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Conservation and breeding of natural silkworm (*Bombyx mori* L.) in Indonesia

R Agustarini, L Andadari, Minarningsih and R Dewi

Forest Research and Development Center, Forestry and Environment Research, Development and Innovation Agency, Jalan Gunung Batu No. 5, Bogor, Indonesia 16118

E-mail: retno.agustarini@gmail.com

Abstract. Natural silk farming is a series of silk agro-industry activities from mulberry cultivation, silkworm maintenance, cocoon production and processing, silk spinning and weaving. Many supporting factors for the development of natural silk in Indonesia. However, the natural silk industry is getting worse with high import values. The main problem is the low quality of silkworm egg seeds. The Forest Research and Development Center (P3H) is a government agency authorized for natural silk commodity development. This study aims to describe the efforts of conservation and breeding of natural silkworm in Indonesia. The material used for conservation is the germ plasma silkworms collection owned by P3H. Whereas for breeding activities, silkworms used are produced from conservation activities. Conservation is carried out using eggs of the same type of strain in the germ-plasma, while breeding is done by crossing various types of moths. The result of conservation is the maintenance of 58 germ plasma silkworms. While breeding results are a type of hybrid caterpillar PS 01, which has a production advantage compared to commercial types CS 03. The dissemination of results such as hybrid PS 01, which has a comparative advantage needs to be improved to help overcome the problem of Indonesian silkworms.

1. Introduction

Natural silk farming is a series of silk agro-industry activities that have a long series of activities, including mulberry planting, silkworm maintenance, cocoon production, cocoon processing, silk spinning and weaving [1]. The natural silk farming activities include four main components. Those are: 1) Moriculture (mulberry cultivation for silkworms' feeding); 2) Sericulture (production process from silkworm eggs, cultivation of silkworm, cocoon formation process and cocoons' harvesting); 3) Filature (the process of improving the quality of silk threads into silk fabric also weaving); and 4) Manufacture (the use of silk fabrics into other derivative products, and marketing) [2]. Silkworm has the Latin name *Bombyx mori*. *Bombyx* comes from the name of a type of fiber-producing insect in the Bomycidae family, and *mori* comes from morus, which means mulberry as its food.

Silk was known since 2500 BC in China during the Han dynasty. At that time, silk was very closely related to the culture and traditions of Chinese aristocrats. Because of the beauty and smoothness of silk, silk manufacturing began to be traded to European and Asian countries and then spread to Indonesia. The Japanese first introduced silk in Indonesia in 1927 in Garut Regency, West Java. Then it expanded to South Sulawesi and Yogyakarta around 1947. Several factors support the

development of natural silk in Indonesia, including regional potential, human resource potential, and market potential.

Indonesia is a tropical country that enables the availability of mulberry leaves as silkworm feed throughout the year. The fact is a supporting factor for natural breeding. Mulberry plants 38% affect the production and quality of the cocoon produced. These environmental factors enable the maintenance of silkworms throughout the year so that they are expected to contribute to the world's needs in the form of cocoons and silk threads. In general, rural communities can manage natural silk farming because technology is labor-intensive and straightforward so that it can absorb high labor. Natural silk farming can be the answer to overcome the problem of poverty and can develop people's economy. Silk products have high economic value, not only favored by the residential community but also in foreign markets. Every year there is an increase in market demand for silk products, both domestic and export so that it can be an opportunity for the development of Indonesian silk.

Supporting condition for natural silk development did not automatically make silk cultivation and its related areas grow rapidly in Indonesia. Data showed that the natural silk sector has declined from year to year (Table 1). Indonesia itself is listed not only as of the number 9 silk-producing country in the world but also based on the 2016 Trade Map data, Indonesia is also listed as the importer of silk thread number 63.

Table 1. Information on the Indonesian natural silk industry.

The activities	**Year**	
	2012	**2016**
Production	20 million tons	6 million tons
Imported yarn and silk fabric	US $ 1.06 million	US $ 1.39 million
Imported silkworm	US $ 32,000	US $ 1,000

Source: inserco, trade map, ministry of industry, data processed

Silk products have declined sharply in recent years, with a continuing downward trend. In 4 years, production decreased by around 60%. However, on the other hand, imports of silk yarn and fabric produced very progressive growth. In 2012, the only US $ 1.06 million increased 31% to US $ 1.39 million in 4 years, and there is an increasing trend. This condition illustrates the imbalance between the upstream and downstream sectors in the Indonesian natural silk industry. The upstream silk sector in this country cannot fulfill the increasing needs of the downstream industry.

