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DETERMINATION OF SILKWORM (*BOMBYX MORI* L.) LARVAE VIABILITY AT THE EGG STAGE

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SUMMARY

Identifying potent and stable silkworm (*Bombyx mori* L.) genotypes among the large population at early stages of development is one of the primary problems in silkworm breeding. For families with resilience to develop under stressful conditions in the embryonic period, the presented experiments transpired to identify the outstanding families during incubation at the highest temperature. During the wintering period of eggs, a sample of 100 eggs came from each breeding family. These 100 eggs sustained incubation at the temperature of +34 °C. In almost every line, the traits recorded for provocative and average incubation, and the viability of the larvae fully corresponded to each other. Breeding families with different characteristics underwent three grades' division regarding egg livability at critical temperatures. The gradations contained families with high, medium, and low livability. Families performed well during provocative. Meanwhile, identification and selection also ensued under standard incubation conditions. Based on preliminary results, the families exhibited the varied response rate to stressful conditions during the embryo period. However, the families with resistance to high temperatures showed the maximum viability potential during the larvae feeding. In particular, L-31 (59.6%) and L-300 (51.4%) lines gave the highest viability rates, and using these lines have served to conduct additional studies. A plan of experiments is in progress for the future to improve these lines to the breeding level. Developing a dietary plan to feed individuals living in warm climates is ongoing, with much care given in a challenging environment.

Keywords: Silkworm (*B. mori* L.), eggs, incubation, larvae, viability, cocoon, shell ratio, heat shock

Key findings: The research involving incubation at a high temperature, newly developed lines of the mulberry silkworm (*B. mori* L.) and the selection of families with high vitality have practical implications. The latest results will enhance the theoretical understanding of sericulture and inspire practical applications, improving the quality and productivity of silk production.

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INTRODUCTION

The world's demand for highly productive cocoons, producing a silk filament with a high metric number (fine silk) is rising. The largest silk-producing countries, such as, China, India, Italy, France, and Japan, have demands mainly directed at the silk of class A, 4A, and 5A. Silk fabrics, surgical (suture) silk filaments, and the natural silk goods are beneficial in the electronics industry, aviation, morphology, and some sectors of the national economy, coming from silk filaments. Relevant scientific and practical problems of world sericulture center on producing silkworm (*Bombyx mori* L.) breeds and hybrids with an increased raw silk yield, superior length and thinness of the silk filament, with better strength. Likewise, adaptation to various climatic conditions and establishing highly effective breeding methods are major considerations.

By screening the silkworm to identify and increase its thermotolerance, developing new breeds and hybrids have emerged (Kumari *et al.,* 2011). The genetic variability has also been distinctive within the silkworm populations, which can play a key role in its further development through breeding (Ruiz and Almanza, 2018). According to the past research, ideal parents require selection and those can effectively obtain the genotypes with the highest heterosis.

Past research identified the response in silkworm to temperature fluctuations at the egg stage, belonging to the Indian breed group, which was useful in breeding for further improvement and obtaining resistance to the high temperature (Jingade *et al.,* 2015). According to past data, the breeds and industrial hybrids developed based on bivoltine forms and appeared more resistant to sudden variations in air temperature and relative humidity (Begum *et al.,* 2001, 2002). Knowingly also, variations in the protein structure dramatically take place with the influence of abiotic stress due to temperature (Mukhopadhyay *et al.,* 2013; Shinde *et al*., 2018), and identify genes against thermal stress factor in the silkworm (Chandrakanth *et al.,* 2015). One can assume adaptation to lethal temperature levels has also occurred in silkworms groups for several generations.

In Chinese sericulture experiments, the selection of the most stable silkworm genotypes proceeded in the breeding material by exposing it to high temperature and infecting with various viruses (Kantaratanakul and Tharvarnanukulkit*,* 1987; Guo *et al.,* 2015). However, the highly heterozygous genetic nature of the hosts makes it difficult to understand the inheritance and expression of these quantitative traits (Duo *et al.,* 2023). Although, as a result of extensive genetic research, the F_1 hybrid (Line 4 x CSR2) resulted (Rao and Umadevi*,* 2012).

The selection of the most stable and robust silkworm genotypes happened even in these latest experiments. These included feeding the larvae strictly with an artificial reduction in feed quantity, maintaining high and low temperatures, as well as, reducing the larvae feeding area in the populations of several breeds (Umarov *et al.,* 2018). Temperature and humidity considerably affect the silkworm's silk production (Ashraf and Qamar, 2023).

