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Study of the relationship between fluorescence type and percentage content of good-quality cocoons of the silkworm *Bombyx mori* L. breeds and hybrids

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Abstract

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Establishing relationship between fluorescence type and percentage content of good-quality cocoons from silkworm *Bombyx mori* L. was the aim of this paper.

The study was conducted during 2016-2020 at the training experimental station of the sub-department “Special branches (Sericulture)”, department “Animal husbandry – Non-ruminant animals and special branches”, Faculty of Agriculture, Trakia University – Stara Zagora. In fulfillment of the purpose and tasks, biological material from 8 breeds, 16 dihybrids and 10 tetrahybrids reared with mulberry leaf was used. The type of fluorescence of the silk sheaths was established when exposed to ultraviolet (UV) rays with a wavelength in the range 254-365 nm.

The analysis of the obtained data shows that the type of fluorescence of the silk sheath, as well as the breed (hybrid), have a highly reliable influence ($p \leq 0.001$) on the variation of the percentage of good-quality cocoons (*an-ras*). Of the three fluorescent groups, that of the yellow-fluorescent cocoons was characterized by the highest mean percentage of good-quality cocoons in both dihybrid (94.75%) and tetrahybrid (95.34%) forms of the mulberry silkworm.

This gives reason to consider that the results obtained by us confirm the established in the scientific literature relationship between ultraviolet fluorescence and the technological qualities of cocoons. This relationship can be used to increase the quality of the obtained production by applying the methods selection and breeding by type of the fluorescence.

Keywords: Bombyx mori L.; breeds; hybrids; fluorescence; good-quality cocoons; an-ras

Introduction

The economic efficiency is an important aspect of the sericulture and is determined by the maximum yield of raw silk from silk sheaths, which largely depends on several factors, but mainly the quality of the cocoons (Nzyoki, 2020).

Jumagulov et al. (2021) studied influence of external environmental factors on the productivity of mulberry silkworm (Bombyx mori L) in different sizes enterprise of the silkworm breeding. The results show that the larger the silkworm breeding enterprise, the more difficult it is to maintain a comfortable temperature, relative humidity and other environmental factors in which silkworms form cocoons.

As cocoon quality contributes to the tune of about 80% of the raw silk quality, good-quality cocoons is essential for the production of quality silk. The quality of silk cocoons depends on many characteristics and each of these measures different aspects of quality of cocoons. The quality of silk cocoons is determined by a number of characteristics and each of them measures different aspects of the production quality (Nagadevara, 2004). Among the various variables, percentage of defective cocoons and silk cocoon formation conditions have a high degree of correlation with raw silk quality (Kumaresan et al., 2010). Mounting should not be delayed when larvae mature as it results in loss of silk because of bad quality cocoons production (Rajan et al., 2000). Even if the silkworm crop is healthy, improper mounting methods, cocooning conditions, mounting density, mounting of immature larvae and bad type of mountages can results in low quality cocoons (Krishnaswami et al., 1973). Thus, the equipment used for supporting the cocoons spinning larvae i.e., the mountages play a vital role in determining quality of cocoons and price fixation at the cocoon market (Sahana et al., 2020). An improper use of mounting structure and lack of care during handling and management of mature silkworms results in formation of defective cocoons accounting to a loss of about 5 to 8 per cent of cocoon yield (Chandrakanth et al., 2004; Grekov et al., 2005).

According to Shivakumar et al. (2016) some of the most important factors affecting cocoon quality, respectively raw silk yield and quality, are mounting time and method, as well as the frame for the cocoons. The type of frames used for cocooning also has an influence, a number of authors claim (Singh et al., 1999; Sangappa et al., 2010; Fayaz et al., 2022).

Alisher et al. (2019) establishes influence of food quality and rearing conditions of silkworms with enriched mulberry leaves on cocoons quality and silk sheaths properties. Kamel (2014) investigated the effect of balanced NPK fertilization of mulberry plantations on silkworm development and productivity. The results obtained show that rearing larvae with leaves from such mulberry trees increases the weight of the raw cocoon, the weight of the silk sheath; shell-to-cocoon ratio, percentage of good-quality cocoons, silk yield, hatchability, thread length and weight.

