

# Scientific-research Center of Georgian Agriculture

## Preservation and improvement of mulberry silkworm genofond breeds

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# Mulberry silkworm genofond

- in 2018 on the base of materials accumulated about history of origin and development of sericulture in Georgia Georgian silk was granted **cultural heritage monument status**.
- Works for preservation and improvement of mulberry silkworm genofond breeds are in progress in the just created laboratory of sericulture, at Tsilkani base of Scientific-research Center of agriculture.
- Genofond stores silkworm breeds which were selected after 1930 (when scientific selection activity was commenced in Georgia) as well as breeds obtained by folk selection. Viability of those breeds is so high that after spreading of silkworm disease “Pebrina” only some breeds of mulberry silkworm were rescued. Irrespective of the fact that they lost industrial significance, thanks to high technological characteristics the breeds are kept in the collection as selection material. Breeds of this group contain::  
“Tetraparkiani qartuli” (that is “TQ”), (white cocoon Georgian), “Kakhuri Tetri” (“Kachetian white”) that is “Telavi”, “Kakhuri Mtsvane”, (“Kakhtian Green”), Kakhuri Narinjisperi” (“Kakhetian Orange-color”), “Tergis Mtsvane” (“Tergi Green”) (N.Shavrov, 1888). Alongside with these breeds the genofond keeps breeds: Indian, Japanese, Chinese, Uzbek, Azerbaijanian, Polish et al).
- From modern Georgian breeds the following ones should be emphasized: Mziuri group -10 breeds. Dighmuri group – 8 breeds and breeds Tao1 and Tao2 labeled in grain phase

# Mulberry silkworm breeding and leaf preparation



# Grain preparation



# Results of Mziuri group silkworm feeding, 2017-2018

| Characteristics                  | years | breeds |      |      |        |      |       |        |       |
|----------------------------------|-------|--------|------|------|--------|------|-------|--------|-------|
|                                  |       | Mz-1   | Mz-2 | Mz-3 | Hereti | Dina | Elita | AISI–2 | Daisi |
| Grain number per gram, pieces    | 2017  | 1553   | 1580 | 1610 | 1610   | 1580 | 1553  | 1635   | 1600  |
|                                  | 2018  | 1553   | 1580 | 1610 | 1610   | 1580 | 1553  | 1635   | 1600  |
| Worm number per gram, pieces     | 2017  | 2323   | 2335 | 2460 | 2460   | 2323 | 22335 | 2470   | 2400  |
|                                  | 2018  | 2323   | 2335 | 2460 | 2460   | 2323 | 22335 | 2470   | 2400  |
| grain vivification,%;            | 2017  | 98.0   | 97.0 | 98.0 | 98.0   | 97.0 | 97.0  | 97,0   | 96,8  |
|                                  | 2018  | 98.0   | 98.0 | 98.0 | 98.0   | 98.0 | 97.0  | 98.0   | 98.0  |
| feeding duration, day;           | 2017  | 33     | 33   | 33   | 32     | 33   | 33    | 32     | 33    |
|                                  | 2018  | 33     | 33   | 33   | 32     | 33   | 33    | 32     | 33    |
| worm viability,%;                | 2017  | 93.0   | 93.5 | 94.7 | 94.0   | 94.0 | 95.5  | 95,5   | 96,0  |
|                                  | 2018  | 95.9   | 95.5 | 96.7 | 95.7   | 96.3 | 96.3  | 97     | 96,5  |
| live cocoon mass, g.;            | 2017  | 2.1    | 2.1  | 2.0  | 2.2    | 2.0  | 2.0   | 2,0    | 2,1   |
|                                  | 2018  | 2.2    | 2.2  | 2.2  | 2.2    | 2.2  | 2.2   | 2,1    | 2,2   |
| shell mass, mg;                  | 2017  | 493    | 491  | 481  | 517    | 460  | 479   | 464    | 484   |
|                                  | 2018  | 546    | 537  | 531  | 553    | 562  | 565   | 535    | 557   |
| silk capacity, %;                | 2017  | 23.0   | 23.0 | 23.2 | 23.5   | 23.0 | 23.3  | 22,8   | 23,3  |
|                                  | 2018  | 24.8   | 24.4 | 24.1 | 25.1   | 25.2 | 25.6  | 25,4   | 25,3  |
| cocoon yield per gram worm, kg;  | 2017  | 4.5    | 4.5  | 4.6  | 4.3    | 4.6  | 4.4   | 4,4    | 4,6   |
|                                  | 2018  | 4.8    | 4.8  | 5.2  | 5.2    | 4.9  | 4.9   | 5.0    | 5.2   |
| inclusive: normal cocoon, %;     | 2017  | 90.0   | 90.0 | 92.0 | 90.9   | 88.7 | 91.5  | 90,0   | 94,6  |
|                                  | 2018  | 89.7   | 91.6 | 92.8 | 91.0   | 90.4 | 91.3  | 91,7   | 90,7  |
| cocoon with twin silkworm,%;     | 2017  | 2.6    | 4.0  | 2.4  | 3.4    | 4.3  | 4.0   | 6,1    | 2,4   |
|                                  | 2018  | 6.1    | 3.9  | 4.0  | 5.6    | 5.8  | 6.1   | 5,3    | 5,8   |
| hollow and defective/infected,%. | 2017  | 6.7    | 6.4  | 5.2  | 5.3    | 6.5  | 4.9   | 4,4    | 4,0   |
|                                  | 2018  | 4.1    | 4.4  | 3.9  | 3.8    | 3.9  | 3.7   | 2,9    | 3,3   |

