Multidirectional Activities for Gene Pool Conservation in GCEARS-PSP

Daniel Severus DEZMIREAN¹, Liviu Alexandru MARGHITAȘ¹, Otilia BOBIȘ², Adriana Cristina URCAN¹, Horatiu DEZMIREAN³, Claudia PAȘCA¹, Adela Ramona MOISE^{1*}

¹Department of Apiculture and Sericulture, University of Agricultural Science and Veterinary Medicine, 3-5 Mănăștur Street, 400372 Cluj-Napoca, Romania

²Life Sciences Institute “King Michael I” of Romania, University of Agricultural Sciences and Veterinary Medicine, 3-5 Mănăștur Street, 400372 Cluj-Napoca, Romania

³Department of Horticulture, University of Agricultural Science and Veterinary Medicine, 3-5 Mănăștur Street, 400372 Cluj-Napoca, Romania

*corresponding author: adela.moise@usamvcluj.ro

Bulletin UASVM Animal Science and Biotechnologies 75(1)/ 2018

Print ISSN 1843-5262; Electronic ISSN 1843-536X

DOI:10.15835/buasvmcn-asb: 000218

Abstract

The Global Centre of Excellence for Advance Research in Sericulture and Promotion of Silk Production (GCEARS-PSP), recognised by ISC since 2014, was created with the main purposes: development of advanced research in sericulture and diagnostic services using modern techniques, achievement of a gene pool of different silkworms races and promotion of silk production. The objective of this study was to characterize the races from the GCEARS-PSP, in order to conserve the most valuable and resistant ones. This characterisation is a part of GCEARS-PSP's strategy for the revival and development of sericulture and silk production in Romania. The GCEARS-PSP represents an important point of reference for Romanian sericulture. The experimental data described showed a good prospective for a proper functioning of the research centre and the obtained results are promising for the next lines of research.

Keywords: *conservation, GCEARS-PSP, silkworm, breeds*

INTRODUCTION

The Global Centre of Excellence for Advance Research in Sericulture and Promotion of Silk Production (GCEARS-PSP), recognised by ISC since 2014, was created with the main purposes: development of advanced research in sericulture and diagnostic services using modern techniques, achievement of a gene pool of different silkworms races and promotion of silk production.

GCEARS-PSP works in UASVM CN with the main aim of Romanian sericulture revival.

Multidirectional approaches for achieving the goals are:

Analyze the sericulture revival's possibilities in Romania;

Standardization of phenotypic characteristics of autochthones races and hybrids and theirs exploitation in MSF (familial sericulture module);

Identification of the areas with sericulture potential in Romania;

Creation of new models for sericulture farms establishment, having a good practical applicability in sericulture entrepreneurship;

Real consultancy and support for the farmers;

Analyze the silkworm's and mulberry's pathogens, using classical and specialized laboratory techniques, including the molecular genetics ones.

The objective of this study was to characterize the races from the GCEARS-PSP, in order to

conserve the most valuable and resistant ones and further to create the next generations of hybrids. This characterization is part of GCEARS-PSP's strategy for the revival and development of sericulture and silk production in Romania.

MATERIALS AND METHODS

Bombyx mori races with white and color silk cocoons from GCEARS-PSP, and with different country origins, were studied for the main race parameters.

During trials, the larvae samples (200 for every race) have been bred under controlled temperature and humidity conditions, using a well knowing and already standardized method (Mărghitaş, 1992; Mărghitaş, 1995; Mărghitaş *et al.*, 2003); the measurements of followed parameters (incubation and hatching of the silkworm eggs, the evolution of the larvae stages, and the biological indices of the crude cocoons) were done on 20 randomized picked up larvae.

The most important materials used in experimental breeding organization are:

- Mulberry plantation, which provides the silkworm food – mulberry leaves; the mulberry leaves from UASVM CN plantation were used;
- The room for silkworm's experimental series;
- Growing modules (MSF- Familial Sericulture Modules);
- Paper and cocoons making materials.

A series of work methods were applied during silkworm's breeding, everyone having important and direct implications in achieving the final goal of experimental trails, meaning the obtainment of high quality and quantity of cocoons. The parameters of temperature and humidity were registered three times per day, and the conditions established before (Bojan *et al.*, 2007) were respected.

