

*Full Length Research Article***Comparison of the Eggs and Cocoons Quality of Some Mulberry Silkworm Hybrid *Bombyx mori* L.**

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ABSTRACT

Sericulture is one of the non-timber forest products (NTFPs) that can help Indonesians live better lives and support environmental improvement. Silk, as a product of sericulture, is one of the five NTFPs that Indonesia has identified as having the ability to boost the nation's economy and alleviate poverty. However, there are several issues with the development of silk in Indonesia, chiefly issues with the quality of silkworm seedlings and low cocoon yield. Crossbreeding is one method used to boost egg output. This study aimed to evaluate the eggs and cocoons produced by various silkworm (*Bombyx mori* L.) crosses. The Pustarhut Laboratory hosted the research from May to August 2022. Twelve treatments were employed in the form of crosses using the Randomized Block Design (RBD) method, and each treatment applied three replicates. Overall, the findings demonstrated that the CE cross ((919 x 927) x (804 x p208)) produced eggs (652 eggs) and cocoons of higher quality than other crossings. However, although the egg numbers of CF cross ((919 x 927) x (p208 x 804)) and FC cross ((p208 x 804) x (919 x 927)) produced 622 and 631 eggs, respectively and were significantly different from CE cross, they still produced egg numbers above 600. In addition, they produced cocoon quality that was not significantly different from CE cross. All finding indicates that silkworms from CE, CF, and FC crosses can be used as an alternative to superior silkworm breeding to increase the productivity of silkworm rearing in Indonesia.

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1. Introduction

Forests play an essential role in poverty alleviation efforts by increasing income, increasing food security, reducing vulnerability, and improving the sustainability of natural resources, which can improve people's welfare (Warner 2000). Wood or non-timber items are both examples of forest products. Non-timber forest products (NTFPs) are biological items other than wood derived from unaltered or managed natural forest environments. Additionally, NTFPs are defined as all items or commodities derived from forest ecosystems that are marketed, consumed on a household scale, or have social, cultural, or religious value (Marshall et al. 2003; Pandey et al. 2016; Shackleton and Shackleton 2004). Sericulture is one of the NTFPs that can improve people's welfare and support environmental improvement in Indonesia. In the Indonesian context, sericulture agribusiness is designated as one of the five priority non-timber forest products

(NTFPs) that have the potential to contribute to the country's economy and overcome poverty (Minister of Forestry of the Republic of Indonesia 2009; Mutiara and NH. 2017; Tenriawaru et al. 2021).

Despite the market's continued openness, Indonesia's sericulture has not been at its best. Even though Indonesia's tropical climate greatly facilitates mulberry cultivation and silkworm rearing, just 5% of the country's annual 900 tons of silk thread need is supplied by local production (Detikfinance 2014). Several conditions must come together to boost silk production, including adequate and high-quality mulberry leaf production, effective rearing techniques, effective marketing, and institutions. The conditions could contribute to the silk industry's ability to obtain high cocoon output and seeds of the necessary quality (Andadari 2016; Santoso 2012). The silkworm hybrid of C301 (Andadari and Kuntadi 2014; Minarningsih et al. 2021), BS 09 (Estetika and Endrawati 2018), PS 01 (Agustarini et al. 2020; Fambayun et al. 2022; Yuniati et al. 2021) are several types of hybrid silkworms from crosses are applied in Indonesia. This hybrid type crosses the existing Chinese and Japanese races. BS 09 and PS 01 are hybrid types of crosses between Chinese race males and Japanese race females, and the only difference is the strain. If BS 09 is a cross between line number 808 × 807 while PS 01 is a cross between 804 × 927.