# Results of Digmuri group silkworm feeding, 2017-2018Y.Y.

| N  | Characteristics                  | years | breeds |      |      |      |      |      |      |      |
|----|----------------------------------|-------|--------|------|------|------|------|------|------|------|
|    |                                  |       | ღ-1    | ღ-2  | ღ-3  | ღ-4  | ღ-5  | ღ-6  | ღ-7  | ღ-8  |
| 1  | Grain number per gram, pieces    | 2017  | 1620   | 1630 | 1645 | 1670 | 1645 | 1645 | 1630 | 1650 |
|    |                                  | 2018  | 1620   | 1630 | 1645 | 1670 | 1645 | 1645 | 1630 | 1650 |
| 2  | Worm number per gram, pieces     | 2017  | 2260   | 2260 | 2258 | 2255 | 2340 | 2330 | 2250 | 2240 |
|    |                                  | 2018  | 2260   | 2260 | 2258 | 2255 | 2340 | 2330 | 2250 | 2240 |
| 3  | grain vivification,%             | 2017  | 98     | 98   | 97   | 90   | 97   | 98   | 98   | 97   |
|    |                                  | 2018  | 98     | 98   | 98   | 97   | 97   | 96   | 97   | 95   |
| 4  | feeding duration, day            | 2017  | 30     | 30   | 32   | 31   | 30   | 32   | 32   | 30   |
|    |                                  | 2018  | 30     | 30   | 32   | 31   | 30   | 32   | 32   | 30   |
| 5  | worm viability,%                 | 2017  | 99     | 91   | 97   | 98   | 98   | 96   | 96   | 95   |
|    |                                  | 2018  | 97.2   | 97.8 | 95   | 95.8 | 96.4 | 93.8 | 97.2 | 94   |
| 6  | live cocoon mass, g.             | 2017  | 2.1    | 2.1  | 2.0  | 2.0  | 2.0  | 2.0  | 2.0  | 2.1  |
|    |                                  | 2018  | 2.1    | 2.1  | 2.2  | 2.1  | 2.1  | 2.1  | 2.1  | 2.1  |
| 7  | shell mass, mg.                  | 2017  | 474    | 468  | 448  | 420  | 438  | 449  | 443  | 490  |
|    |                                  | 2018  | 511    | 515  | 543  | 539  | 526  | 536  | 512  | 519  |
| 8  | silk capacity, %                 | 2017  | 22.5   | 22.2 | 22.4 | 21.0 | 22.0 | 22.4 | 22.1 | 23.3 |
|    |                                  | 2018  | 24.3   | 24.5 | 24.6 | 25.6 | 25.0 | 25.5 | 24.3 | 24.7 |
| 9  | cocoon yield per gram worm,kg.   | 2017  | 4.6    | 4.6  | 4.4  | 4.4  | 4.6  | 4.6  | 4.6  | 4.6  |
|    |                                  | 2018  | 4.6    | 4.6  | 4.7  | 4.6  | 4.6  | 4.5  | 4.5  | 4.4  |
| 10 | inclusive: normal cocoon,%       | 2017  | 93     | 91   | 93   | 94   | 94   | 90   | 90   | 86   |
|    |                                  | 2018  | 92     | 91   | 85.7 | 89.3 | 90.4 | 88.1 | 91.6 | 87.8 |
| 11 | cocoon with twin silkworm,%      | 2017  | 4.1    | 2.7  | 3.7  | 3.7  | 3.6  | 5.0  | 5.2  | 8.6  |
|    |                                  | 2018  | 5.5    | 7    | 9.9  | 6.4  | 6.0  | 5.7  | 6.4  | 7.2  |
| 12 | hollow and defective/infected,%. | 2017  | 3.3    | 4.1  | 3.7  | 2.2  | 2.5  | 4.7  | 4.4  | 5.0  |

## Mulberry silkworm feeding results (2013-2018 yy.)

|           | Breeds                           | Grain<br>vivifi-<br>cation, % | Silkworm<br>viability, % | Length of<br>feeding, day | Live cocoon        |                    | silk-<br>capacity,<br>% |
|-----------|----------------------------------|-------------------------------|--------------------------|---------------------------|--------------------|--------------------|-------------------------|
|           |                                  |                               |                          |                           | Cocoon mass<br>g., | shell mass<br>mg., |                         |
| <u>1.</u> | Telavi(2014)                     | 98                            | 93                       | 27                        | 1,9                | 380                | 20.0                    |
| <u>2</u>  | Telavi(2018)                     | 98                            | 95                       | 27                        | 2.0                | 410                | 20.5                    |
| <b>3.</b> | „Tetraparkiani<br>Kartuli“ 2014) | 97                            | 94                       | 27                        | 1,9                | 410                | 21.5                    |
| <b>4</b>  | „Tetraparkiani<br>Kartuli“ 2018) | 97                            | 96                       | 27                        | 2.0                | 435                | 21.7                    |
| <b>5</b>  | Iulduz(2014)                     | 96                            | 96                       | 30                        | 2.1                | 514.3              | 24.5                    |
| <b>6</b>  | Iulduz(2018)                     | 96                            | 97                       | 30                        | 2.1                | 520.0              | 24.7                    |
| <b>7.</b> | Orzu(2014)                       | 96                            | 98                       | 30                        | 2.2                | 530.3              | 24.1                    |
| <b>8</b>  | Orzu(2018)                       | 96                            | 98                       | 30                        | 2.2                | 540,4              | 24.5                    |