The determination of the main parameters were developed using recognized methods (Dezmirean, 2013; Bentea, 2006). Similar studies regarding biological parameters of *Bombyx mori* development were done by Hussain *et al.*, 2011.

RESULTS AND DISCUSSIONS

The main characteristics followed in the experimental year 2017 were: incubation and hatching of the silkworm eggs, the evolution of the larvae stages, and the biological indices of the crude cocoons with different origin. There are just

a few scientifically reports regarding the European breeds of silkworms, but we have to mention the study of Tzenov *et al.* (2015), which demonstrated the good productive characters values in Bulgarian x Italian hybrids.

All results were statistically processed and are in the range of race parameters, demonstrating that the gene pool of *Bombyx mori* is valuable for the next experimental research and suitable for breeders supply.

Hatching percent

The hatching percent $[(\text{number of hatching eggs} \times 100) / \text{number of eggs/hatching}]$ is an important breed character; it characterizes the race and has an important role in selection and amelioration of silkworm breeds and varieties. In table 1 the results regarding the prolificacy and hatching percent of 24 pure races and hybrids are presented; table 2 summarizes the statistical parameters of its.

The biggest number of eggs per hatching was registered at Chinese race P4/T (668) and the smaller one at the Russian one US4 (512), but the hatching percent depends on the number of hatching eggs, as well.

Anyway, the highest hatching percent at Romanian breeds was registered at AO₃₃ race, even if the average of eggs/number was the smallest one. The prolificity and hatching percent are characters that were studied by other authors at Romanian breeds and hybrids. Comparing these data (Benţea, 2006), we can state that only few differences are registered regarding the hatching percent: at AB a difference of 1.45%, at AC₂₉ a difference of 4.85% and at B₇₅ -0.94%.

With such a little difference of percentage, the hatching percent represents one of the most stable race characters in silkworm characterization, and once again we can state that is one of the selection criteria in amelioration or hybrid's obtainment. This is hardened by the variability coefficient which is very low (0.39 to 3.26%), demonstrating at the autochthonous races, as well as the foreign ones, that the experimental groups were homogeneous.

Anyhow, regarding the foreign races and hybrids studied here, the issue of data comparison remains unsolved, due to the lack of scientifically reports; studying these foreign races founded in GCEARS-PSP gene pool, is one of our objectives for the next years.

Table 1. Prolificacy and hatching percent of studied silkworm breeds

Origin	Breeds	Number of eggs/ hatching	Number of hatching eggs	Hatching percent %	Number of unhatching eggs
Romania	AB	627	597	95.22	30
	AC	609	588	96.55	21
	AC ₂₉	608	581	95.56	27
	B ₇₅	529	499	94.33	30
	AO ₃₃	514	500	97.28	14
	S ₇₆	585	550	94.02	35
China	P4/T	668	638	95.51	30
France	Var	610	587	96.23	23
Uzbekistan	B2	604	579	95.86	25
	S9	531	500	94.16	31
	S21	525	502	95.62	23
	S8	589	559	94.91	30
	B1	520	489	94.04	31
Japan	Kempaku	594	555	93.43	39
	C127	665	645	96.99	20
	C122	612	580	94.77	32
Italy	AJ17	604	580	96.03	24
	AJ2	541	510	94.27	31
Rusia	US4	512	489	95.51	23
	SK2	587	549	93.53	38
	V39	654	624	95.41	30
	S17	579	540	93.26	39
Ucrania	U2	624	587	94.07	37
Indonesia	N1	620	579	93.39	41

Table 2. Averages and dispersion parameters calculated for hatching percent at pure breeds and hybrids