The initial breeding stock sorted in breeding enterprise is usually a mixture of cocoons of different quality. It consists of normal cocoons that are uniform in size, small and large, as well as various types of defective cocoons (ugly, irregular, thin-walled, etc.) that differ in one way or another from normal cocoons in size and mass. The majority of defective cocoons affect not only the quality of the laid eggs, but also subsequently the quality of the raw silk. They are heritable and can be strengthened or weakened in offspring, so they must be carefully scrapped during sorting (Mirzakhodjaev et al., 2020).

The present study aims to determine the influence of some factors on the quality of silk cocoons, such as fluorescence of the cocoons.

Material and methods

The study was conducted during 2016-2020 at the training experimental station of the sub-department "Special branches (Sericulture)", department "Animal husbandry – Non-ruminant animals and special branches" of the Faculty of Agriculture at Trakia University – Stara Zagora. The object of research was the populations of 8 breeds – 1, 3, 4K, 1A, 19, 1013, 20, 1014; 14 dihybrids – 20x1014, 1014x20, 19x1013, 1013x19, 19x1014, 1014x19, 1013x20, 20x1013, 19x20, 20x19, 1013x1014, 1014x1013, S_1xH_2*[1] , H_2xS_1*, $Vr_{35}xM_2$*, M_2xVr_{35}* and 10 tetrahybrid forms of the silkworm *Bombyx mori* L. – (19x1013)x(20x1014), (1013x19)x(1014x20), (20x1014)x(19x1013), (1014x20)x(1013x19), (19x20)x(1013x1014), (1013x1014)x(19x20), (M_2xH_2)x(CH_1xU_1)*, (CH_1xU_1)x(M_2xH_2)*, (G_2xB_2)x(KK_1xH_1)*, (KK_1xX_1)x(G_2xB_2)*.

The incubation of the eggs and the rearing of the silkworms were carried out in previously prepared specialized rooms under established temperature-humidity conditions and feeding regime (according to Ovesenska & Panayotov, 1991). The cocoons, obtained by the technology of replaceable bedding were picked on the 9-10th day from the mass climb of the silkworms on the mountages, sorted and dried in an electric dryer under standard conditions.

The cocoons are sorted according to BDS 20-52-76/1977, being divided into "An-ras" (I, II, III categories), double cocoons and defective cocoons. The nature of the formation of cocoons from two or more silkworms does not allow the application of standard technologies for unwinding the cocoons, the harvested raw material is of much lower quality and this has a negative impact on the formation of the purchase price. For this reason, the presence of double cocoons in the batches is undesirable.

From a total of 39,508 cocoons obtained from all breeds and hybrids, 35,186 cocoons, determined as good-quality, were differentiated into three groups – with violet, intermediate and yellow fluorescence. For this purpose, an ultraviolet lamp with a filter passing UV rays in the range 254-365 nm was used. The content of good-quality cocoons was established as a percentage of the total number of cocoons for each breed or hybrid.

The obtained data were systemized and processed with the respective modules of STATISTICA software of StatSoft and Microsoft Excel 2012.

[1] *The specified hybrid forms were created in the Scientific Center on Sericulture, Vratsa

Results and discussion

Tables 1, 2 and 3 present data differentiated by fluorescence for the content of good-quality cocoons in breeds, di- and tetrahybrids of *Bombyx mori* L, respectively.

From the data presented in Table 1, it can be seen that at breeds the percentage of good-quality cocoons varies between 82.25% – 95.28% in total for all fluorescent groups. The lowest value is in the violet group, at breed 1, which has 13.03% good-quality cocoons less than the breed with the highest percentage (breed 4K, in the intermediate group).