Silkworm cultivation technology has been developed, especially technology that has simple maintenance and low cost. Nevertheless, this information has not been appropriately disseminated to all silk farmers. Silk farmers still use traditional techniques that are low in production per unit area. So the quality of the cocoon does not appropriate with the industry. As a result, yarn import is still ongoing in some areas. The efforts to increase productivity and quality of cocoons need to be continued, one of them is by searching for silkworm seeds that have high productivity. The Center for Forest Research and Development (P3H), the Agency for Research and Innovation, the Ministry of Environment and Forestry, is a government agency authorized in the field of natural silk commodity development, both research and development. One of the tasks of this research center is to manage the sources of state silkworm seeds in the form of plasma banks and also conduct breeding to obtain superior types of silkworms. The 58 collections of pure silkworms that are owned need to be preserved and developed to be able to overcome the problem of the natural beauty of Indonesia. This research aims to describe conservation efforts and natural silk breeding in Indonesia.

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2. Material and methods

2.1. Material

The research activities consisted of 2 main activities, conservation, and breeding. The material used for conservation activities was the collection of pure silkworms owned by P3H. Whereas for breeding activities, silkworms produced from conservation activities were used.

2.2. Study site

This research was carried out in a natural silkworm laboratory (Figure 1) located at the Forest Research and Development Center, Bogor, Indonesia, on Jalan Kreteg No. 4, Bogor, with an altitude of 220 m above sea level and a daily temperature of 26 - 30 ºC. The total area of the silkworm laboratory is 3,500 m2 at an altitude of 250 meters above sea level. This laboratory is a natural silkworm research laboratory that was established in 1994 to support the development of silkworms. The silkworm maintenance area consists of three rooms, each measuring 4 x 8 m. The laboratory is equipped with cold storage for storing eggs (temperature 5 ºC) and storing pupae and moths (temperature 10 ºC). There is also an egg incubation room with a temperature of 25 ºC.

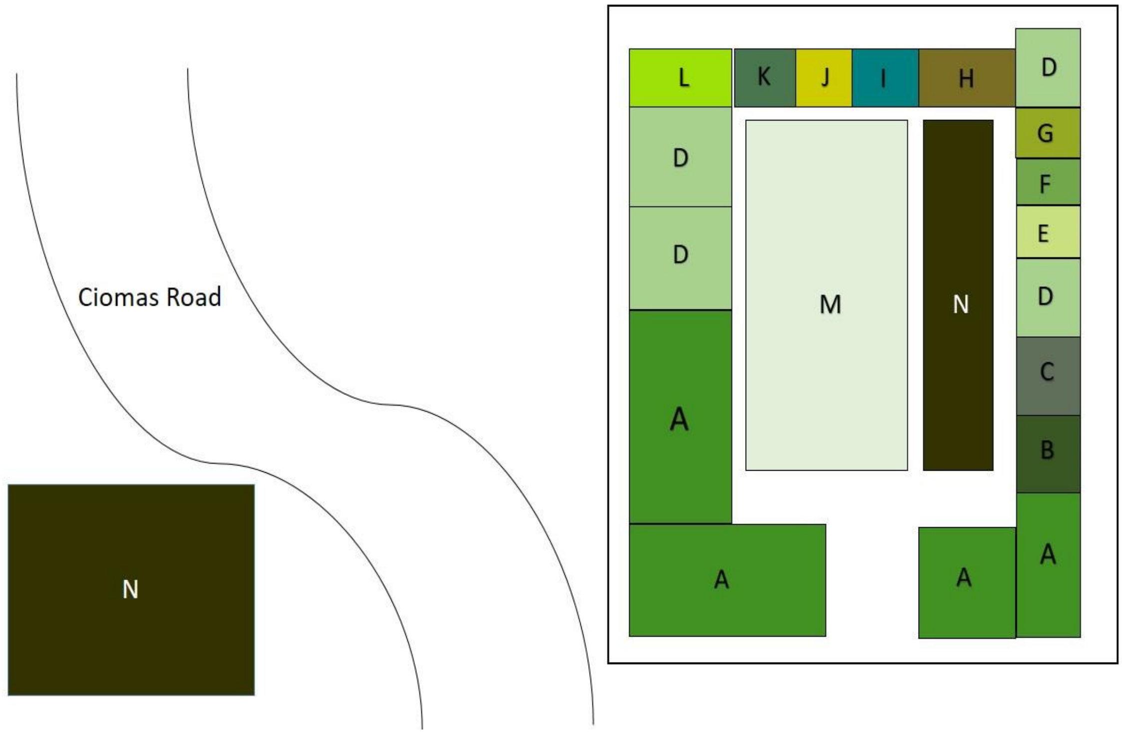

Figure 1. Sketch of silkworm laboratory in FOERDIA.

Remarks

A: Office and researcher workspace	H: Equipment warehouse
B: Egg treatment room	I: Disease examination room
C: Incubation room	J: Kitchen
D: Rearing chamber	K: Cocoon drying room
E: Cold storage	L: Warehouse
F: Generator set chamber	M: Field
G: Medicine and chemicals room	N: Murbei garden

2.3. Methodology

The silkworm's genetic resources are collected in the egg stage from the P3H collection, where it is developed or maintained as a strain or breeding material. During the collection process, to avoid contamination of the main germ plasma bank, disinfection is carried out using a 2% formalin solution

for 5-6 minutes and then washed with water before incubation. The activity carried out aims to carry out conservation and breeding of existing species.

