Creation of new silkworm *Bombyx mori* L. Hybrids with increased unfavorable rearing conditions tolerance by crossing sex-limited for egg color and larval markings breeds

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Abstarct

There is a very close connection between environment and *Bombyx mori* L. in all stages of development. The abiotic factors with the strongest influence on the development of the silkworm are: temperature, humidity, premise ventilation and light. The current study explores the adaptive abilities towards unfavourable conditions of newly created mulberry silkworm hybrids obtained by crossing sex-limited for egg and larval markings breeds. Some biological and technological features of the hybrids tested have been evaluated. Under provocative silkworm rearing regime the newly created hybrids manifest a relatively high vitality of the larvae which exceeds the one in the control and under optimal breeding conditions they have a comparatively high productivity.

Key words: *Bombyx mori* L., sex-limited strains, rearing performance, silkworm

Създаване на нови хибриди буби с повишена толерантност към неблагоприятни условия на отглеждане посредством кръстосване на породи, маркирани по пол в стадий яйце с породи, маркирани по пол в стадий ларва

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Резюме

Връзката между околната среда и *Bombyx mori* L. през всички стадии на развитие е много тясна. От абиотичните фактори най-силно влияние върху развитието на черничевата копринена пеперуда имат температурата, влажността, аерацията на помещенията и светлината. В настоящото проучване са разгледани адаптивните способности към неблагоприятни условия на новосъздадени хибриди черничева копринена пеперуда, получени при кръстосване на породи, маркирани по пол в стадии яйце с породи, маркирани по пол в стадии ларва. Направена е оценка на някои биологични и технологични признаци на изпитаните хибриди. Установено

е, че новосъздадените хибриди имат повишена толерантност към неблагоприятни условия и показват по високи стойности на признаците в сравнение с контролния вариант.

Ключови думи: *Bombyx mori* L., маркирани по пол породи, условия на отглеждане, черничева копринена пеперуда

Introduction

The life of the silkworm passes in the closest connection with the conditions of the external environment. Among the various environmental factors, temperature and humidity of the air, lighting and ventilation of the premises, quantity and quality of food and the rearing space are of the greatest importance for the silkworm development.

The great importance of sex-limited silkworm breeds and lines both for the purposes of heterosis selection and for simplification and improvement of technology for the production of absolutely pure from the initial forms industrial (F_1 hybrid) silkworm eggs requires the development of a number of fundamental tasks of great application importance. They should be related to development and improvement of the known methods as well as exploration of some new methods leading to an increased vitality and productivity of our breeds and silkworm hybrids which will lead to bigger production of cocoons and silk.

The first attempts for sex regulation in *B. mori* L. were made by Tihomirov in 1855 (cited by Astaurov, 1974) who managed to provoke the development of unfertilized eggs through physicochemical reactions. Attempts to induce artificial parthenogenesis in the silkworm have also been made by Astaurov (1940).

According to a number of scientists (Petkov, 1995; Nacheva, 1990; Petkov et al., 1999), the industrial crossbreeding between two or more initial forms (breeds, lines) with different geographical and genetic originis one of the most effective methods for creating high heterosis silkworm hybrids and their use in the production. According to these authors, the superiority in F_1 hybrids over the average parental value of their initial forms in the main productive traits is in the range of 10–30%.

Singh et al. (2009) analyzed the adaptation of the mulberry silkworm and the combination of factors with influence on its physiology.

Rajan and Himantharaj (2005) analyzed the influence of premise ventilation on the development of the silkworm. They concluded that at air velocity of 1.0 m/sec in the 5th larval instar leads to a decrease in larvae mortality and improves food absorption, digestibility, larvae weight, cocoon weight and cocoon formation speed compared to non-ventilated premises.

Khan (2014) analyzed the effect of temperature and humidity on the characteristics of cocoons in India. They recommended that silkworm breeding should be done at 25 °C temperature and 80% humidity.

According to Ramchandra et al., 2001, the best temperature for cocoon formation is 22 °C and 65% humidity. In other studies, Penzaman and Jagdeeshkumar (2010) reported optimal temperatures from 22 °C to 27 °C.