Diagram 1. Changes in cocoon mass and silk-capacity of the breeds to be improved  
(2013-2018 yy)

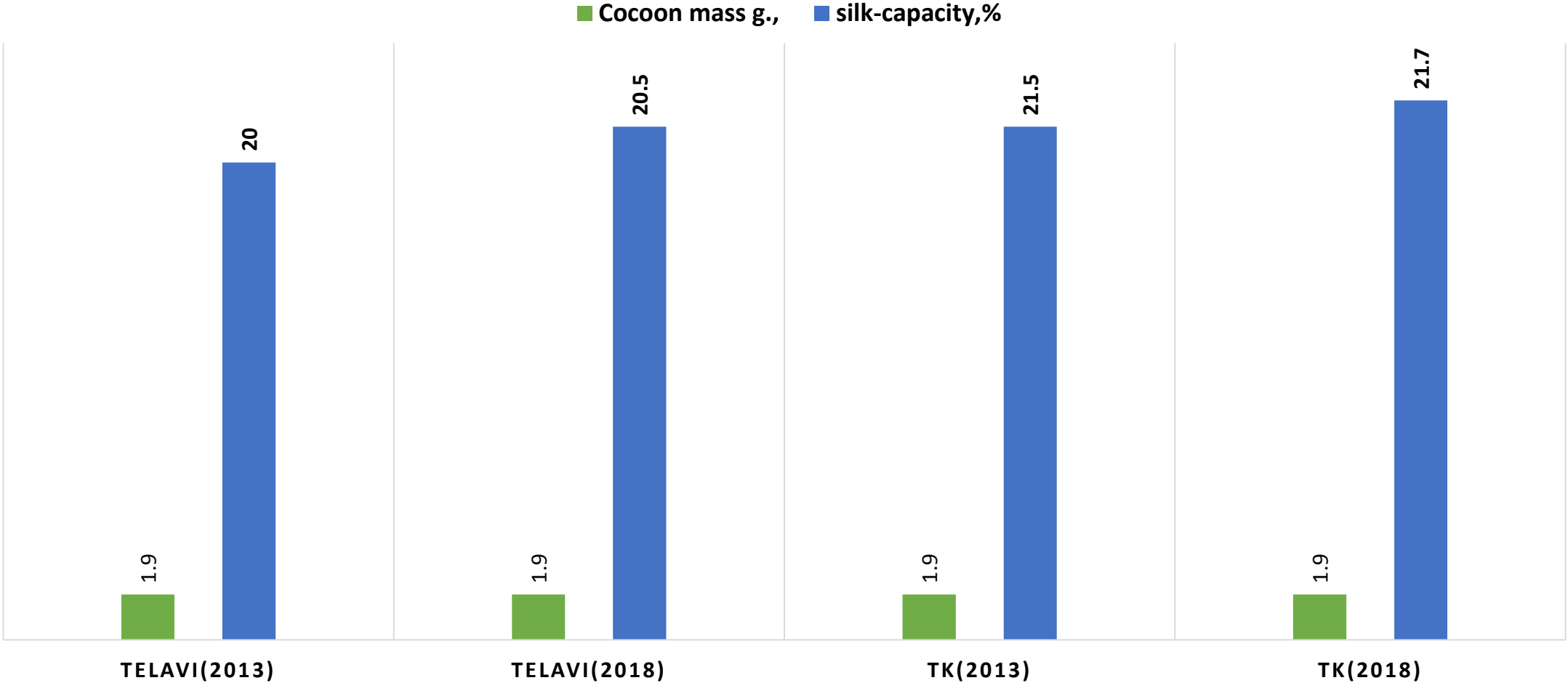


Diagram2. Changes of cocoon shell mass of breeds to be improved  
(2013-2018)

