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Effect of high temperature on mulberry silkworm viability in the embryonic stage of development

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This research paper presents the preliminary results of experiments on the study of the resistance of mulberry silkworm embryos to critical temperatures during egg incubation. The response of breeding lines to critical temperatures at +34ºC was found to be different, according to the study. Thus, in some families of Lines 31 and 301, the revitalization of eggs at high temperatures was from 72.0 % to 84.0 %. However, the number of such resistant breeding families is not large. The different responses of breeding lines to critical temperatures indicate that mulberry silkworm genotypes are resistant to this unfavorable factor to varying degrees.

***Keywords:** Mulberry silkworm, egg, selection, embryo, caterpillars*

INTRODUCTION

The yield of mulberry silkworm cocoons depends on the number of healthy worms and the average weight of the cocoon. Naturally, the preservation of the maximum caterpillar population is dependent on the viability of caterpillars. Studies of mulberry silkworm breeding have shown that one-sided selection aimed at increasing the silkiness of cocoons leads to a decrease in egg revitalization and caterpillar viability (Zhang et al., 2022). If a breeder regularly selects cocoon silkiness, this breeding population will show a dramatic deterioration in caterpillar viability (Umarov et al., 2020). However, breeders face two main problems: increasing the viability of caterpillars and maintaining a high yield and cocoon quality. These issues are among the most difficult problems in mulberry silkworm breeding; breeders try to solve these problems by relying on different methods. As it is known, there are a number of breeds and breeding lines at the Silk Research Institute (Uzbekistan). Some of them have good characteristics of cocoon weight or silkiness, while others have a high potential for caterpillar viability. However, it is difficult to identify breeds with good silkiness and strong caterpillars that are resistant to different climatic conditions.

The seasonal differences in the environmental components considerably affect the genotypic expression in the form of phenotypic output of silkworm crops, such as cocoon weight, shell weight, and cocoon shell ratio (Rahmathulla, 2012). The result indicated a higher level of thiobarbituric acid reactive substances (TBARS, as an index of lipid peroxidation) and total hydroperoxides in the male pupae exposed to high temperatures (40±1°C) (Umarov and Nasirillaev, 2023).

The research design used was a complete randomized design (CRD) with four treatments and five replications, each consisting of 20 larvae. The treatment consists of administering heat shock at temperatures of 34, 38, and 42°C, and without heat shock (ambient temperature). Heat shock was conducted in the 4th instar for 3 hours. The research results showed that heat shock could increase the percentage of mortality, accelerate the larval stage, and reduce larval weight by

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lowering growth; consumption, and digestibility (Tanjung et al., 2017).

The biological architecture determining post-embryonic development and traits is well programmed during the process of morphogenetic movements and organogenesis in the embryo. However, changes in the environmental temperature for a few hours, which is uncommon, affecting the embryo development, protein expression, and hatching of larvae in the Eri silkworm (*Samia cynthia ricini*) remain enigmatic. Hence, for the first time, the eggs of new Eri silkworm breed C2 were exposed to heat shock (HS) temperatures of 35°C, 40°C, and 45°C for 2 hours, not only to measure heat sensitivity but also to uncover differential expression of proteins at a different age of the embryo (Punyavathi et al., 2022).

Therefore, in summer and fall, small, silky-thin cocoons are obtained. At the same time, it is important to try and select breeds and hybrids that are resistant to hot weather conditions and create new breeds and hybrids suitable for the summer and fall seasons. Extreme temperature and humidity conditions resulted in the prolongation of eclosion in silkworm, *Bombyx mori* L. it is extending for two consecutive days. A reduction in eclosion (%) in bivoltine bread NB4D2 was recorded (Reddy et al., 2002). On the other hand, PM (a pure breed of silkworm) and PM × NB4D2 (a hybrid of silkworm) confined their eclosion to a single day (Umarov and Nasirillaev, 2023).