Watanabe (2002) studied the genetically determined resistance of the silkworm against viral diseases. He found that with the exception of resistance to densonucleosis virus (DNV), the resistance of silkworms against other viral diseases is controlled by polygens. Resistance against DNV is controlled by 2 recessive andone dominant gene.

However, according to Li at al. (2001) when studying the resistance of 346 breeds of silkworms against nuclear polyhedrosis virus, it was found that one major gene located in the autosome and several low-effect genes located in the Z chromosome control the NPV (grasery) resistance. In another study of 206 silkworm breeds, the same authors found that

8 of them were not prone to densonucleosis virus.

In recent years, in the selection of the silkworm there has been a special attention to the adaptive ability and use of adaptive potential in creating high vitality hybrids. According to Nagaraju et al. (1996) in cases when the parental breeds, lines and their hybrids are grown under extreme conditions (air temperature above 31 °C) the average values of the main productive features of the hybrids significantly exceed those of the parental forms.

Aim of the study

The aim of the present study is to establish the values of the main productive characters in F_1 hybrids created by crossing between sex-limited for egg color and larval markings silkworm breeds, tested both in conditions of a provocative larval rearing regime and under optimal conditions.

Materials and methods

The study was conducted in the period 2018–2020 in the Scientific Center on Sericulture, Vratsa with the sex-limited for egg color silkworm breeds ME 1 and ME 2 and the sex-limited for larval markings breeds Iva1 and Nova 2.

ME 1 and Iva 1 breeds are mono-bivoltine, white cocoon, Japanese type, characterized by elongated cocoon shape.

ME 2 and Nova 2 breeds are mono-bivoltine, white cocoon, Chinese type, characterized by oval cocoonshape.

The following hybrid combinations have been tested:

♀ ME 1 x ♂ Nova 2;

♀ ME 2 x ♂ Iva 1

Testing was done in 4th and 5th larval instars under the following provocative regime:

Each hybrid was tested under provocative and under optimal silkworm rearing conditions with 2 repetitions of 300 larvae counted after the second moult.

The silkworm larvae rearing took place in the spring (month of May). The larvae were grown according to the standard method for spring cultivation in Bulgaria (Panayotov and Ovesenska, 2002). They were fed ”ad libitum” with leaves of the Bulgarian variety № 24. Apart from rainwater, the mulberry plantations were not additionally irrigated during the spring season. The data obtained were statistically processed according to Lidanski, 1988.

The hybrid Super 1 x Hesa 2 was used as a control.

Таблица 1. Метод на отглеждане
Table 1. Rearing method

Начин на отглеждане на бубите / Breeding mode of the silkworm larvae	Температура, °C / Temperature, °C	Относителна влажност на въздуха, % / Relative air humidity, %	Хранителна площ / Feeding space	Хранителна норма / Feeding amount	Проветряване / Ventilation
Провокационен режим / Provocative regime	28–29	80–90%	11 м^2 за 1 кутийка буби / 11 m^2 for 1 box of silkworms	1 хранене дневно / 1 feeding daily	Плътно затворени прозорци и врата / Tightly closed door and windows
Стандартна технология / Standard technology	23–25	55–70%	22 м^2 за 1 кутийка буби / 22 m^2 for 1 box of silkworms	2 хранения дневно / 2 feedings daily	Отворени два прозореца, а при необходимост и вратата / Two open windows. If necessary open door, too

Results and discussion

The data obtained are shown in Tables 2, 3 and 4.

The data in Table 2 show that under conditions of provocative regime both new hybrids and especially ME 2 x Iva 1 show relatively high vitality of the silkworms which exceeds that of the control. Under optimal silkworm rearing conditions, the new hybrids show relatively high values of the cocoon weight and silk shell weight. According to (Chandrakanth et al., 2015) one of the reasons why the higher temperatures the decrease in silkworms characteristics is due to low nutritional activity at high temperatures.