By studying the viability of fast-growing silkworm larvae over several generations, it was evident a positive association existed between the accelerated rate of development and productivity, as well as, their viability (Nasirillaev *et al.,* 2023; Khudjamatov *et al.,* 2023). However, in almost all the methods, the breeding material received complete revival and care, bringing it to the butterfly stage with significant time and labor (Abdukadirov *et al.,* 2024). Particularly under the conditions of Uzbekistan, the selection of heat-resistant silkworm genotypes of monovoltine breeds during the oviposition period and the development of highly productive and viable breeds were practically crucial (Nasirillaev *et al.,* 2024). Based on the said discussion, the presented research aimed to identify the stable and resistant silkworm genotypes under a critical high temperature at the egg stage among breeding lines.

MATERIALS AND METHODS

The Scientific Research Institute of Sericulture seats in the east of the District Shaikhontokhur, Tashkent City, Uzbekistan (41.33 South latitudes, 69.19429774867733 East longitudes). The said Institute has more than 10 hectares of mulberry plantations and specially equipped larvae-hole laboratories. The mulberry plantations stand on the fertile loamy soil, with the mulberry trees watered from a stream naturally. The experimental cells for keeping silkworm larvae were 12 m × 8 m × 4 m in size. The provision of lighting mode has a natural and electric lighting for 12 hours a day. Breeding experiments conducted in special larvae-holes of the laboratory of "Breeding of the Silkworm" at the Scientific Research Institute of Sericulture apply the guidance document "Basic methodological rules of Breeding of the Silkworm." The feeding of populations of the experimental breeds comprised mulberry leaves of the same quality grown in mulberry plantations of the unitary enterprise "Mulberry Experimental Farm." The experiments proceeded on the silkworm breeding lines, i.e., Line 300, Line 301, Line 30, Line 31, Line 27, and Line 28, which differed in the cocoon's weight and the technological properties of silk filaments.

The selection of breeding clutches of eggs relied on the analysis of reproductive characteristics of the silkworm lines, viz., Line 300, Line 301, Line 27, Line 28, Line 30, and Line 31. A 100-egg sample came from each tribal family. These 100-egg samples bore incubation in parchment bags at the critical high temperature. The number of the families and the breeding lines received markings on the bags. The prepared samples' placement in a TCO-1/80 SPU thermostat underwent reviving at a high temperature of +34 °C (February 10–15, 2024) (Figure 1). The monitoring of thermostat's air temperature and relative humidity occurred four times a day during the eggs' incubation period. When incubated at the high temperature, the eggs' transfer to room temperature every day remained for two hours. This incubation mode continued until the hatching of eggs, and afterward, the calculation of percentage of hatched eggs in each package ensued, with the rate of egg livability determined.

The spring incubation period of the experimental variants provided a uniform temperature (+24 °C–+25 °C) and humidity (75%–80%) treatment. Feeding the silkworm breeding material selected for the experiments commenced in spring, with a number of 250 in each breeding family (Yalgashev *et al.,* 2023). Likewise, released larvae's feeding ensued in optimal hydrothermal conditions (Figure 1). In the experimental lines of silkworm, the data recorded included the biological traits, i.e., viability of larvae (%), the morbidity (%), cocoon weight (g), cocoon shell weight (mg), and shell ratio (%).

Placing of artificial cocoons at the end of the fifth age of the larvae followed, and then, removing them on the seventh day from the day of mass curling. The resulting cocoons reached peeling, and after two days, the cocoons underwent grouping into healthy, twins, and capercaillie. The percentage of viability and morbidity's determination had the ratio of the total number of healthy cocoons against the total number of larvae. Determining the weight of the cocoon and shell and the shell ratio utilized gross weighing 15♀ and 15♂ on an electric scale (Figure 1).

RESULTS

The egg productivity of the mulberry silkworm (*B. mori* L.) is the trait primarily determined and important for the seeding. The silkworm genotypes and the larvae feeding conditions, as well as, the quantity and quality of feed largely influenced fertility traits. Before starting any selection work, validating the traits occurred. These included the number of eggs laid by female butterflies of the selected breed, its weight, the weight of one egg, as well as, the number of unpaired, and withered eggs (physiologically unfit) in the egg casting. Basing on the results, had the selected breed divided into lines with information about the egg productivity. In particular, the Line 300 and Line 301 were in the ranks of the large cocoon, the Line 30 and Line 31 belonged in the medium cocoon, and the Line 27 and Line

Figure 1. The reviving of the silkworm in the thermostat to measure the cocoon weight in silkworm.

28 fell under the small-cocoon breeds obtained in the presented experiments. The gathered egg castings each year received visual sorting first, counting each laid egg individually and identifying physiologically unfit traits, egg laying, and one-egg weight (Table 1).