This is related to the aforementioned scientific problem (Akhmedov, 1998), found in their experiments the effect of contrasting air temperature and humidity on changes in the processes of embryonic development of mulberry silkworm. The experiments substantiated a sharp drop in the percentage of caterpillar revival due to a decrease in the water content of eggs (Bessonova, 1977; Umarov et al., 2023) carried out selection work on reproductive traits in new silkworm lines resistant to hot climates (Bogoslavsky, 2009).

In our opinion, the solution to this problem should be studied starting from the embryonic stage of the development of breeding material, that is, by selecting caterpillars at the last stage of embryonic development. Therefore, it is necessary to establish a relationship between viability at the embryonic stage and in the postembryonic period. In this case, the main goal was to select the most viable genotypes of the breeding material at the embryonic stage. In this direction, V.A.Strunnikov (Strunnikov, 1959) conducted the first studies in the 90s of the twentieth century, and in these experiments, it was found that in families of pure breeds that were well revitalized against high temperatures, the viability of the caterpillar stage was also high.

MATERIALS AND METHODS

Experimental research was carried out in the laboratory of mulberry silkworm breeding at the Research Institute of Silkworm Breeding. The material used in this research was a population of new breeding lines, which differ from each other in cocoon weight and technological characteristics of the sheath. These lines were divided into three groups. The first group included small cocoon lines - Line 27 and Line 28, which have high technological properties of cocoon thread. The second group included medium cocoon lines - Line 30 (Parvoz 1 x Xorij) and Line 31 (Parvoz 2 x Xorij). The third group consisted of lines - Line 300 and Line 301, which produce large cocoons. This material was created in the laboratory "Breeding of Mulberry Silkworms" at the Scientific Research Institute of Sericulture.

The experiment began by analyzing all egg lying obtained during the spring brooding period of 2022. Eggs selected from each line of 100 eggs in February were placed in a thermostat under special laboratory conditions, the temperature was maintained at 34ºC, and only for 2 hours, each morning, the eggs were removed to room temperature during incubation. At the end of incubation, embryos that had reached the blastogenesis stage, the period of embryonic development in the egg, were counted. The next stage of experiments was continued in spring, in March-April. Spring incubation was carried out under moderately optimal hygrothermal conditions (24-25ºC and 75-80%). At the end of each incubation process, the percentage of egg revitalization was determined both in spring and winter.

RESULTS AND DISCUSSION

The mulberry silkworm is such a biological object that some complexity of selection on the viability of caterpillars characterizes it. If breeding families from the same breed are kept under the same optimal conditions, the viability of the breeding material will not differ much from each other. However, viability in lying resulting from the fusion of different parental gametes may also be manifested to different degrees. If a method of selecting strong genotypes (families) from multiple breeding families without caterpillar feeding is developed for breeding and pedigree work, selection for caterpillar viability in the breeding population can be much more effective and low-cost. In addition, by applying this method, it is possible to accelerate the process of selective breeding and achieve the goal. For this selection, various influences must be exerted on the egg later in the wintering of the eggs, when the eggs can already be revitalized. For example, to create extreme factors, eggs were kept in water for some time, exposed to hot water, and exposed to hydrochloric acid.

Table 1. Egg revitalization rates under critical and normal temperature conditions

Selection lines	The number of families analyzed		Percentage of revivability at critical temperature, (gradations)	Distribution of families by gradations	
				Quantity	Percent
Line 28	44	I gr	10.0-21.0	27	61.4
		II gr	22.0-34.0	15	34.1
		III gr	35.0-46.0	2	4.5
Line 27	62	I gr	21.0-30.0	23	37.1
		II gr	31.0-50.0	32	51.6
		III gr	51.0-61.0	7	11.3
Line 30	9	I gr	18.0-31.0	2	22.2
		II gr	32.0-45.0	3	33.3
		III gr	46.0-59.0	4	44.4
Line 31	14	I gr	33.0-49.0	4	28.6
		II gr	50.0-66.0	6	42.9
		III gr	67.0-84.0	4	28.6
Line 300	82	I gr	26.0-42.0	20	24.4
		II gr	43.0-61.0	44	53.2
		III gr	62.0-78.0	18	22.0
Line 301	83	I gr	23.0-39.0	29	34.9
		II gr	40.0-56.0	44	53.0
		III gr	57.0-72.0	10	12.0