Таблица 2. Жизненост и продуктивност на хибриди буби, отгледани при провокационен режим

Table 2. Vitality and productivity of hybrid silkworms grown under provocative regime

Хибрид / Hybrid	Жизненост на бубите, (%) Vitality of the silkworms, (%)	Тегло на пашкул, (mg) Fresh cocoon weight, (mg)
ME 2 x Ива 1 ME 2 x Iva 1	78,33***	1615***
ME 1 x Нова 2 ME 1 x Nova 2	74,00***	1317*
Супер 1 x Хеса 2 Super 1 x Hesa 2	50,50	1409

** – P < 0,05; ** – P < 0,01; *** – P < 0,001*

Data in Table 3 manifest that all three hybrids showed vitality values of over 80% as the hybrid ME 2 x Iva 1 has 3.25% higher values compared to ME 1 x Nova 2 and 9.75% less than the control. Productivity of cocoons and raw silk largely depends on vitality, which is a major biological character.

Comparing data from the vitality indicator in the two breeding regimes we notice the most significant difference in the control Super 1 x Hesa 2 as the difference in the vitality values under optimal conditions and provocative breeding regime is 46% while in the other two hybrids we have a difference below 10%. In ME 2 x Iva 1 the difference observed is 8.42% and in ME 1 x Nova 2 the difference is 9.50%.

Under provocative silkworm rearing regime, higher values were reported for both hybrids compared to the control as the hybrid ME 2 x Iva 1 showed values of 78.33% while the control has values of 50.5%. The difference here is 27.83% and the hybrid ME 1 x Nova 2 – 74% which is 23.5% more than the control variant. Based on this we can conclude that the newly created hybrids survive better under provocative regime compared to Super 1 x Hesa 2.

Considering the data on the cocoon weight in both of the breeding regimes, again the biggest difference is in Super 1 x Hesa 2 with a value of 1031 mg, followed by 862 mg difference in ME 1 x Nova 2 and 567 mg in ME 2 x Iva 1. Here, the research of Khan (2014) confirms that as the

Таблица 3. Жизненост и продуктивност на хибриди буби, отгледани при оптимални условия

Table 3. Vitality and productivity of hybrid silkworms grown under optimal conditions

Хибрид / Hybrid	Произход / Origin	Жизненост на бубите, (%) / Vitality of silkworm larvae, (%)	Тегло на пашкул, (mg) / Fresh cocoon weight, (mg)	Тегло на копринената обвивка, (mg) / Silk shell weight, (mg)	Свиленост, (%) / Silkiness, (%)
ME 2 x Ива 1 / ME 2 x Iva 1	България / Bulgaria	86,75***	2182*	458*	20,99*
ME 1 x Нова 2 / ME 1 x Nova 2	България / Bulgaria	83,50***	2179*	456*	20,93*
Супер 1 x Хеса 2 / Super 1 x Hesa 2	България / Bulgaria	96.50	2440	546	22.38

** – P < 0,05; ** – P < 0,01; *** – P < 0,001*

temperature increases, the weight of the cocoon decreases.

Under normal silkworm rearing conditions, the difference observed between the two new hybrids ME 2 x Iva 1 and ME 1 x Nova 2 is hardly 3 mg while the difference between the control Super 1 x Hesa 2 and ME 2 x Iva 1 is 25 mg and between the control and ME 1 x Nova 2 is 261 mg.

Considering the silk shell weight, we see that we have the highest values in the control variant Super 1 x Hesa 2 with a value of 546 mg. In the two new hybrids we observe a slight difference in the silk shell weight within 0.02 mg. Comparing the first hybrid ME 2 x Iva 1 with the control we see that the difference in the indicator is 88 mg and in the second hybrid ME 1 x Nova 2 we have 90 mg difference.

Data on the average values of the silkiness of fresh cocoons character which characterizes the relative silk content in cocoons show that in all hybrids we have values above 20%. Again the highest values are for the control variant. Here again we have insignificant differences in the range of 1.39 mg in ME 2 x Iva 1 to 1.45 mg in ME 1 x Iva 2. The two hybrids show extremely close values in this indicator as well.

Table 4 presents the data of some technological features of the silk thread in newly created silkworm hybrids.