2.3.1. *Conservation.* The conservation is an effort to preserve and maintain pure strain. The collection of pure silkworm strain in the Plasma Bank is stored in cold storage with a temperature of 5 ° C in the form of eggs. Considering the egg storage time, which has a specific limit to maintain and preserve these strain, periodic maintenance or collection stock needs to be regenerated no later than eight months for dormant species and every two months for non-dormant species. The tropical species must be maintained every two months, while the bivoltine types (Chinese and Japanese races) need to be done every six months.

2.3.2. *Breeding.* This activity is a selection technique to get superior silkworm hybrids from the parent conservation results. All of them are carried out in a maintenance system that consists of maintaining small/juvenile silkworm (process for 12 days) and maintaining large/mature silkworm (12-16 days) so that the total time to harvest is 30-36 days.

The silkworm maintenance process can be seen in Figure 2. The difference between conservation activities from breeding activities is that conservation efforts are carried out using eggs of the same type or strain. As for breeding activities, the aim is to get superior hybrids, which are carried out by crossing various types of moths. For example, the type of Japanese race with the Chinese race.

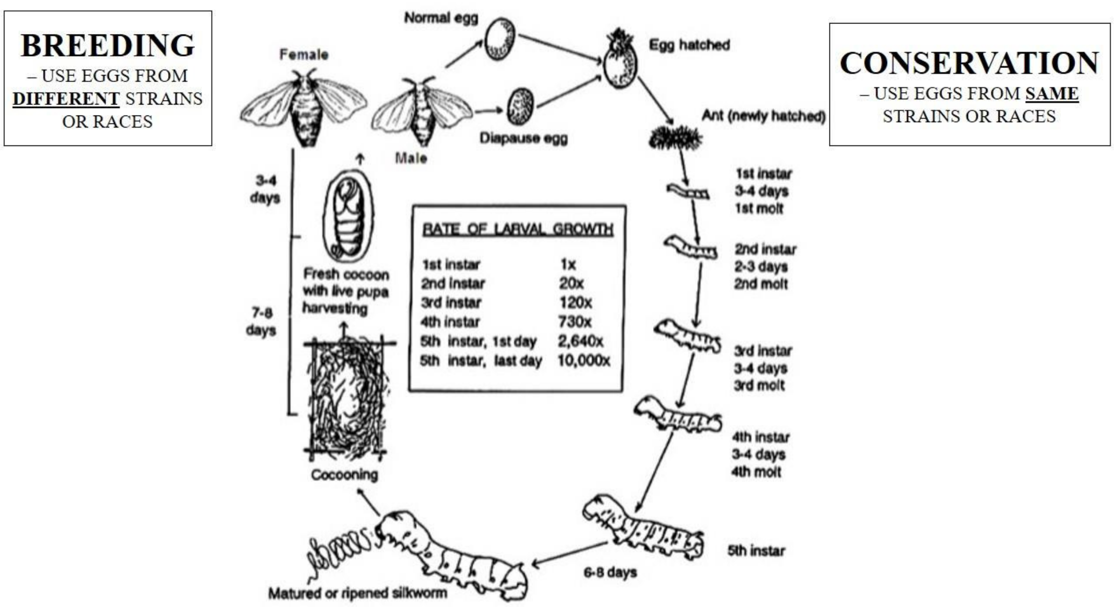

Figure 2. Silkworm maintenance process.

2.4. Maintenance procedures

2.4.1. *Disinfection Research Room.* The rearing chamber is first disinfected using a 5% formalin solution, then closed tightly for 24 hours and reopened for 24 hours before rearing larvae.

2.4.2. *Condition of research room.* During the larval rearing process until the cocoon process, the temperature and humidity of the rearing chamber are controlled in accordance with the temperature and humidity requirements of each instar. Temperature, humidity, and light intensity are recorded three times a day for the maintenance of instar 1(one) to 3 (three), i.e., in the morning, afternoon and evening. The recording is carried out four times a day, during the 4 - 5 instar period, in the morning, afternoon, evening and evening at 08.00, 12.00, 17.00 and 21.00. The temperature is based on two thermometers installed in the rearing chamber, which consist of a room thermometer and a dry-wet

thermometer. The air relative humidity is known from the dry-wet thermometer, while the light intensity is captured through the optical recording of the Lux Hi Tester.

2.4.3. *Egg hatching process.* The eggs from each of the strain crossed and generation are hatched by following the procedures [3 - 5]. Poly eggs are best washed with HCI solution. The eggs hatch 9-11 days after being put in an incubator. The larvae that come out first or hatch on the ninth and tenth days are put in a Petri dish glass and stored in the 50°C refrigerator while waiting for the rest of the eggs to hatch uniformly. After the eggs are hatched uniform, the *hakikate* is done at 09.00.