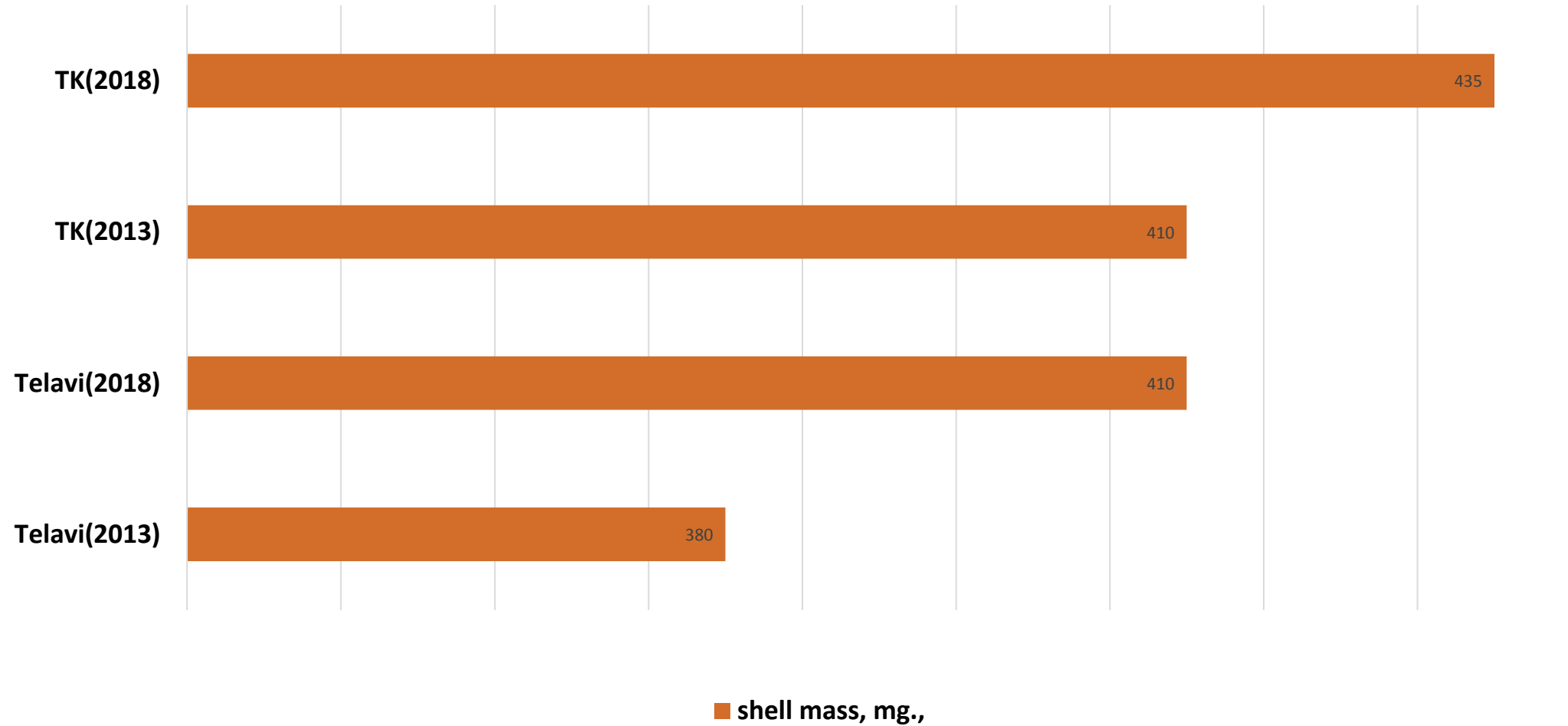
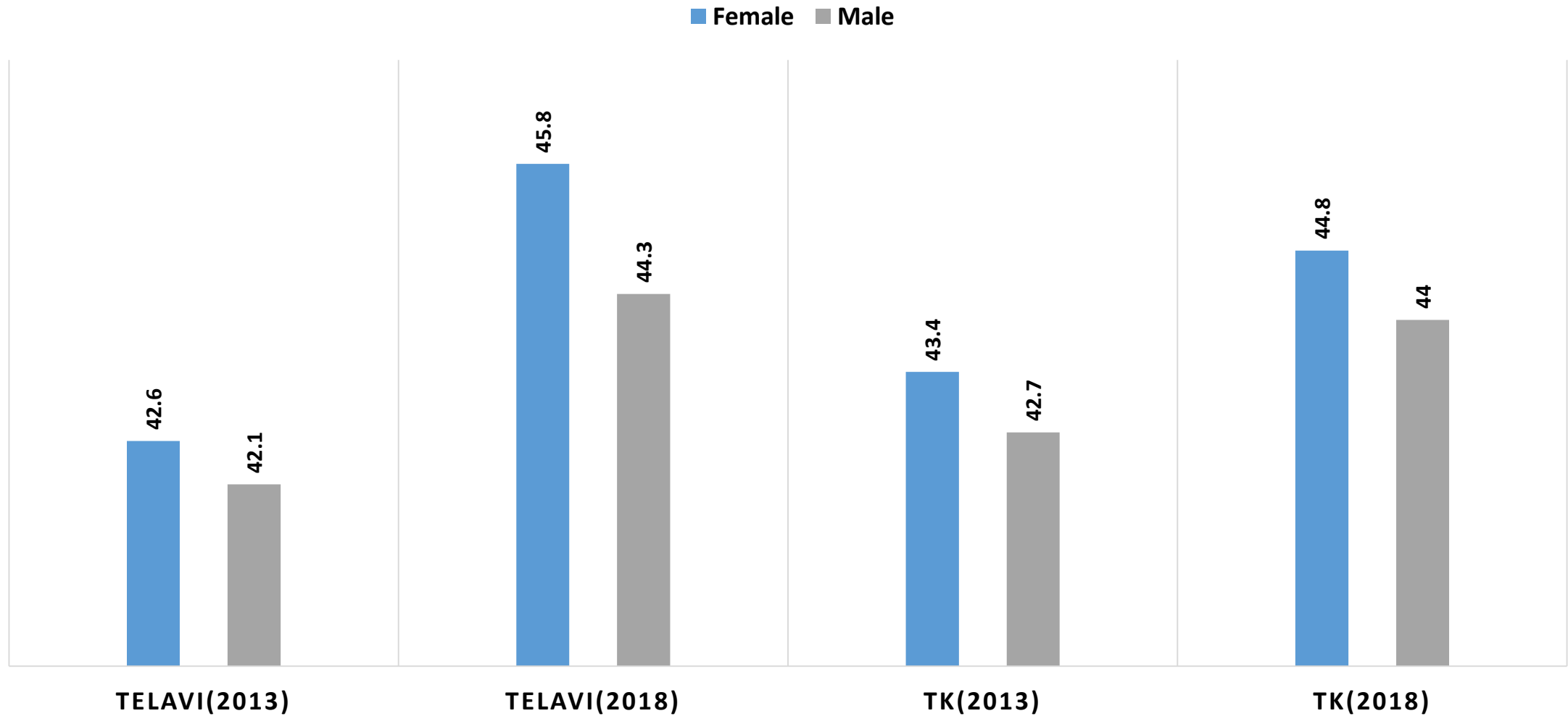


