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Study on the possibilities for creation of new silkworm, *Bombyx mori* L. hybrids with high tolerance towards unfavorable larval rearing conditions by crossing sex-limited for larval markings with hardy silkworm breeds

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Abstract

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The present study aimed to investigate the possibilities for creation of new, tolerant to adverse rearing conditions four-way silkworm hybrids by crossing sex-limited for larval markings with hardy silkworm breeds. The main goal expected of the new hybrids would be an easier and more precise egg production, combined with a higher viability and productivity under adverse rearing conditions, which is the common practice at the field level.

By means of crossing between sex-limited for larval markings silkworm breeds and breeds possessing high sturdiness there were created and tested two new silkworm hybrids. In the conditions of a provocative silkworm rearing regime, they showed considerably high silkworm vitality (HB2xNova2 81.17%), which exceeded that of the control (Super 1 x Hesa 2 – 50.50%), having average and high values of cocoon weight and silk shell weight, as well as high silk filament technological character values at the optimal rearing conditions.

Keywords: silkworm; *Bombyx mori* L; sex-limited breeds; crossing; sturdiness

Introduction

The increase and stabilizing of cocoon yields requires the creation and introduction in practice of highly productive silkworm breeds, lines and hybrids possessing high adaptability and plasticity towards the conditions of the different feeding seasons.

The production of hybrid silkworm eggs, without any contamination with pure parental breeds, technically is a complex task. Difficulties evolve from the fact that silkmoths copulate themselves immediately after their emergence from the cocoons. Thus, some pure-breed silkworm eggs are obtained along with the hybrid ones.

That is why, in order to avoid intra-breed mating it is necessary to separate and isolate preliminarily by gender millions of specimens at their cocoon stage (pupa).

Over the last years the countries developed in sericulture have invested considerable resources in order to solve the problem with the process management related to inheritance and regulation of gender of the mulberry silk moth.

Moorthy et al. (2007) created several bivoltine silkworm lines possessing increased thermo-tolerance by means of back-crossing with polyvoltine breeds.

Rao et al. (2006) tested 31 polyvoltine breeds with regard to their productivity. As a result, 10 of them were defined with the highest productivity being appropriate for their inclusion in selection programs.

On the other hand, in India there have been studied poly-voltine breeds with regard to their thermotolerance. Kumaresan et al. (2012) tested 10 poly-voltine breeds, 4 of which were recommended to be used for rearing in regions with high temperature and low humidity. Another 4 breeds were recommended to be reared in regions with high temperature and high humidity.

In recent years, an important direction in the silkworm breeding is the crossing of monobivoltine with tropical poly-voltine breeds in order to increase tolerance to adverse rearing conditions and stress factors.

According to Chatterjee (1993) in India, the silkworm breeding aiming to improve the overall adaptability of the silkworm includes breeding with reduced feeding amount and rearing space. Breeding of silkworms at high temperatures is also used as a selection method.

The genetic diversity of the silk moth now bred is result of the crossing of breeds of different geographical origins, which have shown a wide variety of qualitative, quantitative and biochemical characteristics. (Nagaraju, 2000; Petkov et al., 2006). According to Xu & O'Brochta (2016), as a result of many years of research, the silkworm (Bombyx mori L.) has become one of the few insects for which the most advanced genetic and selection methods have been developed. Their application not only facilitates the functional genetic research of Bombyx mori, but also contributes to complex genetic modifications of silkworms in order to improve their commercial value, as well as the development and use of similar technologies in other insects.

According to Ghosh (2010) there are differences in response between silkworms rearing in tropical climates and those in temperate climates.

Even though the sex-limited for larval markings silkworm breeds possess a comparatively high productivity and viability at optimal rearing conditions, they are less viable when reared under adverse environment like high temperature and restricted feeding amount during the 4^{th} and 5^{th} larval instars. On the other hand in Bulgaria by crossing between unibivoltine and polyvoltine races two hardy silkworm breeds, VB1 and HB2 were created.

The present study aims to investigate the possibilities for creation of new, tolerant to adverse rearing conditions four-way silkworm hybrids by crossing sex-limited for larval markings with hardy silkworm breeds. The main goal expected of the new hybrids would be an easier and more precise egg production, combined with a higher viability and productivity under adverse rearing conditions, which is the common practice at the field level.

Materials and Methods

The study has been carried out at the Scientific Center on Sericulture – Vratsa in the period from 2018 to 2020.

Two silkworm breeds, Iva 1 and Nova 2 – sex-limited for larval markings and two hardy breeds – VB1 and HB2 were used in the study.

The breeds Iva 1 and VB1 are of the Japanese type, having elongated white cocoons and the breeds Nova 2 and HB2 are of the Chinese type, with white cocoons and oval shape. (Table 1).

The following hybrid combinations were examined:

Iva 1 x VB1
VB1 x Iva 1
Nova 2 x HB2
HB2 x Nova 2
Iva 1 x VB1 x Nova 2 x HB2
Nova 2 x HB2 x Iva 1 x VB1
Super 1 x Hesa 2 – control

Each hybrid was tested at provocative and optimal silkworm rearing conditions in two repetitions with 300 silkworm larvae each 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 & Ovesenska, 2002). They were fed „ad libitum" with mulberry 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, using SPSS- SAS.

Table 1. Rearing method

Technology of silkworm rearing	Temperature, °C	Relative air humidity, %	Feeding space	Feeding norm	Ventilation
Provocative regime	28 – 29	80 – 90%	11 m^2 for 1 box of silkworms	1 feeding daily	The windows and the door closed tightly
Standard technology	23 – 25	55 – 70%	22 m^2 for 1 box of silkworms	2 feedings daily	Both windows open, the door open if necessary

Results and Discussion

The data obtained are presented in Table 2, 3 and 4.