Silkworm breeding involves two techniques: creating pure strains by choosing qualitative and quantitative traits across many generations and choosing appropriate hybrids for commercial use. The artificial cross method comprises single and reciprocal, top, double, and rear crossings (Subantoro et al. 2008). Switching the parents in the cross is essential for successful crosses and the ensuing F1 characters due to the numerous elements that affect the cross, including genetic and environmental factors and the intersecting method. Currently, a single cross is used to create commercial silkworms. With a few exceptions, the single cross is more heterogeneous than the three-way or double cross. However, the three-way cross and double cross can play a crucial role as intermediary technologies in hot or humid environments when the raising of the bivoltine F1 hybrid fails in the field, and the local races produce low-quality animals (Singh and Jayasomu 2002). The findings of the single and double crosses vary depending on the researcher, the material employed, and the available circumstances. There was no difference between single and three-way crossings regarding health, such as pupae percentage and larval mortality rate. The two, however, exhibit heterotic differences under suboptimal maintenance settings (Hemmatabadi et al. 2016). Additionally, Joge et al. (2003) assert that in the situation at hand, the stability of multiple cross hybrids with higher viability is more important than increased productivity. The primary reasons bivoltine crop stability is not reached are the environment, upkeep, and socioeconomic factors. To improve the output of the cocoon and graded raw silk, double-crossing hybrid silkworm breeds must be developed and evaluated. As a result of the efforts of breeders, the current breed has a high percentage of cocoon shells and raw silk (raw silk thread) but is negatively correlated with egg productivity from the parent (Kumari et al. 2011). Therefore, to increase egg production, double crosses are usually used. Egg productivity positively correlates with the number of eggs per parent and the number of parents that can produce large eggs. However, this character has a negative relationship with the cocoon shell and the percentage of filaments. Therefore, selecting pure strains for high egg productivity without reducing the cocoon shell ratio is formidable (Kumari et al. 2011).

The problem in obtaining superior strains is the selection of parents for crosses. The ability to join depends on the interaction of the complex of genes that cannot be determined only by the parents' appearance (Hussain et al. 2011). Therefore, trying as many double crosses as possible is

necessary before determining the best results. This study aims to determine the advantages of several silkworm double cross crosses as an alternative superior silkworm seed. It is hoped that these superior silkworm seeds will further increase the productivity of Indonesian sericulture.

2. Materials and Methods

2.1. Materials

The tools used in this research are silkworm-rearing equipment, seriframe (box cocooning made of plastic), base paper, paraffin paper, egg-laying paper, lime, chlorine, HCl, formalin, and other auxiliary tools. The research material used was silkworm eggs from crosses between strains in the same race, namely the Japanese race with strain numbers 919, 927, and 403, and the Chinese race with strain numbers 930, 804, and p208. The feed used for the silkworm's rearing is mulberry leaves.

2.2. Methods

2.2.1. Crossing activities

The pupae of 6 silkworm strains were selected and differentiated by sex. The female pupa is characterized by a large body, a slightly rounded tail, and an x mark near the tail at the lower end of the abdomen. In contrast, the male pupae are relatively small, the tail is somewhat pointed, and the markings on the bottom of the abdomen are only dots. Each strain was taken as many as 18 cocoons (9 pairs) and left until they turned into moths ready to be crossed.

The silkworm pupae were crossed for 3 and 4 hours in a slightly dark room (not exposed to direct sunlight). After mating, the male and female moths are separated. Then, the female moth is placed in a closed box until it lays eggs.

Silkworm eggs double cross were disinfected with 2% formalin and then dried without direct sunlight. Furthermore, when eggs are dried, it treated with HCl to stimulate the embryo and then left in an incubator for 10 to 12 days at 25°C until they hatch; when the eggs hatch are given sufficient lighting.

After all the eggs hatch, the tiny larvae are transferred to the *sasag* (container for placing larvae). The research design used was a completely randomized design (RCBD). Reared two hundred fifty larvae from each cross were conducted in three replicates. The number of larvae for the study ($3 \times 12 \times 250$) was 9000. The twelve double crosses of silkworms from three strains of Japanese races (919, 927, and 403) and three strains of Chinese races (930, 804, and p208) are as follows (**Table 1**).