Breed/Hybrids	$\bar{X}$	sd	V%	Breed/ Hybrids	$\bar{X}$	sd	V%
AB	95.22	1.76	1.84	B1	94.04	2.28	2.36
AC	96.55	1.23	1.27	Kempaku	93.43	3.04	3.26
AC ₂₉	95.56	2.45	2.57	C127	96.99	2.74	2.89
B ₇₅	94.33	2.95	3.14	C122	94.77	1.53	1.62
AO ₃₃	97.28	3.49	3.26	AJ17	96.03	0.93	0.98
S ₇₆	94.02	1.18	1.23	AJ2	94.27	1.24	1.29
P4/T	95.51	0.37	0.39	US4	95.51	1.60	1.68
Var	96.23	1.68	1.72	SK2	93.53	1.96	2.06
B2	95.86	0.97	1.01	V39	95.41	2.96	2.98
S9	94.16	0.52	0.55	S17	93.26	2.04	2.19
S21	95.62	1.46	1.64	U2	94.07	1.10	1.19
S8	94.91	2.11	2.18	N1	93.39	1.08	1.52

Evolution of larvae stages

For characterize the larvae stage of *Bombyx mori* races and hybrids the main characteristics were the weight of larvae in the Vth instar and the length of it's in the IVth and Vth instars.

The weight of 20 heads/breed or hybrid were determined using an analytical balance; the weight was determined in the 2nd and in the 5th day of the Vth instar and the percent of weight

development was calculated; the results regarding this parameter are presented in figure 1. Due to the fact that the larval stage is pretty long (between 25 and 35 days) and depends by a lot of factors (race/hybrid, food quality and quantity, microclimate conditions), the measurements of weight and length are done two times during the fifth stage of development. The microclimate conditions, especially temperature influence on larvae stages,

Figure 1. Graphical representation of weight development at *Bombyx mori* in Vth instar

Table 3. The average values of the larvae length during IVth and Vth instar

Origine	Breeds	Length (mm)/ IV th stage	Length (mm)/ V th stage	
			2 nd day	5 th day
Romania	AB	47.270 ± 0.133	56.040± 0.789	66.650± 0.828
	AC	46.230 ± 0.544	55.150± 0.312	69.425± 0.696
	AC ₂₉	46.412 ± 0.441	53.199± 0.346	70.250± 0.826
	B ₇₅	44.990± 0.061	56.601± 0.311	67.970± 0.780
	AO ₃₃	46.550 ± 0.122	49.380± 0.211	67.581± 0.305
	S ₇₆	46.430± 0.208	54.271± 0.588	61.919± 0.332
China	P4/T	46.090 ± 0.327	58.110± 0.274	67.752± 0.602
France	Var	49.271± 0.109	54.832± 0.124	68.561± 0.162
Uzbekistan	B2	48.845± 0.657	55.615± 0.215	73.271± 0.823
	S9	48.210± 0.154	54.364± 0.402	72.705± 0.602
	S21	38.031± 0.063	57.020± 0.122	70.130± 0.233
	S8	37.659± 0.056	56.722± 0.128	71.480± 0.616
	B1	45.950± 0.325	58.840± 0.154	73.052± 0.254
Japan	Kempaku	42.580± 0.216	54.952± 0.158	76.381± 0.434
	C127	39.540± 0.119	55.520± 0.111	78.516± 0.525
	C122	46.420± 0.140	55.358± 0.125	71.850± 0.468
Italy	AJ17	47.521± 0.161	56.630± 0.826	74.802± 0.242
	AJ2	48.435± 0.201	54.191± 0.327	70.499± 0.355
Rusia	US4	36.102 ± 0.182	46.916± 0.240	69.396± 0.428
	SK2	46.010± 0.165	55.207± 0.320	69.860± 0.440
	V39	46.555± 0.195	56.590± 0.200	71.554± 0.302
	S17	45.323± 0.128	59.405± 0.412	73.022± 0.409
Ucrania	U2	44.990± 0.208	57.815± 0.234	67.603± 0.509
Indonesia	N1	38.030± 0.054	56.369± 0.124	70.435± 0.268