Diagram 3. PProtein rate in silkworm hemolymph according to gender.



**Diagram 4. Silkworm and silk gland ratio according to gender  
(2013-2018)**

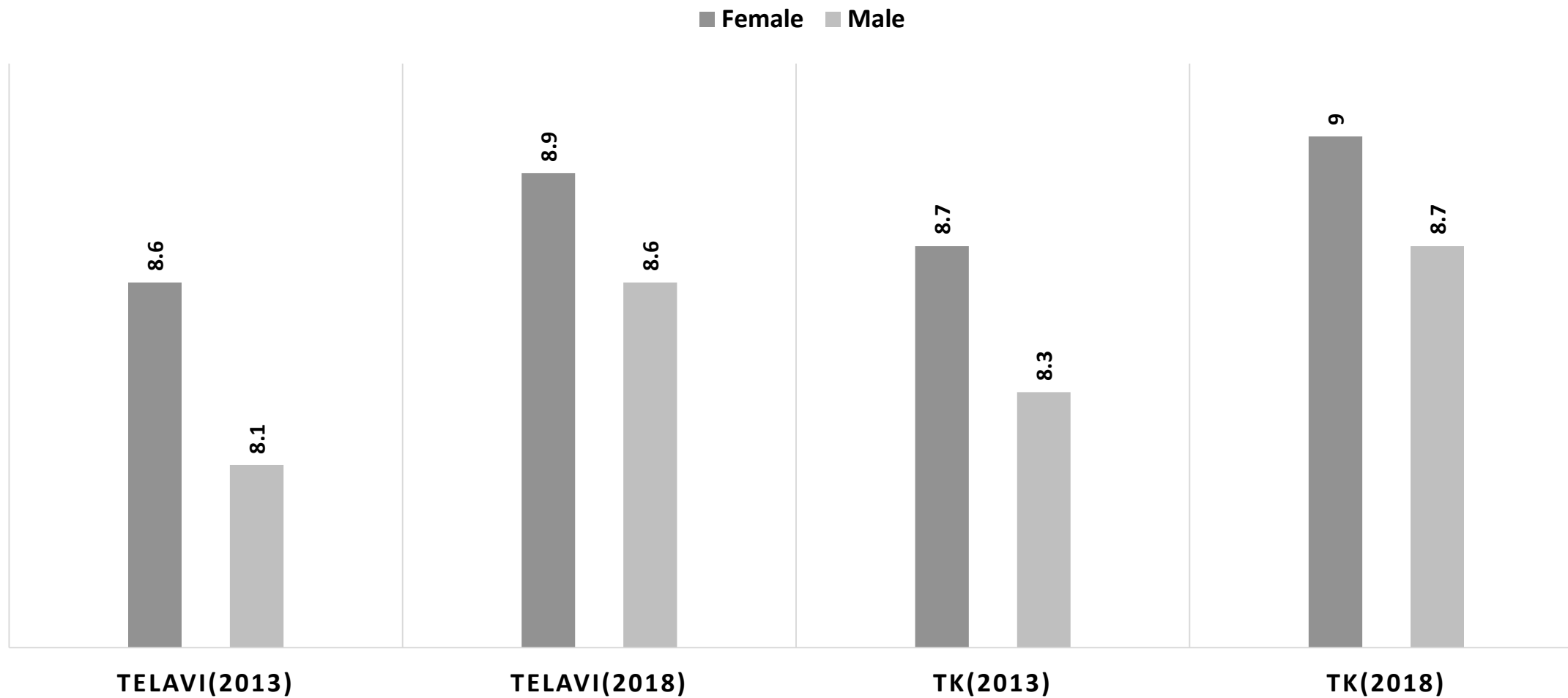
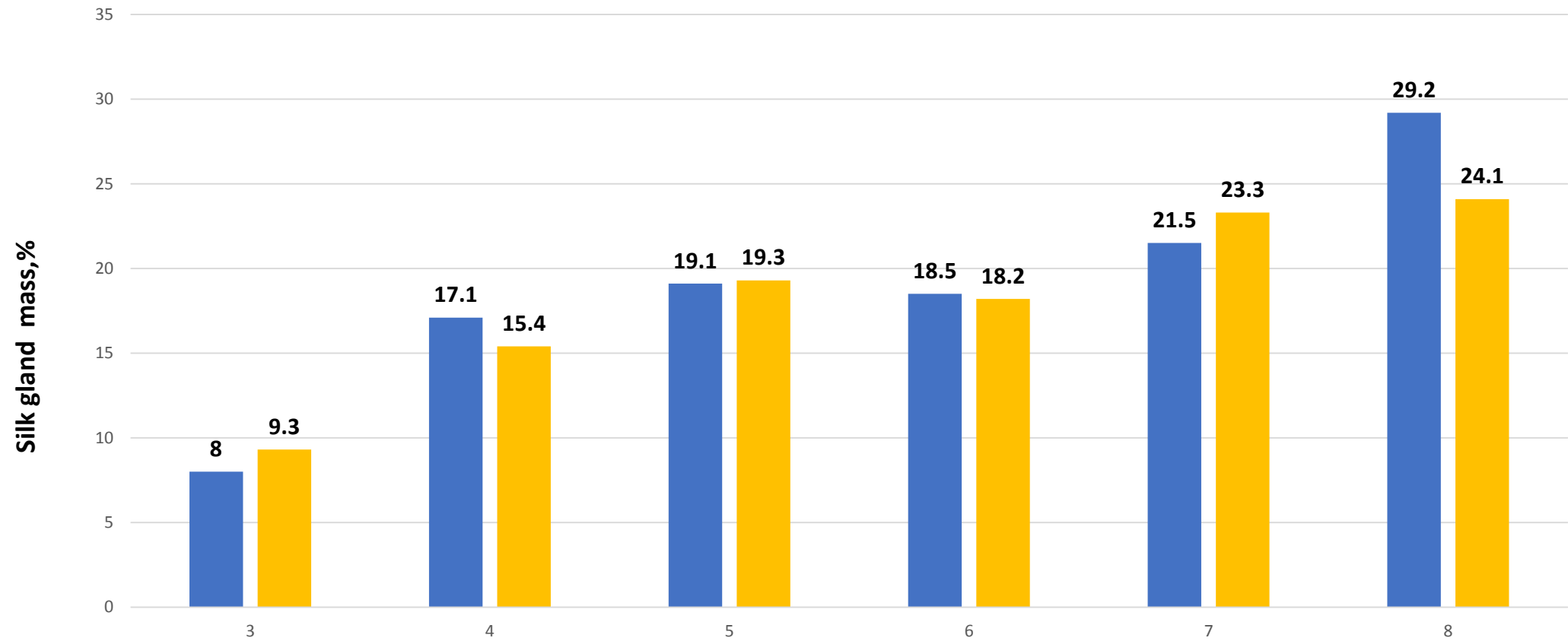