2.4.4. *Maintenance of larvae.* Larval rearing starts from one to three instar larvae. Usually maintained in one rearing bed (*sasag*) according to race and marriage generation (*zuriat*). Small larvae are fed three times a day, i.e., morning, afternoon and evening. In the case of large larvae, when larvae have entered the instar four to five, the larvae need to be raised in a rearing bed with 100 larvae in each rearing bed. Large larvae are fed four times a day, i.e., morning, afternoon, evening and night. Each larva will experience skin replacement but previously disinfected using a mixture of calcium oxide and chlorine with a dose of 95 g of calcium oxide and 5 g of chlorine. Sprinkle entirely on the body parts of the larvae using a fine sieve.

2.4.5. *Larvae feed.* Silkworm food is mulberry leaves. Proximate analysis is performed on feed given at each instar and according to the level of mulberry leaves. In small larvae (instar 1 to 3), the feed is given in the form of sliced mulberry leaves ranging in size from 2 mm to 6 mm. while for large/mature silkworm (instars 4 - 5) were feed by unsliced leaves.

2.4.6. *Cocooning*. At the end of the fifth instar or when the larvae's body looks transparent, larvae are transferred to the grafting device. The larvae are left until they become perfect cocoons, then harvest the cocoons by cleaning the cocoon feathers.

2.4.7. *Moth mating.* Male moths generally come out earlier than female moths, and while waiting for marriage, the male moths are stored in a Thermoline cooler at 10°C. The mating process were carried out in special boxes, covered by plastic funnels to avoid changing of mating partners. The mating lasted for four hours to get high hatchability [6, 7]. After copulation lasts four hours, the male and female moths are separated. Female moths are placed in cylindrical boxes of parallels with a diameter of 4 cm, the height of 1.5 cm with a tool to lay eggs from cement paper, then covered with a black rubber sheet until all-female eggs lay an egg.

3. Result and discussion

3.1. Overview of the maintenance process

Silkworm type was classified based on (1) moulters skin replacement, consisting of silkworms with 3, 4 and 5 skin changes; (2) the change of generation per year (voltinism) in natural conditions consisting of univoltine, bivoltine and multivoltine or polyvoltine that experience a change of generation per year for 1-3 or more generations; (3) places formed (races), namely European, Chinese, Japanese, Indian, or Tropical races [8, 9]; (4) cocoon colors are white and colored cocoon groups [8]; (5) rearing season; (6) specific characters; and (7) feed types and habitats were consisting of wild silkworms and commercial silkworms. Polyvoltine silkworm is known as short-lived species, and short-lived types are usually a feature of the healthy (stiff) silkworm. Tropical races are usually polyvoltine. Polyvoltine type, when crossed with bivoltine type will produce healthier and more disease resistants generation.

Silkworms are among the insects that undergo the complete metamorphosis process. They also include poikilotherm animals whose life cycle is influenced by environmental factors such as temperature, light, and humidity [9, 10]. As in the case of a typical Lepidopteran insect, the silkworm passes through four distinct stages, i.e., egg, larva, pupa, and adult during its life cycle, the duration of which may last for six to eight weeks depending upon race characteristics and climatic conditions. The

process of metamorphosis runs in 2 levels, namely regressive and progressive metamorphosis. Regressive metamorphosis is the stage of rejuvenation and destruction of larval body tissues. In contrast, progressive metamorphosis is the stage of re-formation of tissues and other organs from the form of pupae into moths [11].

The length of the rearing period depends on the genetic quality and feed [12], race, season and maintenance condition [12, 13]. The period of development from hatching to commercial or cocoon production is 30-35 days [14]. Multivoltine races found in tropical areas have the shortest life cycle with the egg, larval, pupal, and adult stages lasting for 9-12 days, 20-24 days, 10-12 days and 3-6 days, respectively. In uni/bivoltine races, the egg period of the activated egg may last for 11-14 days, the larval period 24-28 days, the pupal period 12-15 days and the adult stage 6-10 days [13].

Silkworms have speedy growth following a very dramatic linear curve. Growth can reach 10,000 - 12,000 times [15] between the initial weight before cocooning and the weight of newly hatched larvae. *Bombyx mori* L. is only depend on mulberry leaf (*Morus* spp.) and is an essential factor in natural breeding [16, 17]. Mulberry leaves, which have high quality, quantity, and productivity as silkworm feeding source will affect the production and quality of the cocoon produced [18 - 20].

The feeding quantity required by each instar is different. At the time of small larvae or instar, one to three young mulberry leaves in needed which are limp, rich in water, high protein, carbohydrates, and minerals. At this stage, the feed is provided in the form of small slices of mulberry leaves. Whereas for large larvae or instars of four to five mulberry leaves with little water content but rich in protein are needed without slicing them.

Larval rearing is the most critical stage in sericulture. During the larval life, the worm molts or casts off its skin four times to be able to grow. The larval life is divided into five distinct stages or instars, which are referred to popularly as five different ages (instars). The first three instars are referred to as "young ages" and the fourth and fifth instars as "late ages." The duration of the different instars and molting periods for the different races is as follows:

Table 2. Duration of different instars and molting periods.

