{jkl}
31*heat*m	1.312^{m}	0.260^{mno}	19.833^{ijk}
31*cold*f	1.267^{o}	0.232^{q}	18.333^{nop}
31*cold*m	1.295^{n}	0.208^{st}	16.056^{s}
32*control*f	1.383^{j}	0.299^{hij}	21.640^{e}
32*control*m	1.258^{op}	0.291^{j}	23.123^{bc}
32*heat*f	1.340^{l}	0.265^{lmn}	19.766^{ijk}
32*heat*m	1.233^{rs}	0.256^{no}	20.183^{fg}
32*cold*f	1.286^{n}	0.218^{rs}	16.976^{r}
32*cold*m	1.197^{t}	0.217^{rs}	18.166^{op}
103*control*f	1.265^{o}	0.242^{pq}	19.173^{klm}
103*control*m	1.165^{v}	0.264^{lmn}	22.653^{cd}
103*heat*f	1.250^{pq}	0.221^{r}	17.646^{pqr}
103*heat*m	1.163^{v}	0.242^{pq}	20.773^{fgh}
103*cold*f	1.224^{s}	0.194^{u}	15.903^{s}
103*cold*m	1.150^{w}	0.215^{rst}	18.700^{lmno}
104*control*f	1.392^{j}	0.302^{hi}	21.706^{e}
104*control*m	1.263^{op}	0.292^{ij}	23.146^{bc}
104*heat*f	1.261^{op}	0.258^{mno}	20.430^{ghi}
104*heat*m	1.180^{u}	0.251^{op}	21.313^{ef}
104*cold*f	1.122^{x}	0.209^{st}	18.613^{lmno}
104*cold*m	1.087^{y}	0.206^{t}	19.003^{lmn}
151*control*f	1.544^{f}	0.334^{e}	21.646^{e}
151*control*m	1.465^{h}	0.346^{d}	23.606^{ab}
151*heat*f	1.505^{g}	0.278^{k}	18.513^{mno}
151*heat*m	1.360^{k}	0.280^{k}	20.596^{fgh}
151*cold*f	1.457^{hi}	0.217^{rs}	14.900^{t}
151*cold*m	1.244^{qr}	0.210^{st}	16.896^{r}
153*control*f	1.643^{d}	0.358^{c}	21.803^{e}
153*control*m	1.554^{ef}	0.369^{b}	23.740^{ab}
153*heat*f	1.558^{e}	0.319^{g}	20.496^{ghi}
153*heat*m	1.450^{i}	0.302^{hi}	20.863^{fg}
153*cold*f	1.465^{h}	0.272^{kl}	18.596^{lmno}
153*cold*m	1.366^{l}	0.232^{q}	17.380^{qr}
154*control*f	1.775^{a}	0.389^{a}	21.936^{de}
154*control*m	1.663^{c}	0.398^{a}	23.930^{a}
154*heat*f	1.723^{b}	0.345^{d}	20.030^{hij}
154*heat*m	1.560^{e}	0.332^{ef}	21.293^{ef}
154*cold*f	1.663^{c}	0.293^{ij}	17.613^{pqr}
154*cold*m	1.446^{i}	0.262^{lmn}	18.130^{opq}
SEM	0.0021	0.0018	0.1307
P-value	<0.0001	<0.0001	<0.0001

Note: m: Male, f: Female; $^{a\text{-}q}$ Different superscript letters in the same column indicate significant differences according to Tukey's HSD test at $P < 0.05$ level