Diagram 5. Growth of silk gland mass in the fifth instar of a worm, according to days and gender.



## Silk worm colorful breeds



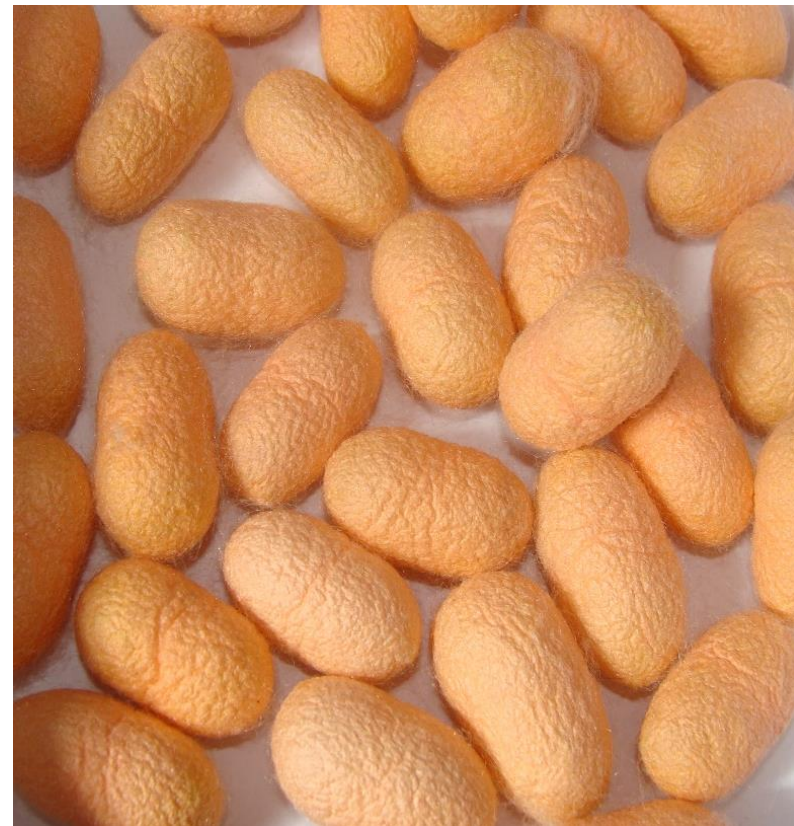
## Kaxuri mtsvane



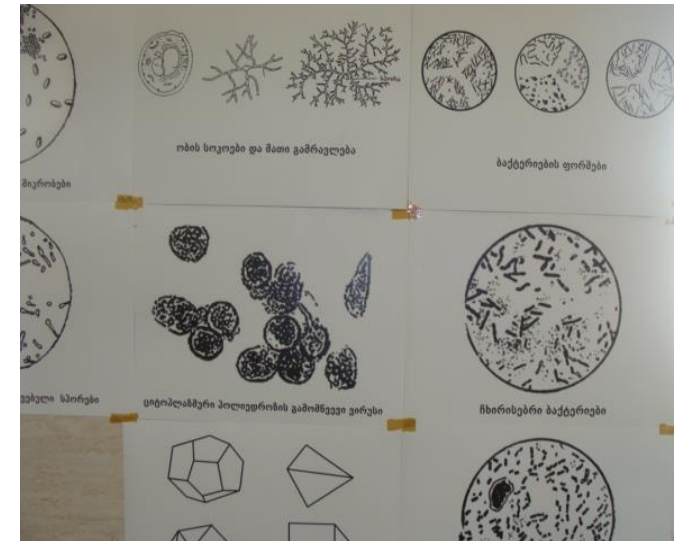
# Orange



# New combination



# Dry moth microanalysis



Researches were performed in connection with identification of mulberry silkworm disease “nuclear polyhedrosis” in early (grain) phase and we revealed DNA causing disease in worm hemolymph as well as in grain.

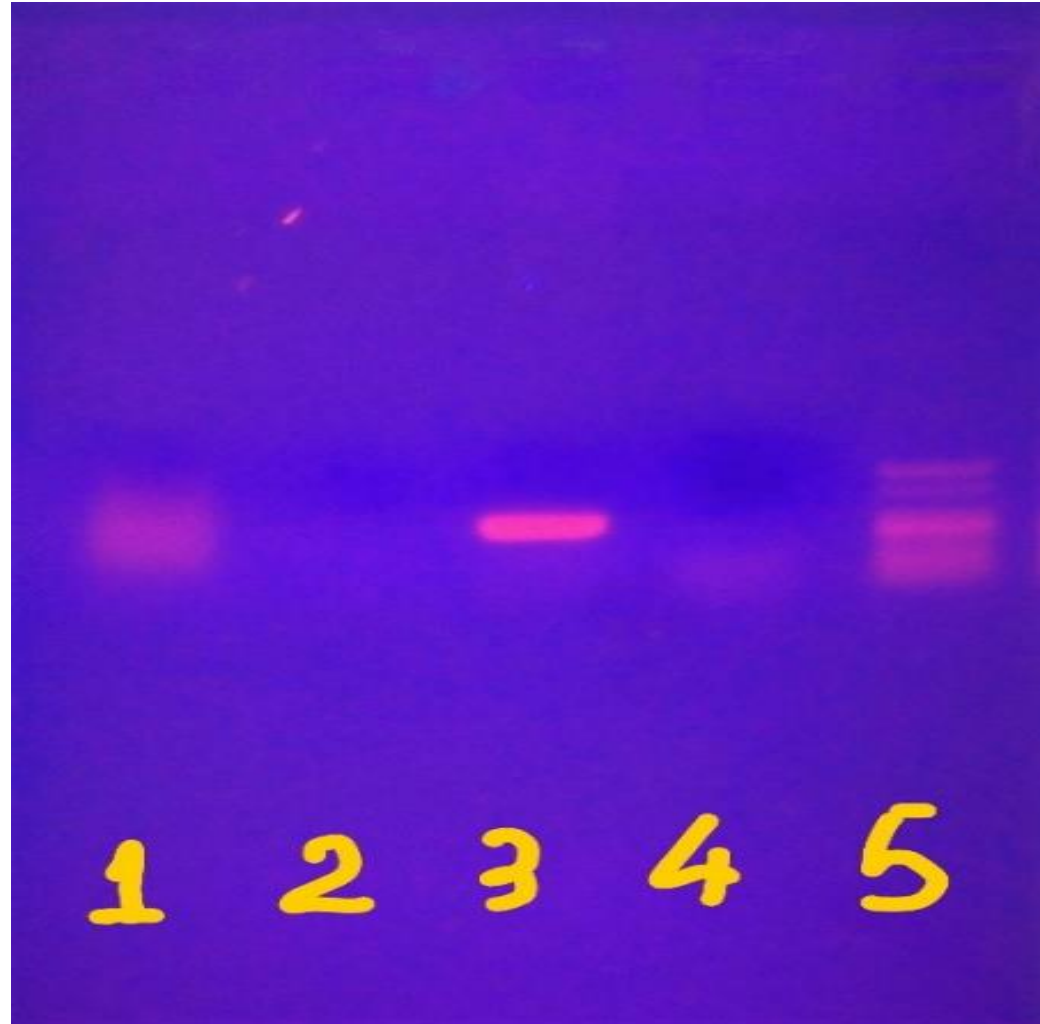
## **Electrophoresis of polyhedrosis virus DNA in 1% agarose gel (arrow shows DNA band)**

- 1. DNA preparation treated by 300 mcg/ml K-proteinase.**
- 2. DNA preparation treated by 500 mcg/ml K-proteinase.**
- 3. Preparation treated by 1% SDS**



## PCR analysis of DNA isolated from grain.

1. DNA of infected grain (isolated by a normal method)
2. DNA of infected grain (dissolved in TE buffer)
3. Virus DNA
4. DNA of healthy grain - control
5. Marker



# Carrying out polymerized chain reaction by BmNPV polyhedrine gene primers

For identification of polyhedrosis virus in infected mulberry silkworm grain by PCR analysis to construe 3 pairs of primers by the program Primer –BLAST.