Maintenance stage		Multivoltine races		Uni and bivoltine	
		Duration	**Temperature and humidity conditions**	**Duration**	**Temperature and humidity conditions**
I	Instar	3 days	27°C	3 days	27°C and 85 % R.H.
I	Molt	20 hours	80 – 85 % R.H.	20 hours	
II	Instar	2 days		2 days	
II	Molt	20 hour		20 hour	
III	Instar	3 days		3 days	25°C and 30 % R.H.
IV	Molt	1 day		1 day	
IV	Instar	4 days	25-26°C	5 days	22-24°C and 75 % R.H.
V	Molt	1 day	70 - 80 % R.H.	1 day	
V	Instar	6-7 days		9 - 10 days	20-23°C and 70 % R.H.
Total duration		22-23 days		26 – 27 days	

Each instar can be broadly divided into two phases, i.e., the feeding phase and the molting phase. After feeding voraciously and having attained full growth for the particular instar, the worm loses its appetite, and the larvae prepare to molt and cast off its old skin. Before each molting, the larvae stop feeding and rests with its head held up. The radiant body skin gradually becomes translucent, loose and wrinkled, and the worm becomes dull in appearance and wanders about in search of a resting place.

The molting periods last for 20 hours being shortest in the second molt, followed by the first, third and fourth. The resting for molting is often referred to as "going to sleep" and the coming out of the worm from molt as "waking up." During the molting period, the worms should not be disturbed so that the process of molting is not interfered with, and uniform molting is ensured.

After the silkworm passes through four molts, it reaches the fifth and final instar when it attains its maximum weight a day before maturity and before it stops feeding. At its maximum weight, it is about 10.000 times its weight at the time of hatching, and this phenomenal growth takes place within the short span of 20-25 days. When the worm is sufficiently mature for mounting, it loses its appetite, stops feeding, and excretes soft feces with high moisture content. At this stage, because of its massive increase in size, the silk gland is visible through the body integument and, therefore, the thorax and body segments of the mature larvae appear translucent. The silk glands are enlarged as to account for nearly 40 percent of the body itself. The characteristic of the ripened worm and serves as guidance for picking the mature worms for mounting. The mature worms become very restless and raise their heads in search of support to be able to start spinning.

The spinning of the cocoon starts almost immediately after mounting for 48-72 hours, spinning is completed by the mature worm. In another day or two, the worm transforms itself into the pupa within the cocoon. The pupal period may last for 8-14 days after which the adult moth emerges slitting through the pupal skin, and piercing the tough cocoon shell with the aid of the alkaline secretion (cocoonase- a protease enzyme) that softens the tough cocoon shell.

Adult moths are ready to copulate as soon as they emerge from pupae and females then lay eggs. The life of an adult moth is concise, lasting only 3-10 days, depending on race and season. Adult moths do not eat and fly. Female moths are more massive and generally slow, while males are somewhat smaller and more active. Females of the multivoltine variety can lay eggs on average by around 400 eggs. However, the average number for bivoltine uni and bivoltine moth varieties is around 500-600 eggs.

3.2. Conservation

The primary purpose of germplasm maintenance is to maintain germplasm collection properly. These collections itself is in the form of pure strain silkworms or geographical races. The pure strain is generally uniform for morphological characters and show minimum variations for quantitative traits in populations. In contrast, geographical races show wide variations in morphological and quantitative nature.

The Silkworm plasma bank in P3H currently has a collection of 58 pure silkworm lines, from approximately ten countries. The majority of lines as many as 54 strains come from the Tropical Race, China, and Japan, each race has advantages and disadvantages.

Japanese races usually have gray eggs, white eggshells, more massive eggs, freckled skin complexion, color between pink belly segments, longer silkworm, stronger silkworm, bean-shaped cocoons. Chinese races generally have greenish-colored eggs, yellow eggshells, plain silkworm patterns, color between blue belly segments, shorter silkworm age, oval/oval rounded cocoon shapes. Meanwhile, tropical races have white egg skin color, plain silkworm pattern, strong silkworm/disease resistance, short silkworm period, small cocoon, cocoon shell hard to spin. The advantage of Tropical Race is that although it has poor productivity and fiber quality, it has high adaptability to environmental conditions that are not suitable [21]. In contrast, Japanese and China races produce cocoon production and yarn quality, which are higher in sub-tropical conditions and show low tolerance and adaptation to tropical situations [22]. Information on P3H silkworm germplasm collections can be seen in Table 3.

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Table 3. Information on P3H silkworm germplasm.