Data averaged across all breeds showed the highest mean percentage (90.38%) of good-quality cocoons in the intermediate fluorescence group. They are superior than the group of violet (87.63%) and yellow (88.93%) by 2.75 and 1.45%, respectively. Of the violet-fluorescent cocoons, the highest % of good-quality cocoons (92.41%) is observed in breed 1013, and in the case of yellow – breed 4K (91.29%), the difference between the two being 1.12%. In the cocoons with yellow fluorescence, the lowest variation of the traits was observed – from 0.01% to 5.67%, while in the cocoons with violet and intermediate they were from 0.17% to 10.16% and from 2.28 % to 8.39% (Table 1).

The data presented in Table 2 show that the proportion of good-quality cocoons formed by the mulberry silkworm dihybrids, analyzed in the present study varies from 83.05% in

Table 1. Content of good-quality cocoons (%) in breeds

N	Breed	Violet cocoons fluorescence			Intermediate cocoons fluorescence			Yellow cocoons fluorescence		
		Total cocoons (no.)	An-ras (no.)	x̄±SE (%)	Total cocoons (no.)	An-ras (no.)	x̄±SE (%)	Total cocoons (no.)	An-ras (no.)	x̄±SE (%)
1.	**1**	2 597	2 136	82.25±3.17	3 111	2 774	89.17±4.10	3 557	3 170	89.12±3.57
2.	**3**	716	615	85.89±12.57	946	822	86.89±11.78	663	588	88.69±8.79
3.	**4K**	994	903	90.85±12.97	1 250	1 191	95.28±11.50	505	461	91.29±4.81
4.	**1A**	2 707	2 231	82.42±5.16	2 928	2 641	90.20±3.64	1 585	1 357	85.62±3.80
5.	**19**	6 427	5 912	91.99±5.66	–	–	–	–	–	–
6.	**1013**	7 325	6 769	92.41±5.07	–	–	–	–	–	–
7.	**20**	–	–	–	–	–	–	11 709	10 471	89.43±6.04
8.	**1014**	–	–	–	–	–	–	12 366	11 060	89.44±5.42
Total		**20 766**	**18 566**	**x̄=87.63±2.66**	**8 235**	**7 428**	**x̄=90.38±1.51**	**30 385**	**27 107**	**x̄=88.93±3.22**

Table 2. Content of good-quality cocoons (%) in dihybrids

N	Hybrid	Violet cocoons fluorescence			Intermediate cocoons fluorescence			Yellow cocoons fluorescence		
		Total cocoons (no.)	An–ras (no.)	x̄±SE (%)	Total cocoons (no.)	An–ras (no.)	x̄±SE (%)	Total cocoons (no.)	An–ras (no.)	x̄±SE (%)
1.	**20x1014**	–	–	–	–	–	–	2 029	1 833	90.34±8.80
2.	**1014x20**	–	–	–	–	–	–	1 386	1 264	91.20±10.08
3.	**19x1013**	2 757	2 487	90.21±718	–	–	–	–	–	–
4.	**1013x19**	2 489	2 223	89.31±7.20	–	–	–	–	–	–
5.	**19x1014**	719	640	89.01±3.37	1 421	1 266	89.09±6.50	821	755	91.96±3.31
6.	**1014x19**	227	216	95.15±9.71	651	629	96.62±5.04	452	431	95.35±3.81
7.	**1013x20**	452	423	93.58±2.54	919	849	92.38±6.51	629	594	94.44±5.55
8.	**20x1013**	143	136	95.10±7.21	414	390	94.20±7.60	437	423	96.80±14.16
9.	**19x20**	1 185	1 030	86.92±2.75	2 430	2 210	90.95±5.30	1 018	956	93.91±3.71
10.	**20x19**	711	664	93.39±4.69	1 427	1 334	93.48±8.48	804	755	93.91±9.18
11.	**1013x1014**	1 798	1 556	86.54±4.28	2 618	2 384	91.06±4.62	1 477	1 353	91.60±2.57
12.	**1014x1013**	339	309	91.15±4.23	836	784	93.78±6.60	550	531	96.55±6.69
13.	**S_1xH_2**	820	681	83.05±16.77	941	856	90.97±18.57	719	696	96.80±21.62
14.	**H_2xS_1**	559	478	85.51±8.67	497	480	96.58±8.36	336	334	99.40±8.85
15.	**$Vr_{35}xM_2$**	961	864	89.91±20.15	156	151	96.79±6.43	–	–	–
16.	**M_2xVr_{35}**	506	443	87.55±6.42	394	382	96.95±4.81	431	429	99.54±9.52
Total		**13 666**	**12 150**	**x̄=89.74±2.74**	**12 704**	**11 715**	**x̄=93.32±2.50**	**11 089**	**10 354**	**x̄=94.75±2.52**