Table 1. The twelve double crosses of silkworms from 3 strains of Japanese and 3 strains of Chinese races

No.	Code	Double cross	No.	Code	Double cross
1	AB	(927 × 919) × (930 × 403)	7	AF	(927 × 919) × (p208 × 804)
2	BA	(930 × 403) × (927 × 919)	8	BC	(930 × 403) × (919 × 927)
3	AD	(927 × 919) × (403 × 930)	9	CE	(919 × 927) × (804 × p208)
4	DA	(403 × 930) × (927 × 919)	10	EC	(804 × p208) × (919 × 927)
5	AE	(927 × 919) × (804 × p208)	11	CF	(919 × 927) × (p208 × 804)
6	EA	(804 × p208) × (927 × 919)	12	FC	(p208 × 804) × (919 × 927)

2.2.2. Silkworm rearing and cocooning

Larvae in each *sasag* were given the same feed thrice daily at 08.00, 12.00, and 17.00. Each larva will molt each time it increases *instar*. If the larvae have shown signs of cocooning, the larvae begin to stop eating, secrete silk fibers, make their body transparent and slightly yellowed, and the larvae walk to the edge. Transferring the larvae that will become cocoons to the cocooning place is done by hand and moved one by one so that the mature larvae are selected. Cocoon harvesting is done after seven days of cocooning.

Harvesting, processing, or handling is done by cleaning the cocoons. Cocoon cleaning is done by cleaning the cocoons from the fine fibers on the surface of the cocoons. After cleaning, the cocoons were selected by separating good quality and defective ones.

2.2.3. Data collection

Data collection was carried out after cocoon harvesting. The normal cocoons were obtained from each replication and taken from ten male and ten female cocoons to distinguish the sex of the pupa in the cocoon by cutting the cocoon's shell and removing the pupa. The male pupa will look smaller than the female, and the end of the last segment will look sharper. On the other hand, the female pupa will be larger because there are eggs in the pupa's stomach, the end of the last segment is blunt, and there are cross marks.

From each replication, ten female and ten male cocoons were taken randomly. Furthermore, each female and male cocoon was weighed using a weighing balance with an accuracy of 0.001 g. After weighing, the pupae were removed from the cocoons and weighed the cocoon's shell. The results of weighing male and female cocoons are averaged and then tested for variance. Furthermore, the parameters observed in this study include prolificity (number of eggs laid per female parent), egg hatchability, cocoon yield, percentage of defective cocoons, cocoon weight, the weight of the cocoon shell, and cocoon shell percentage. Egg hatchability and cocoon yield are expressed by hatch percentage and cocoon percentage, respectively.

$$\text{Hatch percentage (\%)} = \frac{\text{Number of eggs hatched}}{\text{Number of eggs}} \times 100 \% \quad (1)$$

$$\text{Cocoon percentage (\%)} = \frac{\text{Number of silkworms that survive to form cocoons}}{\text{Number of silkworms reared}} \times 100 \% \quad (2)$$

2.2.4. Data analysis

Variance analysis was conducted to determine the effect of the silkworm double crosses on the parameter observation. Data analysis was performed using JMP Start Statistics 8 and further tested with the Tukey test to determine the difference between the treatments.

3. Results and Discussion

3.1. Eggs Quality

Egg quality is highly determined by hatching percentage, the number of eggs, and cocoon yield. These parameters are essential in the silkworm cultivation business (Andadari and Kuntadi 2014; Rajanna et al. 2008; Sing et al. 2015). The egg quality of the twelve double crosses of silkworms can be seen in **Table 2**.