In the spring of 2022, family-to-family care operations took place, analyzing the eggs laid by their butterflies. The total number of families analyzed was 689, with the highest values found for the number of healthy eggs laid by butterflies from Line 30 and Line 31 (711–764 units), while the proportion of physiological unfit eggs was 0.7%–0.8% in Line 301, Line 300, and Line 31. A total of 294 families were options for breeding work, and 722–755.2 units comprised Line 30 and Line 31, and 664–701 units presented Line 300 and Line 301 of eggs in laying selected families. The egg weight was 402–418 mg in Line 300, Line 301, and Line 31.

Table 1. The number of eggs in casting of selected silkworm lines prepared for the spring of 2022.

Traits	Line 300	Line 301	Line 27	Line 28	Line 30	Line 31
Number of healthy eggs	659.4±7.5	701.0±7.46	624.0±6.45	618.0±12.5	711.0±18.2	764.0±16.1
Percentage of physiological broken eggs (%)	0.8±0.1	0.7±0.1	1.3±0.08	1.25±0.16	1.5±0.3	0.7±0.12
Healthy egg weight (mg)	403.5±6.23	418.0±4.57	349.0±3.8	316.0±6.25	382±11.0	402.0±7.43
One egg weight (mg)	0.612±0.003	0.597±0.003	0.558±0.002	0.512±0.003	0.546±0.01	0.530±0.02

Silkworm selection based on the larvae viability is difficult to find the stable, healthy, and well-responsive families for viability in the next generation, which may not show higher values, and both healthy and weak individuals may occur in one family. In addition, suitable and viable families were choices after feeding larvae, cocoon feeding, and transformation into pupae. Many larvae need feeding to analyze the maximum number of families to select a family with high viability, and then, breeding work can produce some results. Families showing almost the same results at the same time cause a complex situation with the selection of the most tolerant individuals. Considering this, Strunnikov (1959) suggested it can be beneficial to determine whether the families are stable or weak at the embryonic stage.

Concerning modern synthetic products obtained from various silkworms breeds, it is also possible to determine a wide variability and tolerance to the extreme conditions during embryo diapause. In the embryonic period, the high viability can result from affecting the eggs with the highest critical temperature. For the presented experiments, researchers selected the breeding lines with different genotypes isolated from a population of diverse breeds of silkworm. Moreover, determining the revival degree continued by incubating egg samples from the same lines at the high temperature. Distinguishing the degree of viability of families at 34 °C, the eggs' subsequent incubation in the spring ensued at optimal temperature and humidity conditions. The released larvae fed from the same families help identify the viability in the postembryonic stage, the percentage of morbidity, and the productivity of cocoons (Table 2).

Comparing the five silkworm families showed the best recovery in each line with the percent recovery under normal conditions and the viability of the larvae (Table 2). According to results, the provocative and average incubation data and the larvae viability fully correspond in almost every line. In Line 300, with the high temperature, the percentage of livability for four families was 65.0%, 67.0%, 64.0%, and 71.0%, accordingly, compared with 99.8%, 99.6%, 100.0%, and 99.45%, respectively under normal conditions. In the same families, the percent viability and morbidity of larvae were significantly higher (94.8%, 94.8%, 94.4%, 96.6%, and 2.1%, 1.7%, 2.5%, 2.1%, respectively). The above-mentioned positive relationship was also evident in other silkworm lines.

The families of the six breeding lines with different genotypes incurred three-grade classification based on the traits' recovery at the high temperature. However, the families with the low traits belonged in the grade-I, the families with moderate liveliness in the grade-II, and the families with the high liveliness went in the grade-III. Within each line, identifying the families belonging to three different grades also prevailed, with the average values of recovery and variability calculated for each line (Table 3).

The variability in the percentage of vivacity in the provocative conditions is an essential criterion for breeding the silkworms. Thus, the detection of recovery traits' variability coefficient at the unfavorable high temperature occurred in the six different lines. The highest variability appeared in the Line 28 (36.0%), while in other lines, the said variability ranged from 24.6% to 34.1%. In the populations of the studied silkworm breeding

Table 2. Breeding traits of the line families (F1 2023, spring).