No.	Race	Egg Color	Eggshell Color	Larvae pattern	Age of Larvae	Cocoon shape	Cocoon color	Voltinimse	Pure strain	Cocoon Weight (g)	Cocoon Skin Weight (g)	Percentage of skin cocoon (%)
1.	Japan	Grey	White	Spots	Long	Beans	White/ Yellow	Bivoltin	102	1.77	0.35	20.20
2.	Japan	Grey	White	Spots	Long	Beans	White/ Yellow	Bivoltin	106	1.63	0.30	18.53
3.	Japan	Grey	White	Spots	Long	Beans	White/ Yellow	Bivoltin	107	0.89	0.10	11.24
4.	Japan	Grey	White	Spots	Long	Beans	White/ Yellow	Bivoltin	108	1.52	0.35	22.98
5.	Japan	Grey	White	Spots	Long	Beans	White/ Yellow	Bivoltin	109	1.31	0.24	18.35
6.	Japan	Grey	White	Spots	Long	Beans	White/Yellow	Bivoltin	110	1.29	0.24	18.75
7.	Japan	Grey	White	Spots	Long	Beans	White/Yellow	Bivoltin	P110	1.42	0.22	15.30
8.	China	Greenish	Yellow	Plain	Medium	Round/Oval	White	Bivoltin	202	1.50	0.33	22.11
9.	China	Greenish	Yellow	Plain	Medium	Round/Oval	White	Bivoltin	207	1.40	0.27	19.74
10.	China	Greenish	Yellow	Plain	Medium	Round/Oval	White	Bivoltin	208	0.70	0.15	20.79
11.	Japan	Grey	White	Spots	Long	Beans	White/Yellow	Bivoltin	306	1.63	0.32	19.43
12.	China	Greenish	Yellow	Plain	Medium	Round/Oval	White	Bivoltin	307	1.76	0.31	17.69
13.	China	Greenish	Yellow	Plain	Medium	Round/Oval	White	Bivoltin	401	1.65	0.27	16.80
14.	China	Greenish	Yellow	Plain	Medium	Round/Oval	White	Bivoltin	402	1.39	0.28	20.24
15.	Japan	Grey	White	Spots	Long	Beans	White/Yellow	Bivoltin	403	1.19	0.21	17.76
16.	Japan	Grey	White	Spots	Long	Beans	White/Yellow	Bivoltin	503	1.51	0.30	20.22
17.	China	Greenish	Yellow	Plain	Medium	Round/Oval	White	Bivoltin	601	1.50	0.26	17.49
18.	China	Greenish	Yellow	Plain	Medium	Round/Oval	White	Bivoltin	702	1.56	0.32	20.91
19.	China	Greenish	Yellow	Plain	Medium	Round/Oval	White	Bivoltin	708	1.47	0.30	20.54
20.	China	Greenish	Yellow	Plain	Medium	Round/Oval	White	Bivoltin	802	1.42	0.28	19.46
21.	Japan	Grey	White	Spots	Long	Beans	White/Yellow	Bivoltin	803	1.80	0.39	21.96

No.	Race	Egg Color	Eggshell Color	Larvae pattern	Age of Larvae	Cocoon shape	Cocoon color	Voltinimse	Pure strain	Cocoon Weight (g)	Cocoon Skin Weight (g)	Percentage of skin cocoon (%)
22.	China	Greenish	Yellow	Plain	Medium	Round/Oval	White	Bivoltin	804	1.75	0.38	22.12
23.	Japan	Grey	White	Spots	Long	Beans	White/Yellow	Bivoltin	807	1.38	0.27	19.84
24.	China	Greenish	Yellow	Plain	Medium	Round/Oval	White	Bivoltin	902	1.59	0.32	20.27
25.	China	Greenish	Yellow	Plain	Medium	Round/Oval	White	Bivoltin	905	1.23	0.25	20.32
26.	Japan	Grey	White	Spots	Long	Beans	White/Yellow	Bivoltin	911	1.42	0.26	18.84
27.	Japan	Grey	White	Spots	Long	Beans	White/Yellow	Bivoltin	915	1.18	0.19	16.28
28.	China	Greenish	Yellow	Plain	Medium	Round/Oval	White	Bivoltin	916	1.56	0.34	21.71
29.	China	Greenish	Yellow	Plain	Medium	Round/Oval	White	Bivoltin	918	1.48	0.29	20.01
30.	Japan	Grey	White	Spots	Long	Beans	White/Yellow	Bivoltin	919	1.36	0.23	17.27
31.	Japan	Grey	White	Spots	Long	Beans	White/Yellow	Bivoltin	921	1.54	0.31	20.39
32.	Japan	Grey	White	Spots	Long	Beans	White/Yellow	Bivoltin	922	1.50	0.28	19.22
33.	Japan	Grey	White	Spots	Long	Beans	White/Yellow	Bivoltin	923	1.39	0.26	18.89
34.	Japan	Grey	White	Spots	Long	Beans	White/Yellow	Bivoltin	924	1.35	0.26	19.61
35.	Japan	Grey	White	Spots	Long	Beans	White/Yellow	Bivoltin	926	1.33	0.26	19.90
36.	Japan	Grey	White	Spots	Long	Beans	White/Yellow	Bivoltin	927	1.81	0.37	20.49
37.	China	Greenish	Yellow	Plain	Medium	Round/Oval	White	Bivoltin	930	1.61	0.28	17.68
38.	Japan	Grey	White	Spots	Long	Beans	White/Yellow	Bivoltin	931	1.40	0.26	19.00
39.	China	Greenish	Yellow	Plain	Medium	Round/Oval	White	Bivoltin	P208	1.46	0.26	18.30
40.	China	Greenish	Yellow	Plain	Medium	Round/Oval	White	Bivoltin	Poly 2	1.69	0.35	21.06
41.	Tropical	Yellow	White	Plain	Pendek	Lancip	White/Yellow	Polyvoltin	Poly 1	1.19	0.19	16.40
42.	Tropical	Yellow	White	Plain	Pendek	Lancip	White/Yellow	Polyvoltin	Poly pp	1.10	0.18	16.93
43.	Tropical	Yellow	White	Plain	Pendek	Lancip	White/Yellow	Polyvoltin	Poly zk	1.17	0.19	16.60
44.	Tropical	Yellow	White	Plain	Pendek	Lancip	White/Yellow	Polyvoltin	Poly zp	1.10	0.18	16.76
45.	The Other	Grey	White	Spots	Long	Beans	White	Bivoltin	x2-01 X x2-04	1.47	0.28	19.35
46.	The Other	Grey	White	Plain	Long	Beans	White	Bivoltin	x2-02 X x2-03	1.63	0.33	20.46