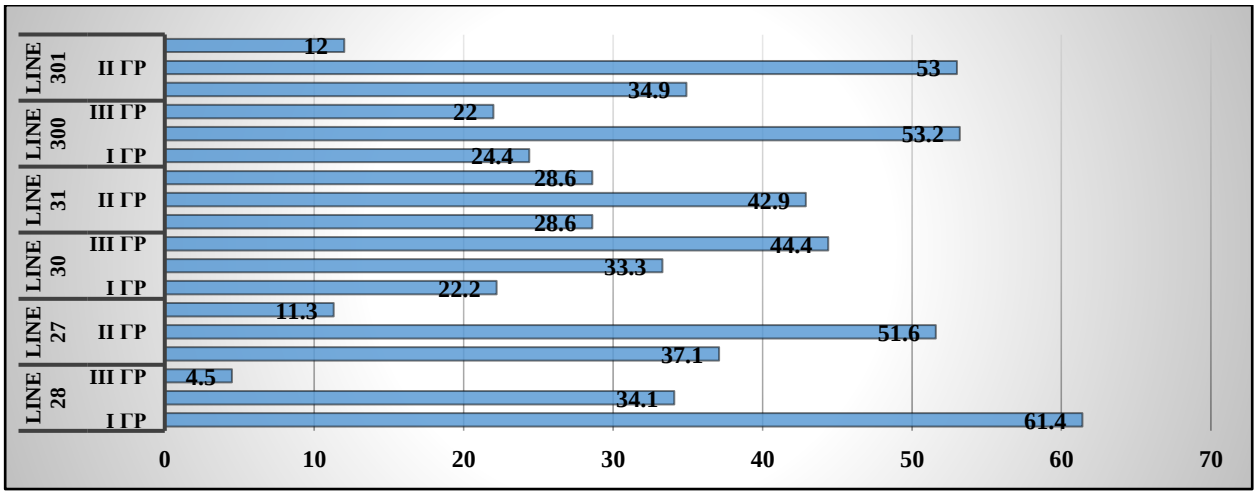

Fig. 1. Egg revitalization rates at critical and normal temperature conditions (Table 1).

The main objective was to identify strong genotypes that retain their embryonic viability under such a variety of severe exposures. Several experiments have found that incubation at high critical temperatures can allow the selection of resistant families among breeding silkworm breeds and lines. With this in mind, we conducted experiments aimed at identifying the strongest, most stable families. Families of breeds with different biological and technological properties under conditions of high-temperature exposure (+34ºC) maximally preserve their potential during embryonic development. The comparative performance of families of lines – Line 27, Line 28, Line 30, Line 31, Line 300, and Line 301 incubated at high critical temperatures is summarized in Table 1 and Fig. 1.

From the data in Table 1, it can be seen that some families of Line 31, Line 300, and Line 301 lines were revitalized from 72.0% to 84.0%. Within each breeding line, families' revivability at high temperatures was divided into three gradations. At the same time, the gradations for each line were made individually. For example, if we compare the relatively low-enlivened Line 28 with the well-enlivened Line 31, we can see that in Line 28, Gradation I indicators are distributed between 10.0-21.0%; Grade II, 22.0-34.0%; and Gradation III, 35.0-46.0%.

The most important thing for us in this experience was how the breeding families of each line would be graded. That is, how many families revitalize well or poorly at critical temperatures? The table clearly shows that only 4.5% of the 44 families in Line 28 were revitalized in the Gradation III section, and this figure was 44.4% in Line 30. The proportion of well-animated families in the III gradation of other lines was 11.3%; 12.0%; 22.0%; and 28.6%, respectively.