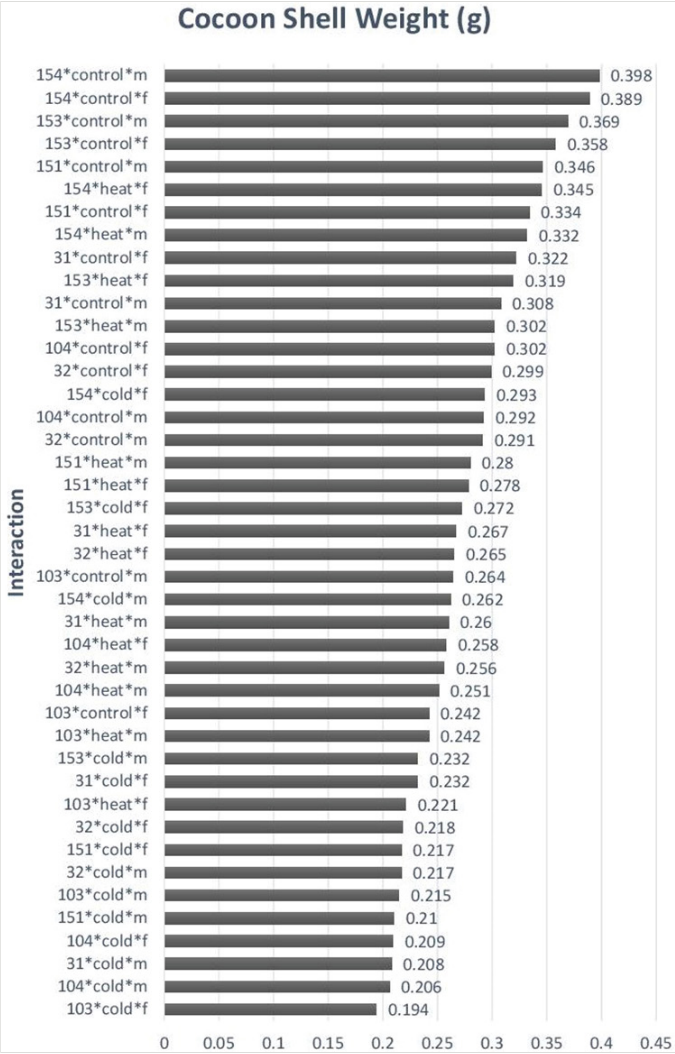

Figure 2. Sorted averages of the lines for the cocoon shell weight (g) under control and stress groups and sex status

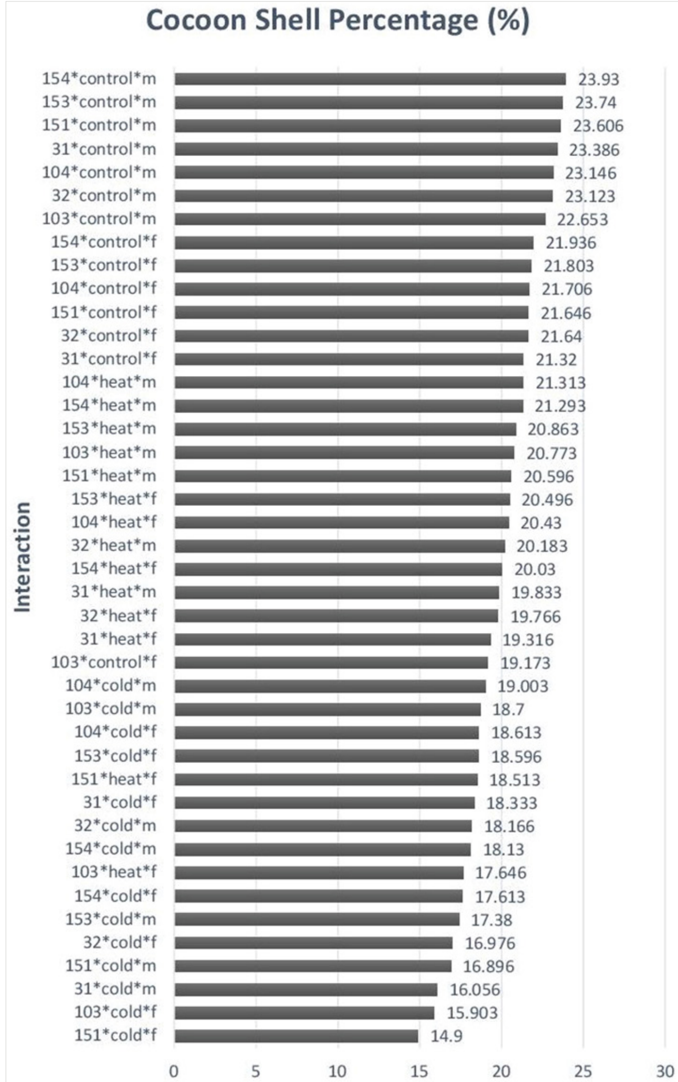

Figure 3. Sorted averages of the lines for the cocoon shell percentage (%) under control and stress groups and sex status

Important economic traits of silkworms are affected by environmental factors such as temperature, relative humidity, light and nutrition. The silkworm is one of the cold-blooded animals and temperature has a direct effect on physiological activities and as a result has a direct relationship with the growth of silkworms. An increase in temperature increases physiological activities and a decrease in temperature decreases physiological activities. High-temperature fluctuations reduce the growth and development of silkworms (Rahmathulla, 2012).

Heat tolerance is one of the important traits in silkworm breeding to select the best hybrids for different rearing regions, especially in incompatible environmental conditions. Thermal tolerance is especially emphasized in silkworm breeding programs to select silkworm species with better adaptation to diverse environmental conditions, especially in tropical regions such as southern India, or summer and autumn in China. To determine the level of thermal tolerance, thermal shock treatment is used based on the percentage of losses, and this can show thermal tolerance in different strains/breeds of silkworms and other insects (Loeschcke and Sorensen, 2005).