From the data in Table 2 it is evident that in the conditions of a provocative regime the 6 new hybrids show considerably high vitality of silkworms, exceeding that of the control. The highest value of the indicator "vitality" is seen for HB2xNova2 – 81.17%, which is with 30.67% more than the control hybrid Super 1 x Hesa 2. The hybrid Nova2 x HB2 also shows a considerably high value – 77.67%. (Table 2)

Taking into account the cocoon weight character, the highest values are reported for the hybrid HB2xNova 2 – 1731 mg, which is with 322 mg higher than the control, and with 409 mg more than Nova2 x HB2 (1322 mg). These results are consistent with the research of Duanpen et al., 2015 who concluded that increase of the temperature leads to cocoon weight decrease.

The control Super1 x Hesa2 and VB1xIva1 have the lowest vitality indexes – 50.50% and 51.17% respectively, and Nova2 x HB2 and VB1xIva1 manifest the lowest cocoon weight.

At the optimal silkworm rearing conditions (Table 3) the new hybrids possess lower values of cocoon weight and silk shell weight compared to the control. Nevertheless, silkworms show high values of vitality at the provocative regime. It makes an impression that the parent forms of both four – way hybrids have cocoon weight and silk shell weight values without significant difference from the F1 hybrid, which secures high values of seed cocoons and silkworm eggs.

Table2. Vitality and productivity of silkworm hybrids at a provocative silkworm rearing regime

Hybrid	Silkworm vitality (%)	Cocoon weight (mg)
VB1xIva1	51.17	1337*
Iva1 x VB1	68.17***	1442
Nova2 x HB2	77.67***	1322**
HB2xNova2	81.17***	1731***
I1xVB1 x N2xHB2	63.50***	1522*
N2xHB2 x I1xVB1	70.33***	1363*
Super 1 x Hesa 2	50.50	1409

*P < 0.05; **P < 0.01; ***P < 0.001

Hybrids showing vitality rate over 85% are Iva1 x VB1 and Nova2xHB2 x Iva1xVB1 with 88.25% and 88.00%, correspondingly. Those, having vitality rate around 95%, are HB2xNova2 – 94.50% and the control Super 1 x Hesa 2. The difference between them is barely 1%.

Out of all new hybrids, HB2xNova2 has the highest vitality rate at both larval rearing regimes. At the provocative regime it shows values of 81.17%, at optimal rearing conditions it shows 94.50%. There is 13.33 % difference between both regimes.

Taking into account the indicator "cocoon weight", the highest values are seen again for the control, followed by Iva1 x VB1. The hybrid showing the lowest values at both larval rearing regimes is Nova2 x HB2.

Data related to the weight of silk shell indicate high values for the control – 546 mg, followed by HB2xNova2 – 522 mg. The difference between both hybrids, HB2xNova2 and the control, is barely 24 mg. VB1xIva1 hybrid has the lowest values – 447 mg. It shows 99 mg less than the control variant.

Data pointed out in Table 4 report that the new hybrids, reared at optimal conditions have values of the technological features of silk thread, which are close to Super 1 x Hesa 2 hybrid.

Regarding both hybrids created via straight and reverse interbreeding, N2xHB2 x I1xVB1 is with the highest values, and it is the closest to the control. Regarding the indicator "raw silk percentage", the hybrid N2xHB2 x I1xVB1 even exceeds the control by 3,24%. For the indicator "weight of all silk products" there is difference of 0,71 mg between the control and the hybrid with the lowest value – I1xVB1 x N2x-

Table 3. Vitality and productivity of silkworm hybrids at optimal rearing conditions

Breed	Silkworm vitality (%)	Cocoon weight (mg)	Weight of silk shell (mg)	Silkiness (%)
VB1xIva1	91.50*	2290*	447***	19.52***
Iva1 x VB1	88.25***	2338	496*	21.21*
Nova2 x HB2	92.00*	2180**	480*	22.02
HB2xNova2	94.50	2259*	522	23.11*
I1xVB1 x N2xHB2	91.25**	2367	496*	20.95*
N2xHB2x I1xVB1	88.00***	2277*	467**	20.51**
Super1 x Hesa 2	96.50	2440	546	22.38

*P < 0.05; **P < 0.01; ***P < 0.001

Table 4. Silk filament technological characters of newly created silkworm hybrids

Breeds, hybrid	Weight of dry cocoon, mg	Filament length, m	Weight of silk thread, mg	Weight of all silk products, mg	denier	Reelability,%	Raw silk percentage, %
Super1xHesa2	1130	1249	391	433	2.82	90.30	34.60
I1xVB1 x N2xHB2	965**	1104*	335**	362**	2.73	92.54**	34.72
N2xHB2 x I1xVB1	1007*	1289	381	423	2.66*	90.07	37.84***

*P < 0.05; **P < 0.01; ***P < 0.001

HB2. The weight of dry cocoon for I1xVB1 x N2xHB2 is under 1000 mg. The difference between both tested hybrids is 42 mg and 165 mg between the control and I1xVB1 x N2xHB2.

The analyses also confirm the research done by Kalpana & Reddy, 1998 who concluded that the hereditary potential and its ecological plasticity largely determine the qualities of a breed or hybrid.

Conclusions

By means of crossing between sex-limited for larval markings silkworm breeds and breeds possessing high sturdiness there were created and tested two new silkworm hybrids. In the conditions of a provocative silkworm rearing regime they show considerably high silkworm vitality with 30,67%, which exceeds that of the control, having average and high values of cocoon weight and silk shell weight, as well as high silk filament technological character values at the optimal rearing conditions.

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