the violet-fluorescent cocoons of dihybrid C1xX2 to 99.40% in the yellow-fluorescent cocoons of the same hybrid, and the difference between the two groups of cocoons being 16.35%.

Among the violet-fluorescent with the highest rate (95.15%) of good-quality cocoons, dihybrid 1014x19 stands out, which has up to 12.10% higher percentage of good-quality cocoons compared to the other dihybrids in the group. In the group of intermediates with the highest value of the characteristic is dihybrid M_2xVr_{35} (96.95%), where the differences with the others are in the range from 0.16% to 6%. Overall, the yellow-fluorescent fraction had the highest mean percentage (94.75%) of good-quality cocoons, surpassing the mean percentage of intermediate (93.32%) and violet (89.74) by 1.43% and 5.01%, respectively %) (Table 2). Superiority of the cocoons with yellow fluorescence compared to those with violet has also been established by a number of other authors (Ajisawa, 1968; Keping et al., 1988; Zhengcheng et al., 2002) and the mentioned results are explained by the relatively stronger polarity and hydrophilicity of the yellow-fluorescent pigments.

The analysis of the data presented in Table 3 on the percentage of good-quality cocoons (*an-ras*) at the different fluorescent groups of the tetrahybrids shows a variation of the trait similar to that presented in the previous two tables, with the values in the range of 82.59% (1013x19/1014x20, violet fluorescence) to 98.51% (G_2xB_2/KK_1xH_1, yellow fluorescence). The reported difference 15.92% is 2.89% more and 0.43% less than the differences between the lowest and the highest value of the trait in breeds and dihybrids, respectively. The cocoons with yellow fluorescence are characterized by the highest average % *an-ras* (95.34%). They were 2.02% and 6.18% higher average proportion of good-quality cocoons than that of intermediate and violet, respectively (Table 3). According to Zhang et al. (2010) yellow-fluorescent cocoons have better silken unwinding than violet cocoons and cocoon fluorescence is heritable. Therefore, using breeding methods, the production of more individuals forming yellow-fluorescent cocoons will increase the percentage of good-quality cocoons.

The data on Table 4 show that the studied factors have a statistically significant influence on the analyzed trait. Both the breed (hybrid) and the type of silk sheath fluorescence

Table 4. Analysis of variance of effect of the following traits on the percentage of good-quality cocoons

Sources of variation	df	F	P
Breed/Hybrid	33	2.39	***
Fluorescence	2	19.3	***
Breed/Hybrid and Fluorescence	36	0.90	–