Table 2. Eggs quality of the twelve double crosses of silkworms

Double cross code	Fecundity (Number of eggs)	Eggs hatchability (%)	Cocoon yield (%)
AB	561 ^{de}	96,25 ^a	90,50 ^a
BA	586 ^{bc}	93,24 ^a	91,50 ^a
AD	522 ^f	94,75 ^a	92,00 ^a
DA	528 ^f	95,59 ^a	94,70 ^a
AE	510 ^f	93,26 ^a	90,00 ^a
AF	572 ^{cd}	97,35 ^a	94,65 ^a
EA	590 ^{bc}	97,78 ^a	95,00 ^a
BC	549 ^{de}	90,54 ^a	96,00 ^a
EC	565 ^{cd}	94,85 ^a	92,80 ^a
CE	652 ^a	97,42 ^a	90,77 ^a
CF	622 ^b	90,38 ^a	93,60 ^a
FC	631 ^b	95,84 ^a	91,25 ^a

Notes: Means followed by the same letter are not significantly different at $P \leq 0.05$, as determined by Tuckey’s test.

The availability of silkworm eggs in sufficient quantities and of good quality is one factor that needs attention to maintain the continuity of sericulture activities because it is one of the materials necessary in the silkworm breeding process. The number of eggs per parent is often a goal in breeding and even a reference factor for the success of a nursery business of silkworms. Fecundity is the average number of eggs laid per female parent after crossing.

Table 2 and **Fig. 1** showed that the treatment of the cross resulted in a significant difference ($P \leq 0.05$) among treatments in the fecundity value. The CE treatment showed the highest yield and significantly differed from other treatments, producing 652 eggs. However, although the CF and FC treatments had fewer eggs and were significantly different from the CE treatment, both produced eggs above 600. This value significantly differed from the other nine treatments that produced eggs below 600; the highest value was 586 eggs from BA cross, and the lowest was 510 eggs from AE cross. Although the other nine crosses produced egg numbers below 600, the fecundity of this hybrid is better than the study of [Sangle et al. \(2022\)](#), where double hybrid studies produced a fecundity value range of 458–484.

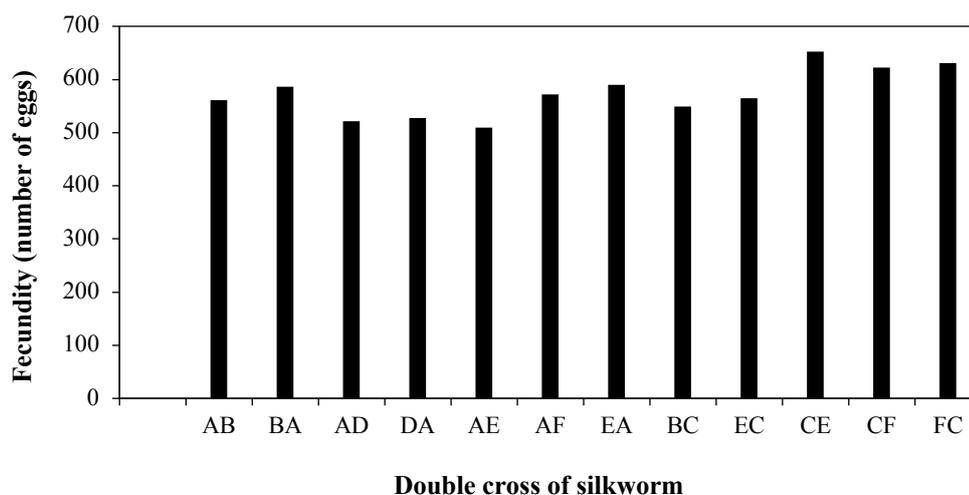


Fig. 1. Fecundity of the twelve double crosses of silkworms.

The number of eggs produced in addition to the double cross method also depends on several factors, including the silkworm rearing, the quality of the leaves as silkworm feed, the silkworm

race, and the mating length. For example, [Abdelmegeed \(2015\)](#) stated that mating times from 0.5–12 hours have high egg fertility, averaging 97.33–99.50% under laboratory conditions of 25°C. In this study, silkworm cocoons were crossed for 3 and 4 hours in a slightly darkened room.

A high hatching percentage is essential and is often used as a benchmark for seed quality. The hatching percentage can see in **Table 1** and **Fig. 2**.