(<https://www.ncbi.nlm.nih.gov/tools/primer-blast/> ).

To construe primers we took a zone of polyhedrine gene of mulberry silkworm nuclear polyhedrosis virus.

| Oligo Name | Sequence                  | Length |
|------------|---------------------------|--------|
| Ten2       | GCGAGGAACTTGTAGCACCT      | 20     |
| Ten1       | GCGCAAGAAGCACCTAATCG      | 20     |
| OL2        | GGGCGTGTGGGTTTGAG         | 17     |
| OL1        | ACAAGAGGAGAAGCAATGGG      | 20     |
| TB2        | GCTGTCCTCAACAAAACGGG      | 20     |
| TB1        | AACGATACAAATTAACCATCTCGCA | 25     |

## Carrying out polymerized chain reaction by BmNPV polyhedrine gene primers

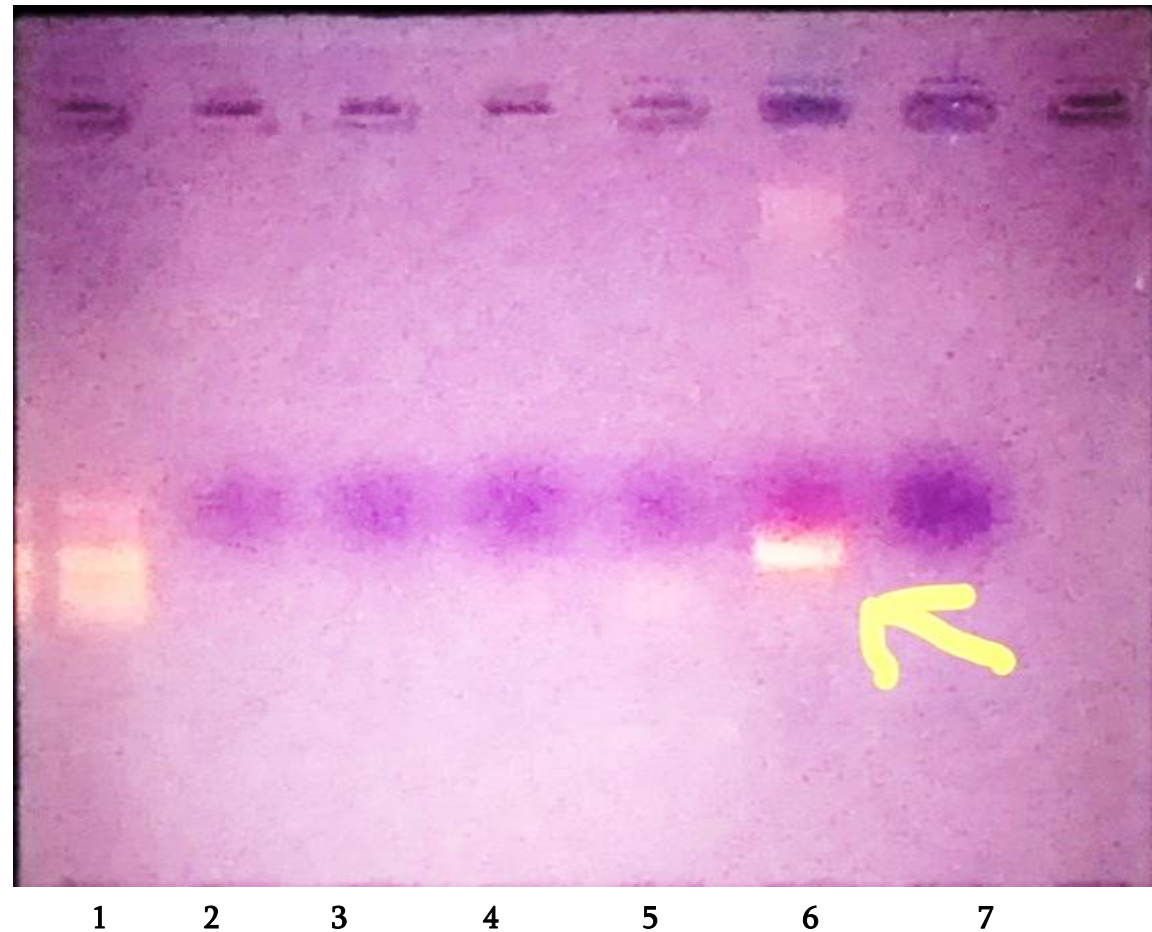
### PCR parameters

1. 95 - 4 min
2. 95 - 30 sec
3. 55 - 45 sec      30 cycle proceeded at the 2, 3, 4.
4. 72 - 3 min
5. 72 - 7 min

DNA marker (DNA Ladder 500 bp); 2-5 primers Ten1-2 and TB1-2;

6. Primer - OL1-2

*Note: virus DNA is not added to control samples 3,5,7.*



Alongside with scientific works annually we used to consuct trial feedings of silkworm and cocoon harvesting in Akhmeta municipality.

### **Silk cocoon harvested in Akhmeta municipality**



## Georgian silk fabrics





**Thank you !**