No.	Race	Egg Color	Eggshell Color	Larvae pattern	Age of Larvae	Cocoon shape	Cocoon color	Voltinimse	Pure strain	Cocoon Weight (g)	Cocoon Skin Weight (g)	Percentage of skin cocoon (%)
47.	The Other	Green	White	Zebra	Pendek	Oval	Yellow	Bivoline	Bk 01	1.93	0.34	17.75
48	The Other	Green	White	Zebra	Pendek	Oval	Yellow	polyvoltine	Bk 02			
49.	The Other	Green	White	Zebra	Pendek	Oval	Yellow	polyvoltine	BK 03 PZP X PZP	1.16	0.19	16.23
50.	The Other	Grey	White	Spots	Long	Beans	White	Bivoltin	C2	1.57	0.32	20.72
51.	The Other	Green	White	Zebra	Pendek	Oval	Yellow	polyvoltine	Pp804	1.35	0.22	16.91
52.	The Other	Green	White	Zebra	Pendek	Oval	Yellow	polyvoltine	Pp927	2.07	0.36	17.47
53.	The Other	Green	White	Zebra	Pendek	Oval	Yellow	polyvoltine	Pzp601Xpzk	1.13	0.18	15.89
54.	The Other	Green	White	Zebra	Pendek	Oval	Yellow	polyvoltine	PZP61	1.84	0.34	18.63
55.	The Other	Green	White	Zebra	Pendek	Oval	Yellow	polyvoltine	PZP804	1.83	0.34	18.89
56.	The Other	Green	White	Zebra	Pendek	Oval	Yellow	polyvoltine	PZP927	1.83	0.33	18.26
57.	The Other	Green	White	Zebra	Pendek	Oval	Yellow	polyvoltine	PZPP01 X PZP927	1.70	0.33	19.95
58.	The Other	Grey	White	Zebra	Medium	Oval	Yellow	bivoltine	Thazp61 dan 61thazp	1.59	0.27	17.00

3.3. Breeding

The collection of pure strain owned by P3H can be used to (1) provide genetic stock, (2) produce hybrids (commercial eggs), (3) create new strains and to improve existing strains. The existence of pure strain by P3H has become the stock to produce commercial egg hybrids so that they can help solve the problem of Indonesian natural silkworm. Desired hybrids of superior silk eggs include disease-free seeds (especially Pebrine disease), high egg production, high percentage of hatched eggs (>90%), steady silkworm growth, disease resistant, and high productivity and quality cocoon.

Essential activities as a basis for livestock breeding [18] that are applied in the maintenance of silkworms are selection, in addition to mating, to improve the genetic quality of livestock [19]. Selection plays a role in changing the frequency of genes that regulate several qualitative and quantitative traits [20]. One of the most popular selection methods is a mass selection [21], by selecting the best performing individuals. The combination of the mating system of unrelated and related individuals, accompanied by a planned and steady selection program, will result in the formation of new strains that can produce quality cocoons and silk fibers.

The success of a crossbreeding program depends on the genetic material of the individual being crossed, and the knowledge of the genetic architecture of each individual's nature, the methods of mating and the mating system used. These factors determine the strength of the combination and the desired characteristics in a generation. Reciprocal repetition method is an effective method for selecting better crossover results regardless of dominant, excessive, and epistatic effects that are considered as major factors in heterosis. This method is also effective for enhancing traits with high heritability that depend on the role of gene additives that increase the general level of combination at the crossing process if the parent stock with good character is crossed, hybrids are generally good. If the parent character is not good, but in reality, the hybrid shows good results, it shows that the specific combining power is high.