*** $p \leq 0.001$

Table 3. Content of good-quality cocoons (%) in tetrahybrids

N	Hybrid	Violet cocoons fluorescence			Intermediate cocoons fluorescence			Yellow cocoons fluorescence		
		Total cocoons (no.)	An-ras (no.)	x̄±SE (%)	Total cocoons (no.)	An-ras (no.)	x̄±SE (%)	Total cocoons (no.)	An-ras (no.)	x̄±SE (%)
1.	**19x1013** **20x1014**	1 490	1 271	85.30±1.90	2 368	2 126	91.09±2.38	1 735	1 607	92.62±2.55
2.	**1013x19** **1014x20**	425	351	82.59±3.78	809	738	91.22±5.34	310	285	91.94±4.31
3.	**20x1014** **19x1013**	449	385	85.75±2.19	1 778	1 652	92.91±7.92	577	527	91.33±3.87
4.	**1014x20** **1013x19**	535	481	89.91±3.82	1 019	948	93.03±6.00	698	655	93.84±3.36
5.	**19x20** **1013x1014**	240	214	89.17±5.06	375	345	92.25±10.91	327	314	96.02±15.95
6.	**1013x1014** **19x20**	218	209	95.87±5.63	434	412	94.93±5.12	187	183	97.86±15.49
7.	M_2xH_2 CH_1xU_1	297	270	90.91±7.67	790	734	92.91±4.11	346	339	97.98±9.15
8.	CH_1xU_1 M_2xH_2	328	295	89.94±10.05	286	270	94.41±11.63	189	183	96.83±4.43
9.	G_2xB_2 KK_1xH_1	619	553	89.34±17.45	116	110	94.83±5.27	134	132	98.51±6.89
10.	KK_1xH_1 G_2xB_2	475	441	92.84±25.30	399	389	97.49±22.63	28	27	96.43±1.57
Total		**5 076**	**4 470**	**x̄=89.16±2.41**	**8 374**	**7 724**	**x̄=93.32±2.50**	**4 531**	**4 252**	**x̄=95.34±1.90**

have a highly reliable influence (p≤0.001) on the variation of the percentage content of good-quality cocoons. While the interaction between the two factors has no effect. Zhonghuai et al. (1997) and Aihua et al. (2005) investigated the relationship between cocoon fluorescence type and breed, hybrid, sex, mounting environment and fluorescence inheritance pattern. Genetic analyzes show that cocoon fluorescence is closely related to and influences the quality of the silk cocoon formed.

The summary analysis of data on the percentage of good-quality cocoons (*an-ras*) in fluorescence-differentiated cocoons of the studied breeds, di- and tetrahybrids showed the lowest trait variation among breeds, with the difference between the lowest and highest trait values is 3.32% and 2.89% lower compared to di- and tetrahybrids, respectively.

The highest percentage of an-ras (99.40%) was obtained from the individuals with yellow fluorescence of the cocoons in dihybrid X2xC1, and the lowest (82.25%) – with violet fluorescence in breed 1. Investigating the phenotypic characteristics of the genetic resources, Petkov et al. (2006) found that the percentage of good-quality cocoons obtained from breeds grown in Bulgaria ranges from 85.39 to 96.21%.

In the breeds with the highest average % of good-quality cocoons, the group of intermediate-fluorescent cocoons (90.38%) stands out, while in hybrid forms (di and tetra) – the group of cocoons with yellow fluorescence (94.75% and 95.34%).

In general, with the highest average % of good-quality cocoons (95.34%), the group of yellow-fluorescent cocoons in the tetrahybrids is distinguished, which superior the other values averaged by fluorescent groups by 0.59% to 7.71%. With the lowest average share (87.63%) of good-quality cocoons, the fractions with violet fluorescence formed by the breeds are characterized.

The results obtained by us confirm the established relationship between ultraviolet fluorescence and the technological qualities of cocoons (Adsizawa, 1968, Aoki et al., 1986; Chang & Nahm, 1988; Ajisawa, 1998; Yu et al., 2008; Panayotov, 2014; 2016; Panayotov & Guncheva, 2015) and enrich the scientific information regarding the influence of the type of fluorescence on the quality of production.

Conclusions

In 69.41% of all breeds and hybrids of the silkworm included in the study, more than 90% content of good-quality cocoons was observed. In the breeds, the percentage is 37.5%, while for di- and tetrahybrids it is significantly higher – 76.92% and 76.67%, respectively.

The yellow-fluorescent cocoon group was characterized by the highest mean percentage of good-quality cocoons in both the dihybrid (94.75%) and tetrahybrid (95.34%) forms of the mulberry silkworm.

The type of fluorescence of the silk sheath, as well as the breed (hybrid), have a highly reliable influence (p≤0.001) on the variation of the percentage of good-quality cocoons.

This gives us reason to believe that the application of correct and purposeful methods of selection and breeding by type of the fluorescence the percentage of good-quality cocoons in the production can be increased significantly.

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