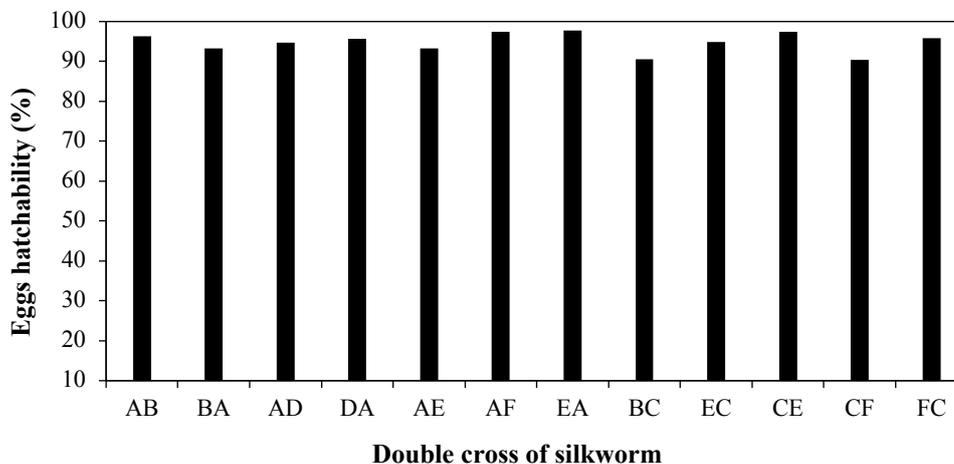


Fig. 2. Eggs hatchability of the twelve double crosses of silkworms.

Fig. 2 shows a slight difference between the hatching of one cross and another. Visually, the hatching percentage varies from 90.38–97.78%, but there was no significant difference between one cross and another (**Table 2**). Therefore, it is indicated that the cross type does not influence the hatching percentage. Hatchability was not significantly different from the twelve crosses. All eggs produced almost uniform hatchability under the same conditions. [Munemanik et al. \(2018\)](#) found that double hybrid silkworms performed better hatching percentage than single hybrid reared under the same conditions. Likewise, [Sangle et al. \(2022\)](#) found that double hybrids produced a better hatching percentage than single hybrids. However, [Hussain et al. \(2011\)](#) explained that a good egg occurs when the temperature, humidity, and incubation time are according to optimal needs. Hatching eggs requires a temperature of 25°C with a humidity of 75–80%. Likewise, according to [Gowda and Reddy \(2007\)](#), environmental conditions will affect the performance of bivoltine hybrids of silkworm *Bombyx mori* L. Meanwhile, according to [Hemmatbadi et al. \(2016\)](#), genetic and environmental factors significantly affect the productivity of a variety, so to increase the quantity and quality of silk, in addition to improving environmental conditions such as nutrition, sanitation, management, and rearing conditions but also must follow the fundamental steps in improving genetic and breeding.

In general, the hatching percentage produced in this study was quite good. The hatching percentage ranged from 90% to 98%. The value indicates that the environmental conditions at the study site are suitable for rearing *Bombyx mori* L. silkworms to increase egg production.

The cocoon yield is the number of cocoons produced from the total number of larva rearing. Based on statistical tests, there was no significant difference between treatments (**Table 2**). It was due to the same rearing technique given to each treatment. All treatments showed promising results, averaging above 90% (**Fig. 3**). Cocoon yields of more than 90% have commercial value, as cocoon production is strongly influenced by cocoon yield ([Rahma et al. 2017](#)).