CONCLUSIONS

For the first time in mulberry silkworm breeding, the effect of stress temperatures on the viability of embryos in breeding populations was detected. The fact that breeding lines respond differently to critical temperature levels indicates that mulberry silkworm genotypes are resistant to an unfavorable factor, such as varying degrees. Our initial experimental results confirm that Line 30 and Line 300 are resistant to critical doses of temperature during embryonic development.

REFERENCES

Akhmedov N.A. (1998) Contrast temperature and moisture effect on the revival of silkworm seeds. *Silk* (Tashkent), **4:** 22-23.

Bessonova M.A. (1977) The influence of temperature, duration of copulation, and repeated use of males on the quality of grenades. *Trudı SANIIŞ* (Taşkent), **11:** 185-193.

Bogoslavsky B.B. (2009) Forecasting the productivity of adaptive abilities of silkworm breeds and fungi using enzyme systems and protein spectra. *Autoref. of PhD Thesis*, Stavropol, p. 3-4 (in Russian).

Punyavathi, Kumar K.H., Moatemjen S., Mahadevegowda L.G., Boregowda M.H. (2022) Changes in the embryonic protein profile and hatching as a response to thermal stress in the Eri silkworm, *Samia cynthia ricini. Journal of Applied Biology & Biotechnology*, **10(03):** 120-126; doı: 10.7324/JABB.2022.100316.

Rahmathulla V.K. (2012) Management of climatic factors for successful silkworm (*Bombyx mori* L.) crop and higher silk production: A review. *Hindawi Publishing Corporation Psyche*. **121234**, 12 p.; doi:10.1155/2012/121234

Reddy P.L., Naik S.S., Reddy N.S. (2002) Implications of temperature and humidity on the adult eclosion patterns in silkworm *Bombyx mori* L. *Journal of Entomological Research*, **26(3):** 223-228.

Strunnikov B. (1959) New in silkworm biology. *Materials of meeting on silkworm breeding*. Moskva, p. 30-34. (in Russian).

Tanjung M., Tobing M.C., Bakti D., Ilyas S. (2010) Growth and development of the silkworm (*Bombyx mori* L.) C301 with heat shock treatments. *Bulgarian Journal of Agricultural Science*, **23(6):** 1025–1032.

Umarov S., Nasirillaev B. (2023) Creation of heat-resistant, high-product breeds and hybrids of mulberry silkworm (*Bombyx mori* L.). In: *E3S Web of Conferences*. EDP Sciences, **371:** 1-9; doi: 10.1051/e3sconf/202337101028.

Umarov S., Nasirillaev B., Batirova A. (2022) Mulberry silkworm (*Bombyx mori* L.) hybrids in Uzbek selection and, viability, cocoon productivity, and reproductive indicators in summer and fall seasons. *In: IOP Conference Series: Earth and Environmental Science*, **1068(1):** 012-021; doi: 10.1088/1755-1315/1068/1/012021.

Umarov S.R., Nasirillaev B.U., Rajabov N.O., Jumaniyozov M.Sh., Batirova A.N., Khudzhamatov S.Kh. (2020) Embryonic and post-embryonic viability of Second generation (F2) silkworm breeds and lines obtained under unfavorable stressful conditions. *International Journal of Scientific Technology Research (India)*, **3:** 863.

Umarov Sh.R., Nasirillaev B.U., Batirova A.N., Akhmedova M.D. (2023) Egg specialty of promising silkworm new systems resistant to heat. *Journal of livestock and breeding work* (Tashkent), **1(29):** 40-41.

Zhang Y-L., Huang Y., Wang P-Y., Li Q., Bi L-H., Zhao A-C., Xiang Z-H., Long D-P. (2022) Very early corona treatment-mediated artificial incubation of silkworm eggs and germline transformation of diapause silkworm strains. *Front. Bioeng. Biotechnol*., **10:** 35-43; doi: 10.3389/fbioe.2022.843543.

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