Considering each indicator separately, we notice that the two new tested hybrids showed very similar values in dry cocoon weight with a difference of 2 mg between them. This leads to a similar difference with the control variant Super 1 x Hesa 2 respectively 233 mg difference in ME 1 x New 2 and 235 mg difference in ME 2 x Iva 1.

In terms of silk thread length, Super 1 x Hesa 2 again retain the highest indicators compared to the other two hybrids with values of 1249 mg. The difference between it and ME 1 x Nova 2 is 113 mg. The difference between Super 1 x Hesa 2 and ME 2 x Iva 1 is 150 mg and the difference between the tested two new hybrids is 37 mg. The biggest difference is between Super 1 x Hesa 2 and ME 2 x Iva 1. The thread length is also the indicator that showed the biggest difference between the two newlyested hybrids. In terms of silk thread weight, all analyzed hybrids showed values below 400 mg with the biggest difference between the control and ME 2 x Iva 1 of 61 mg. The difference between Super1 x Hesa 2 and ME 1 x Nova 2 is 40 mg. The difference between the two new hybrids is 21 mg.

All silk products' weight reported in the studies was 433 mg for Super 1 x Hesa 2, 387 mg for ME 1 x Nova 2, 387 mg and 369 mg for ME 2 x Iva 1. The difference observed between the two new hybrids is 18 mg. The biggest difference is again observed between Super 1 x Hesa 2 and ME 2 x Iva 1 of about 64 mg followed by a 46 mg difference between Super 1 x Hesa 2 and ME 1 x Nova 2.

In the study of the two hybrids, created by sex-limited breeds crossing, we also collected data on the thickness of the silk thread. Here we have low

Таблица 4. Технологични признаци на копринената нишка при новосъздадени хибриди буби
Table 4. Technological features of the silk thread in newly created hybrid silkworms

Хибрид / Hybrid	Тегло на сух пашкул, mg / Dry cocoon weight, mg	Дължина на нишката, m / Filament length, m	Тегло на копр. нишка, mg / Silk filament weight, mg	Тегло на всички копр. продукти, mg / All silk products weight, mg	Denier	Размотваемост, % / Reelabilty, %	Лабораторен рандеман, % / Raw silk percentage, %
Супер 1 х Хеса 2 / Super 1 x Hesa 2	1130	1249	391	433	2,82	90,30	34,60
ME 1 x Нова 2 / ME 1 x Nova 2	897***	1136*	351*	387*	2,78	90,70	39,13***
ME 2 x Ива 1 ME 2 x Iva 1	895***	1099**	330**	369**	2,70	89,43	36,87***

** – P < 0,05; ** – P < 0,01; *** – P < 0,001*

values of indicator variation from 2.82 denier to 2.70 denier or a difference in the range from 0.04 denier to 0.12 denier.

The indicators analyzed so far also confirm the studies of (Kumar et al., 2015) where at a breeding temperature of about 25 °C we have higher values of the indicators compared to silkworms raised at higher temperatures.

With the silk thread reelability character expressed as a percentage, ME 1 x Nova 2 have 0.4% higher values compared to the data of Super 1 x Hesa 2 and both of the above hybrids have similar values. Only ME 2 x Iva 1 showed values slightly below 90% or 89.43% which is 0.87% less than the control variant and 1.27% less than ME 1 x Nova 2.

The yield of raw silk as a technological feature expresses the percentage ratio of the weight of the unwound silk thread towards the weight of the dry cocoons.

As a rule, this feature is also of some importance for the formation of the overall productivity of raw silk from the cultivation of a hybrid silkworm.

The highest values were shown by the hybrid ME 1 x Nova 2 – 39.13% which is 4.53% more than the control variant and 2.26% more than ME 2 x Iva 1. In the other tested hybrid ME 2 x Iva 1 we observed 2.27% more than the control.

Conclusions

By crossing between sex-limited for egg color and larval markings silkworm breeds, new hybrids of silkworms ME 1 x Nova 2 and ME 2 x Iva 1 were created and tested. Under provocative silkworm rearing regime the newly created hybrids manifest a relatively high vitality of the larvae which exceeds the one in the control and under optimal breeding conditions they have a comparatively high productivity.

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