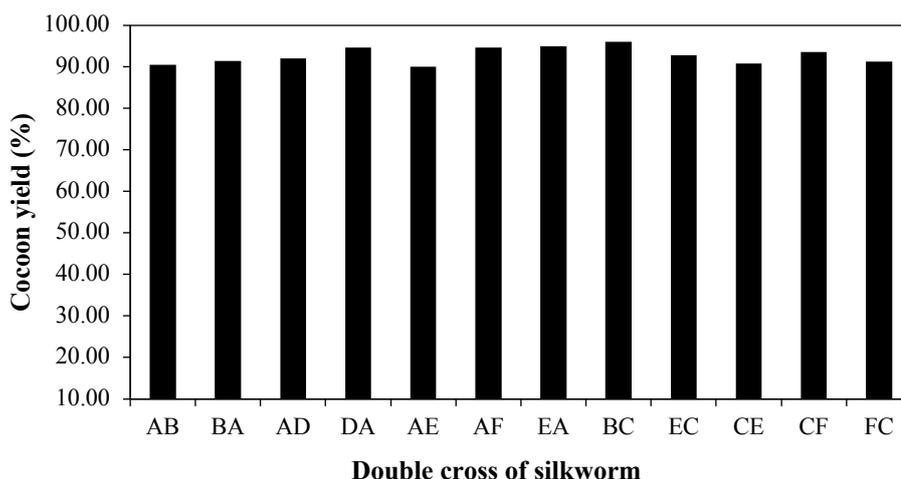


Fig. 3. Cocoon yields of the twelve double crosses of silkworms.

The results showed that in the parameter of egg quality, the twelve double crosses influenced the number of eggs. In contrast, the hatching percentage and cocoon yield did not show significant differences. However, the resulting values in hatching percentage (above 90%) and cocoon yield (above 90%) in the twelve double crosses are categorized as good and can be recommended to have commercial value and potential to be selected in breeding programs to obtain superior seed.

3.2. Cocoon Quality

The cocoon is where the pupa changes into an imago or adult moth. The cocoon silkworm rearing will determine the number of results received. The cocoons' weight determined the quality of the cocoons, the cocoon shell, and the percentage of defective cocoons. A sound-quality cocoon is one of the pupae inside the cocoon that are still alive, the cocoon shell is hard and clean, and the color and shape of the cocoon are like that of the silkworm races reared. The cocoon weight parameter determines the cocoon quality. The cocoon quality improves if the cocoon weight is high (Nursita 2011), and the cocoon weight positively correlates with the cocoon yield per box (Andadari et al. 2013). The cocoon quality of the twelve double crosses of silkworms can be seen in Table 3.

Table 3. Cocoon quality of the twelve double crosses of silkworms

Treatment	Defective cocoon (%)	Cocoon weight (g)	Cocoon shell weight (g)	Cocoon shell ratio (%)
AB	26,4 ^b	1.15 ^a	0.24 ^a	18,61 ^a
BA	24,8 ^c	1.14 ^a	0.23 ^a	18,67 ^a
AD	24,0 ^d	1.28 ^a	0.24 ^a	18,79 ^a
DA	24,8 ^c	1.29 ^a	0.24 ^a	18,81 ^a
AE	23,2 ^e	1.26 ^a	0.21 ^a	19,30 ^a
AF	21,6 ^f	1.31 ^a	0.25 ^a	18,38 ^a
EA	14,4 ⁱ	1.29 ^a	0.25 ^a	19,45 ^a
BC	29,6 ^a	1.37 ^a	0.26 ^a	18,62 ^a
EC	16,8 ^h	1.28 ^a	0.24 ^a	19,19 ^a
CE	17,6 ^g	1.32 ^a	0.26 ^a	19,47 ^a
CF	21,6 ^f	1.37 ^a	0.21 ^a	19,34 ^a
FC	13,6 ^j	1.36 ^a	0.25 ^a	19,04 ^a

Notes: Means followed by the same letter are not significantly different at $P \leq 0.05$, as determined by Tuckey's test.