Egg laying number	Origin of the families No.	Livability in provocative incubation (%)	Livability during average incubation (%)	Larval viability (%)	Percentage of morbidity (%)	Cocoon weight (g)	Cocoon shell weight (g)	Shell ratio (%)
Line 300								
25	11x1	65.0	99.8	94.8	2.1	2.27±0.01	0.492±0.002	21.6±0.18
62	2x10	67.0	99.6	94.8	1.7			
257	8x4	64.0	100.0	94.4	2.5			
386	12x6	71.0	99.4	96.0	2.1			
Line 301								
6	1x9	71.0	99.4	95.2	1.3	2.18±0.06	0.448±0.01	20.7±0.23
31	9x1	65.0	99.8	98.0	1.2			
76	3x1	51.0	99.8	92.0	3.8			
202	2x5	54.0	99.5	96.0	1.3			
Line 27								
15	10x3	27.0	94.6	93.9	4.6	1.76±0.05	0.447±0.01	25.3±0.13
48	3x10	22.0	96.0	96.4	1.7			
73	5x9	47.0	93.6	96.1	2.4			
125	9x5	22.0	94.0	97.1	1.2			
Line 28								
34	8x3	28.0	97.1	98.01	1.6	1.78±0.04	0.453±0.01	25.4±0.35
41	5x7	33.0	95.6	97.6	2.0			
50	5x7	10.0	98.8	92.9	5.5			
77	7x5	16.0	93.5	94.4	2.1			
Line 30								
1	-	36.0	97.3	93.6	1.7	2.04±0.04	0.443±0.01	21.8±0.44
2	-	27.0	94.5	95.2	1.3			
6	-	32.0	95.6	93.6	3.4			
9	-	54.0	98.2	86.8	9.2			
Line 31								
4	-	63.0	97.4	93.6	5.4	2.04±0.03	0.437±0.01	21.5±0.03
18	-	60.0	96.0	94.4	2.5			
28	-	84.0	98.0	95.6	2.9			
32	-	60.0	98.1	92.8	3.9			

Table 3. Assessment of various traits during revival of the breeding silkworm families at a high temperature (2023, spring).

Selection lines	Analyzed families (#)	Gradations in livability in provocative conditions	The limit of gradations	Number of families in grades (#)	A trait of the liveliness of families in provocative conditions, $\overline{X} \pm S\bar{x}$ (%)	Cv (%)
Line 300	82	I grade	26-42	20	51.4±1.41	25.0
		II grade	43-61	44		
		III grade	62-78	18		
Line 301	82	I grade	23-39	29	44.6±1.28	26.0
		II grade	40-56	43		
		III grade	57-72	10		
Line 30	9	I grade	18-31	2	40.2±4.5	34.1
		II grade	32-45	3		
		III grade	46-59	4		
Line 31	14	I grade	33-49	4	59.6±3.8	24.6
		II grade	50-66	6		
		III grade	67-84	4		
Line 27	62	I grade	21-30	23	35.5±1.33	29.5
		II grade	31-50	32		
		III grade	51-61	7		
Line 28	44	I grade	10-21	27	20.8±1.13	36.0
		II grade	22-34	15		
		III grade	35-46	2		

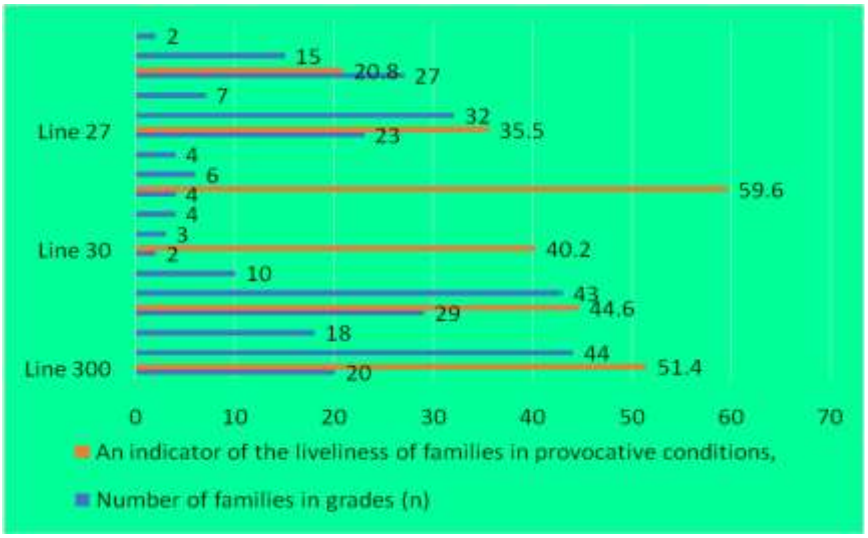

Figure 2. Assessment of traits of the revival of the breeding silkworm families at a high temperature (2023, spring).

lines, a considerable variation existed in the breeding material for selection based on the percentage of revival in provocation conditions (Figure 2).