Table 4. The biological indices of crude cocoons

Origine	Breed	Weight of crude cocoons (g)	Silk incartment (g)	Longitudinal and transversal axes ratio
Romania	AB	1.677 ± 0.023	0.360± 0.011	1.825
	AC	1.495 ± 0.019	0.352± 0.010	1.678
	AC ₂₉	1.586 ± 0.025	0.345± 0.019	2.285
	B ₇₅	1.725 ± 0.051	0.402± 0.015	1.569
	AO ₃₃	1.225 ± 0.021	0.385± 0.011	1.578
	S ₇₆	1.185 ± 0.021	0.342± 0.015	1.728
China	P4/T	1.802 ± 0.033	0.364± 0.011	1.809
France	Var	1.639 ± 0.031	0.442± 0.015	1.812
Uzbekistan	B2	1.705 ± 0.005	0.391± 0.035	1.830
	S9	1.701 ± 0.024	0.342± 0.015	1.827
	S21	1.599 ± 0.015	0.592± 0.017	1.800
	S8	1.662 ± 0.045	0.502± 0.017	1.836
	B1	1.622 ± 0.043	0.360± 0.015	1.845
Japan	Kempaku	1.569 ± 0.034	0.365± 0.005	1.814
	C127	1.917 ± 0.020	0.383± 0.025	1.847
	C122	1.457 ± 0.042	0.523± 0.007	1.768
Italy	AJ17	1.625 ± 0.030	0.401± 0.006	1.828
	AJ2	1.836 ± 0.026	0.353± 0.065	1.829
Rusia	US4	2.066 ± 0.031	0.502± 0.007	1.835
	SK2	1.419 ± 0.032	0.478± 0.017	1.721
	V39	1.581 ± 0.033	0.425± 0.006	1.736
	S17	1.603 ± 0.041	0.347± 0.005	1.815
Ucrania	U2	1.650 ± 0.032	0.351± 0.005	1.924
Indonesia	N1	1.385 ± 0.033	0.342± 0.005	1.865

were seriously investigated by Hussain *et al.* (2011) in Pakistan and Chanda *et al.* (2013) in India, countries with a long history in sericulture.

Other parameter very important for the larvae stage is the length; the measurements were done for 20 larvae from every experimental group, for three times, using an electronic sliding and the result are presented in table 3. The biggest value for this parameter is obtained at the Russian race US4; this fact demonstrate once again, that the hatching percent (which at this race was not the biggest one, having only the smaller number of eggs per hatching) is not directly correlated with the length of larvae's.

Correlation between hatching percent, larvae weight and length in the 5th day of Vth instar were done, and the results showed that the first two parameters are negatively correlated (-0.13), and between the last two ones a positive correlation exist; this result is in accordance with all other authors which studied before these characteristics (Bura *et al.*, 1995; Babu *et al.*, 2001; Bențea *et al.*, 2003; Bențea *et al.*, 2004).

Biological indices of crude cocoons

The most important parameters of crude cocoons for the race's characterization are the weight of crude cocoons, the weight of silk incartment (the weight of cocoons without chrysalides), and the longitudinal and transversal axes of cocoons. These are parameters that have an important role in technological aspects of silk production and the production of raw silk is directed influenced by those (Singh *et al.*, 1990); the longitudinal and transversal axes of cocoons has an metric role in determination of the cocoons shape (Nishioka *et al.*, 2013), which is one of the breed's character in silkworm selection; the shape and the size of cocoons determine the silk quantity and a positive correlation of shell percent with spinning yield was observed by Sonwalkar (2001); the results of experimental trials obtained in 2017 in GCEARS-PSP are presented in Table 4.

Regarding the weight of crude cocoons, the results have ranged from 1.185g (at Romanian S₇₆ race) to 2.066g at US₄ Russian race. The best weight of silk incartment was registered at Uzbekistanian race S₂₁ (0.592g) and the most rounded shape of

cocoons was determined at Romanian B₇₅ race (1.569).

CONCLUSIONS

The GCEARS-PSP represents an important point of reference for Romanian sericulture.

The parameters followed by the research team of GCEARS-PS and presented here are incubation and hatching percent of the silkworm eggs, the evolution of the larvae stages, and the biological indices of the crude cocoons. All these characters are necessary for the characterization of gene pool founded in the custody of the centre, but there are not exhaustive ones, and for this reason more scientifically determinations, including the molecular genetics characterization are needed and have to been done in the near future.

The experimental data described here shows a good prospective for a proper functioning of the research centre and the obtained results are promising for the next lines of research.