Statistical tests showed that treatments affected cocoon quality. The average percentage of defective cocoons was significantly different ($P \leq 0.05$) between the cross factors. According to Tukey's test, the highest percentage of defective cocoons were obtained from BC treatment. All treatments showed a high percentage of defective cocoons (above 8%), which was class D. The high percentage of defective cocoons, besides differences in treatment, also because the temperature during maintenance is high, up to 31°C, while the recommended temperature during cocoon is between 23-24°C. Mulberry silkworm is susceptible to environmental factors such as temperature and humidity fluctuations (Ghazy et al. 2017). According to Tanjung et al. (2017), high temperatures will cause faster larvae stage period, reduced larvae weight and nutritional index value and cocoon productivity, and cause the failure of imago development. Defective cocoons obtained are generally double cocoons. A double cocoon is more oversized because two or more silkworms form a cocoon together. Silkworms will produce more double cocoons at high temperatures. Other defective cocoons obtained were thin-skinned, thin cocoons at the ends, and dirty inside (Suresh et al. 2011). Therefore, the breeding program must continuously produce varieties suitable for climatic fluctuations (Ghazy et al. 2017).

The cocoon weight is the total weight of the cocoon (cocoon shell with pupa). The weight of the cocoons is obtained from good quality cocoons. The greater the weight of the cocoons, the better the cocoon quality because it determines the results of fiber weight and fiber length. Based on statistical tests, the cocoon weights produced from the twelve crosses were not significantly different, but the BC, CF, and FC crosses showed the highest yields, with an average cocoon weight of 1.4 g.

The cocoon shell is a collection of fibers secreted by the silkworm that envelops the pupa and provides shelter for the pupa. The cocoon shell of the silkworm is composed of the proteins fibroin and sericin. The cocoon shell determines the number of threads produced. The heavier of cocoon shell will have, the longer the filament. Based on statistical tests, the weights of cocoon shells made from the twelve crosses were not significantly different.

The cocoon shells will determine the percentage of raw silk in the spinning. The percentage of the cocoon shell was influenced by the cocoon's weight and the cocoon shell's weight. Based on statistical tests, the percentage of cocoon shells produced from the twelve crosses was not significantly different, which means the rate of cocoon shells was not affected by the twelve crosses.

4. Conclusions

Twelve crosses between silkworms in double crosses of 3 Japanese strains and 3 Chinese strains produced hybrids with varied characters. Observing egg quality on the parameters of the fecundity of eggs, hatching percentage, and cocoon yield showed that the twelve double hybrids produced good quality. The highest fecundity of eggs was obtained from the CE cross ((919 × 927) × (804 × p208)), which was 652 eggs, while the lowest was 510 eggs from the AE cross ((927 × 919) × (804 × p208)). For cocoon quality parameters, such as defective cocoon, cocoon weight, cocoon shell weight, and cocoon shell ratio showed that the parameters of cocoon weight, cocoon shell weight, and cocoon shell ratio were not statistically significantly different. The real difference was seen in the defective cocoon, where the BC cross ((930 × 403) × (919 × 927)) produced the highest defective cocoon of 29.6%, while the lowest was the FC cross ((p208 × 804) × (919 × 927)) which produced defective cocoon of 13.6%. The high percentage of defective cocoons from

the BC cross will be detrimental to the silk farming business as it will significantly reduce the selling value of cocoons. Overall, the CE cross was better for egg and cocoon quality than the others. Besides producing the most eggs, it has a higher hatching percentage and cocoon yield of 97.42% and 90.77%, respectively. Therefore, the CE cross can be used as alternative superior silkworm breeds to increase the productivity of silk cultivation in Indonesia. However, the FC cross ((p208 × 804) × (919 × 927)) can also be recommended as a superior silkworm breeder because it produces a high egg number (631 eggs), hatching percent (95.84%), cocoon yield (91.25%), cocoon shell ratio (19.04%), and low percentage of deformed cocoons (13.6%). Likewise, the CF cross can be recommended as superior silkworm breeders. Although the percentage of defective cocoons was higher (21.6%) than the CE and FC crosses, it had high fecundity (622 eggs), hatching percent (90.38%), cocoon yield (91.25%), and cocoon shell ratio (19.04%). From the study results, it can be concluded that the CE, CF, and FC crosses are the best double crosses that can be used as an alternative to using superior seeds in silkworm breeding. However, conducting trials in various locations is necessary to obtain optimal results.

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