In the maintenance of silkworms, the selection is conducted in stages on the parent strain starting from the egg, caterpillar, cocoon, pupae, and moth stages, where only good individuals are selected for seedlings. The purpose of selection in each period is different. Selection on egg stages is to get a high number of eggs per parent, uniform hatching and a good percentage of hatching. While selection on the silkworm stage aims to obtain steady growth, silkworm shortage, and high maintenance yield, the thing to remember in the selection must pay attention to economically significant properties, influenced by genetic and environmental factors. Therefore, in the maintenance of selected strains, environmental effects must be kept as small as possible. At the same time, genetic variation must be analyzed and evaluated in order to select specific strains efficiently. When an egg is obtained from a superior parent, the cocoon of the parent and oval-shaped parent lines are crossed to test the nature of the hybrid because even though the pure line is superior, it does not always produce a good quality hybrid [1].

This method is also used to conduct silk breeding activities using pure strain collection owned by P3H. This silkworm variety greatly influences the quality of the cocoon produced, given that cocoon is the main target of natural production. Cocoon quality (cocoon weight, cocoon shell ratio, and defective cocoon ratio) influences its price determination. The pupa weight and the ratio of pupa to defect are influenced by how the silkworm is maintained. Meanwhile, the ratio of pupa skin is affected by the type of silkworm seedling. The cocoon skin ratio is an essential factor because it is closely related to silk yarn yield. Factors that influence the ratio of cocoon skin are gender, varieties of silkworms, and maintenance and food adequacy. Male cocoons usually have a ratio of cocoon skin 2-3% higher than female cocoons, and good silkworm varieties have a ratio of cocoon skins of 22-25%. Food adequacy is crucial so that the silkworms will be disease resistant and have high egg production

Silkworms have properties that are sensitive to environmental conditions. So that during the breeding process, it is recommended to make a new strain that combines the superior characteristics of several races and strains by environmental conditions and the desired character.

One of the superior products of P3H from crossing and selection is the hybrid silkworm PS 01. This hybrid is a result of crossing from parent 804 x 927 with the Japanese female race and Chinese

male race. This type is better because it can produce cocoons with filament lengths up to 1003 m with a rolling power of ≥ 90%, but it is also suitable to be maintained in low-lying areas with an altitude of 100-200 m above sea level. Cocoon production that ≥ 35 kg/box will have a cocoon skin ratio of ≥ 20%. Characteristics of the species have blue egg color, whitish-yellow eggshell color, freckle larvae pattern, larvae age 23-25 days (long), oval-shaped cocoon, white cocoon color, and bivoltine type voltinism. This variety has been launched formally by Minister Decree No. 794/Menhut-II/2013. More clearly can be seen in figure 3.

Figure 3. P3H superior silkworm hybrid, PS 01: (a) Silkworm, (b) cocoon, (c) yarn.

This hybrid has been applied to several silk development sites under the guidance of P3H, including Pati, Soppeng, Sukabumi, Boalemo, and Garut. Based on farmer information, farmers stated that PS 01 cocoon production was higher than commercial C3 01 hybrid types from Perhutani (Table 4). If the hybrids already produced are capable of being applied by silk farmers, it is not impossible that the problems of Indonesia's natural breeding, especially low-quality cocoons, can be overcome.

Table 4. Comparison of PS 01 hybrid applications and C3 01 commercial hybrids.

Application Location	Hybrid Silkworm Type	Producer	Cocoon production (kg/box)
Pati – Regaloh	PS 01	P3H	35 – 40
(Jawa Tengah)	C3 01	Perhutani	25 – 30
Soppeng	PS 01	P3H	35 – 40
(Sulawesi Selatan)	C3 01	Perhutani	25 – 30
Sukabumi	PS 01	P3H	40
(Jawa Barat)	C3 01	Perhutani	30
Boalemo	PS 01	P3H	40 – 45
(Gorontalo)	C3 01	Perhutani	35
Garut	PS 01	P3H	40
(Jawa Barat)	C3 01	Perhutani	30 - 35

Note: box = the size of the place of silkworm eggs, one box of 25,000 eggs

4. Conclusion

Conservation efforts while maintaining the existence of a collection of both silkworm and mulberry must be carried out for future breeding activities. Efforts need to be conducted to minimize the factors that cause damage to the collection of pure strains, that caused the silkworm is not maintained as scheduled. These factors include non-technical factors (equipment damage on cold storage, electricity power off) and technical factors (pest and disease attacks during maintenance, as well as erratic

climate change conditions affecting the silkworm cultivation process). The result of conservation is the maintenance of 58 germplasm silkworms.

The increase of collection numbers in Bank Plasma is also needed to increase the diversity of species as materials for crossbreeding activities to get improved hybrids that have superior characters to answer the challenges of existing natural silk development. The stages in the germplasm collection need to be conducting, starting from exploration, selection, refining, and conservation. The breeding results are a type of hybrid caterpillar PS 01, which has a production advantage compared to commercial types CS03. The dissemination of results such as hybrid PS 01, which has a comparative advantage needs to be improved to help overcome the problem of Indonesian silkworms.

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