One of the breeding goals considered in modern programs for industrial insects, including silkworms, is the ability to withstand environmental fluctuations. Based on this, it is even possible to produce new varieties that are specific to each geographical and climatic region (Hosseini Moghaddam et al., 2008). In this regard, many studies have been conducted to investigate the resistance and performance of different silkworm strains to environmental stresses. In a previous study by Kumari et al., (2011), to identify potential bivoltine silkworm strains specific for thermal tolerance to high temperature, the fifth instar larvae of bivoltine strains was subjected to a high temperature of 36 ± 1 °C with a relative humidity of 50 ± 5 % for six hours during a day until spinning for three consecutive generations. Highly significant differences were found for all studied traits in the investigated groups. Studied silkworm strains were categorized into three groups including susceptible, moderately tolerant, and tolerant based on pupation rate or survival rate under thermal stress.

Furthermore, based on the performance of nine studied traits such as larval weight, cocoon yield by number and weight, pupation, cocoon weight and shell weight, cocoon shell percentage, filament length and denier, three strains of BD2-S, SOF-BR, and BO(2) were selected based on the potential for thermotolerance (Kumari et al., 2011).

In other studies carried out in this field (Wanule and Balkhane, 2013), the effect of temperature changes on the reproductive behavior, egg laying and longevity of silkworm moths (crossbreed of PM × CSR_2) was investigated and the results showed that at a temperature of 40 ± 5 °C, high losses in larvae were observed and their butterflies were not able to lay eggs. At a temperature of 30 ± 1 °C, the lowest number of eggs was observed and they would die within 72 hours. Also, at a low temperature of 10 ± 1 °C, the diapause mechanism was activated and a delay in egg laying was observed (Wanule and Balkhane, 2013).

In another study by Chandrakanth et al., (2015) on 20 Indian silkworm breeds reared at different temperatures (25 ± 1 °C, 32 ± 1 °C, and 36 ± 1 °C) for six hours during a day from the third day of the fifth instar until spinning, significant statistical differences were found for six studied traits including pupation percentage, larval weight, cocoon yield per 10,000 larvae by weight, cocoon weight, cocoon shell weight, and cocoon shell percentage. In terms of pupation rate, SK4C and BHR3 were introduced as thermotolerant strains and 20 studied breeds were clustered in four groups including susceptible, tolerant, and two moderately tolerant (Chandrakanth et al., 2015).

In a recent study, genetic differences between two parental lines of Iranian silkworm (dumbbell and oval-shape cocoons) in response to high (37 ± 1 °C) and normal (24 ± 1 °C) temperatures were investigated and performance for the best cocoon weight, cocoon shell percentage, larval viability, and pupal viability traits were evaluated. The heat treatment applied in this study (37 °C) was able to determine genetic differences in heat tolerance between studied silkworm parental lines (Asadpour Ardehjani et al., 2023).

In the investigated conditions at low temperatures as well, the effects of keeping silkworm larvae (polyhybrid strain [107.109(108.110)]) in the temperature of 5 °C on some economic traits, in the form of seven treatments including no cold stress (control) and cold stress equivalent to 48, 96, 144, 192, 264 and 480 hours were also studied. Afterwards, the larvae of each treatment were reared under standard conditions and according to common instructions after applying cold stress, their economic traits were recorded. The results of abovementioned study showed that cold stress had a significant effect on most economic traits (larval weight, length of larval periods, molting, feeding and the total number of cocoons, the number of best and average cocoons and cocoon weight, weight of male and female cocoons, cocoon shell weight, cocoon shell weight of female, cocoon shell weight of male, female pupa, and male pupa). A significant decrease was observed compared to the control treatment. Also, the results obtained showed that there was a significant increase compared to the control treatment in the characteristics of larval mortality percentage, double cocoon weight, number of cocoons per liter, cocoon shell percentage of females, and cocoon shell percentage (Bizhannia et al., 2005).

Conclusion

Performances of the seven Iranian commercial silkworm lines including 31, 32, 103, 104, 151, 153, and 154 for cw, csw, and csp, as the most important economic traits in silkworm breeding, were affected by heat and cold stress conditions. Lines 154 and 153 showed higher efficiency than other studied lines and they can be used with higher priority in the production of commercial hybrids in terms of resistance to temperature stress, production, and economic statistics.

Acknowledgements

The authors would like to acknowledge the financial support of University of Guilan and Iran Silk Research Center for this research under grant number 15P/124646-1400/10/06.

CRediT Authorship Contribution Explanation

Ramin Abdoli: Writing – original draft, Supervision, Methodology, Formal analysis, Conceptualization, Performed most of the experiments, Writing – review & editing. **Farjad Rafeie:** Writing – original draft, Supervision, Methodology, Formal analysis, Conceptualization, Performed most of the experiments. **Reza Sourati Zanjani:** Methodology, Resources. **Navid Ghavi Hossein-Zadeh:** Data Curation, Validation, Writing – review & editing. **Jalal Jalali Sendi:** Methodology, Formal analysis. **Faezeh Ramezani Pastaky:** Resources, Performed some of the experiments.

Declaration of Competing Interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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aCS89_17