DISCUSSION

Temperature fluctuations during cultivation led to the death of silkworm larvae, with the same result also confirmed in the past research carried out by Sinha and Sanyal (2013). The lower and upper temperature limits were +17 °C and +33 °C, respectively. The recommended temperature for healthy development of silkworm eggs ranged from +20 °C to +26 °C, and for optimal productivity, the required temperature range is 23 °C to 28 °C. However, the temperatures above +30 °C negatively affected the larvae. Considering this, in the presented studies, the incubation proceeded at the operating temperature of +34 °C.

In this direction, previous studies revealed obtaining the egg on the first day of laying and selecting the genotypes that set the maximum number of eggs on the first day (Nasirillaev *et al.,* 2017). Breeding work also materialized in adverse, stressful conditions to enhance the productive characteristics of the generations. Notably also, a sudden increase in temperature leads to a decrease in reproductive traits and a violation of the natural state of food proteins, resulting from stressful conditions of the embryo inside the eggs. Lines 1 and 2 were visible with an average of 456 and 522 eggs, respectively. The number of families selected for breeding work was 294, and the number of eggs in laying selected families was 722–755.2. In some other experiments in Uzbekistan with a strongly continental climate, healthy eggs came from large cocoon breeds, ranging from 626 to 679 (Umarov *et al*., 2022). Additionally, the presented results supported earlier findings by studying the comparative performance of robust and productive bivoltine hybrids of *Bombyx mori* L. under the high temperature (Kumar *et al*., 1989).

The Indian scientist obtained three groups of silkworms as a result of using several valuable traits at the temperature of +36 °C±1 °C and relative humidity (50%±5%), as the traits of thermal stability, i.e., susceptible, moderately tolerant, and tolerant (Kumari *et al*., 2011). It has been prominent that the silkworm strains BD2-S, SOF-BR, and BO2 have the potential of thermal tolerance. Kumar (2002) also identified only heat-resistant lines in the same experiments, however, in addition to the lines, we selected their families with three different grades in the latest studies.

The results showed the more stable and viable silkworm families appear at the embryonic stage of development, and will be stable and viable in the postembryonic period. A study mentioned the silkworm has received strong influences from temperature and humidity to grow and develop (Tanjung *et al.,* 2017). The outcomes further revealed resistance to high temperatures varies in different silkworm lines at the embryonic period (Manjunatha *et al*., 2010). By comparing the average revivability index at high temperature for different line families, the highest level was notable in Line 31 (59.6%) and Line 300 (51.4%). In the Line 27 and Line 28, the said trait emerged at a low level compared with other lines. It was also evident that the breeding lines with six selected genotypes considerably differed from each other in reproductive characteristics, signs of revival, viability, and productivity of cocoons.

The need to change environmental conditions from season to season, temperature, and relative humidity control for stable cocoon production requires attention (Rahmatulla, 2012). It was also noteworthy that in extreme climatic conditions, the entire cocoon decreases by 1.11–1.39 g, depending on the characteristics of the silkworm breed (Umarov and Nasirillaev, 2023). The cocoon's performance traits were 2.27 g in Line 300 and 1.76 g in Line 27 (Table 2). By comparing the results in the presented experiment, the obtained cocoons' weight was a result of caring for larvae eggs animated by heat that fully meets the standards.

According to past research, the mulberry silkworm commercial lines decreased from 23.93% to 17.64% by exposing to heat shock in Iran (Abdoli *et al*., 2024). In the promising studies, the shell ratio of the silkworm was in the range of 20.7% to 25.4% (Table 2). Given that Iran and Uzbekistan have similar climatic conditions, the presented higher results indicated the conduct of experiments is in the right direction.

The tropical multivoltine breeds of *Bombyx mori* (Pure Mysore, C. Nice, and Nistari) appeared more tolerant to the high temperature (Rahmathulla, 2012). By breeding the hybrid CSR18 × CSR19 in summer, it did not provide the expected result in warm climate. Singh and Kumar (2010) has developed the hybrid CSR46 × CSR47, also found highly productive. However, the viability was significantly lower (46.74%). Past studies reported heat shock proteins (HSP) differed significantly for thermal tolerance in different silkworm breeds (Manjunath *et al.*, 2010). In the progressing studies, the viability coefficients under the influence of heat shock were 59.6% and 20.8% in Line 31 and Line 28, respectively. By comparing all those traits, the above data was also valid.

CONCLUSIONS

The viability of the silkworm (*B. mori* L.) in the larval period determines the productivity and yield of the cocoon. The silkworm families found resistant to high temperatures during embryonic development could demonstrate higher viability potential during the periods of larvae feeding, which is also the primary source of enhanced yield per unit of fed larvae.

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