REFERENCES

1. Babu MR, Lakshmi H, Prasad J (2001). Expression of hybrid vigour for silk productivity in silkworm, *Bombyx mori* L. Journal of Experimental Zoology, 4: 317- 321.
2. Bențea M (2006). Caracterizarea unor hibrizi de viermi de mătase (*Bombyx mori* L.) crescuți în zona Transilvaniei. Teză de doctorat. USAMV CN.
3. Bențea M, Mărghitaș L. Al, Dezmirean D, Matei A, Szendrei I (2003). Research on the prolificity and the hatching of the breed and hybrid combination of *Bombyx Mori* L. Bulletin USAMV, 59: 284.
4. Bențea M, Mărghitaș L. Al, Dezmirean D, Matei A, Vlaic B (2004). Research upon some morphological traits in *Bombyx mori* L. Bulletin USAMV-CN, 60: 268-273.
5. Bențea M, Mărghitaș L. Al, Dezmirean D, Matei A, Vlaic B (2005). Cercetări asupra productivității unor hibrizi ai viermelui de mătase. Bulletin UASVM- CN, 61: 242 – 246.
6. Bențea M, Mărghitaș L. Al, Dezmirean D, Matei A, Vlaic B (2005). Evolution of some morphological traits in *Bombyx mori* L. Proceedings of the XXXX Croatian Symposium on Agriculture Opatija.
7. Bojan C, Mărghitaș L. Al, Dezmirean D, Teleky O, Moise A (2007). Qualitative characters study for silkworm hybrids. Bulletin UASVM- CN, 64:1-2.
8. Bura M, Acatincăi S, Pădeanu I (1995). Viermii de mătase, biologie și creștere, Ed. Helicon, Timișoara.
9. Dezmirean DS (2013). Curs de biotehnologii în apicultură și sericultură. Ed. AcademicPres Cluj Napoca.
10. Hussain M, Khan SA, Naeem M, Aqil T, Khursheed R, Mohsin A (2011). Evaluation of silkworm lines against variations in temperature and RH for various parameters of commercial cocoon production. Hindawi Publishing Corporation, Psyche. Volume 2011, Article ID 145640, 11 pages, doi:10.1155/2011/145640.
11. Mărghitaș L. Al, Dezmirean D, Pașca I (2003). Sericultură, Ed. Mediamira, Cluj- Napoca.
12. Mărghitaș L. Al, Sabău A (1992). Curs de sericultură, Tipo Agronomia Cluj- Napoca.
13. Mărghitaș L. Al (1995). Creșterea viermilor de mătase, Ed. Ceres, București.
14. Matei A (1994). Studiul comportării unor rase de viermi de mătase de proveniență ucrainiană în condițiile României. Rev. de Medicină veterinară și creșterea animalelor, București, 4-5: 2-4.
15. Nishioka T, Mase K, Kajiura Z, Morishima M, Kudoh T (2013). Shape of Silkworm cocoon changes with size in some races. Journal of Insect Biotechnology and Sericology 82: 55-59.
16. Pașca I (2004). Cercetări privind influența condițiilor de mediu din Zona Transilvaniei asupra unor rase și hibrizi românești de viermi de mătase ai dudului (*Bombyx mori* L.) Teză de Doctorat Cluj- Napoca, 2004.
17. Singh T, Sinha Deo SN, Das Mamoni (1990). Heterosis in silk productivity as a breeding index in *Bombyx mori* L. Silk Inform. Bull 3: 1- 3.
18. Sonwalkar TN (2001). Hand Book of Silk Technology, New Age International (P) Limited, New Delhi, 14-21.
19. Subhra C, Lal Mohan S, Nirvan KD, Niharendu BK, Bharat BB (2013). Correlation among Shell Percent, Cocoon yield and reeling parameters of multi x bi cocoons under different agro-climatic conditions of west bengal, India. International Journal of Industrial Entomology, 26: 74-80.
20. Tzenov P, Cappellosza S, Grekov D (2015). Creation and Testing of Silkworm, *Bombyx mori* L. F1 Hybrids between Bulgarian and Italian Breeds. Proceedings of 7th BACSA International Conference "Organic Sericulture – Now and the Future" "ORGASERI" 2015, Sinaia, Romania, 132-137.