

# Breeding of a Natural Green Cocoon Quaternary Hybrid Combination Xiangcailu No. 1 for Spring Rearing

Hong XUE<sup>1</sup>, Junwen AI<sup>1</sup>, Yanghu SIMA<sup>2\*</sup>, Xingjian HE<sup>1</sup>, Changwen LIU<sup>1</sup>, Ying ZHENG<sup>1</sup>, Yong LIU<sup>1</sup>, Yun TANG<sup>1</sup>

1. Sericultural Research Institute of Hunan Province, Changsha 410127, China; 2. Soochow University, Suzhou 215123, China

**Abstract** The breeding activities aimed to create a natural green cocoon variety for spring rearing with the characteristics of stable natural cocoon color, easy rearing and good silk quality. Four different lines from the excellent silkworm variety Jingsong × Haoyue were taken as recurrent parents, using the natural green cocoon variety G 9202 as donor parent. After other excellent parents were introduced to cross with these different lines, four new breeding materials were selected by recurrent backcross breeding. The characters of these breeding materials showed not only natural green cocoon but also excellent comprehensive traits. A naturally colored cocoon quaternary hybrid with excellent practical characters was selected based on multiple hybrids. It is suitable for spring rearing in Yangtze valley which includes Hunan Province. It was approved by Hunan Provincial Crop Variety Approval Committee and designated Xiangcailu No. 1 in 2016.

**Key words** Silkworm; Xiangcailu No. 1; Recurrent backcross breeding; Natural green cocoon; Spring rearing; Easy rearing; Excellent silk quality

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Sericulture is one of the great inventions of the Chinese people. Our ancestors created a profound silk civilization for the world, and they also left behind rich silkworm germplasm resources for human beings. The history of man's use of natural colored cocoons is almost consistent with the history of the use of white silkworm cocoons<sup>[1-2]</sup>. Compared with ordinary white silk, natural colored silk has higher special functions of warm keeping, moisture absorption, air permeation, resistance to bacteria and oxidation, and ultraviolet proofing<sup>[3-5]</sup>. Under the background of strengthening structural reform on the supply side, diversified development of natural colored silk has huge market value and promotion potential. Unfortunately, due to the fact that the basic varieties of natural colored cocoons are generally characterized by small individuals, weak body, thin cocoon shell, poor silk quality and low practicality, directly use of the natural resources results in low yield, short silk length and low efficiency. Therefore, it is necessary to make innovative and practical transformation of this type of resources<sup>[2]</sup>. To this end, Sericultural Research Institute of Hunan Province collaborated with Soochow University to carry out research on the genetic laws of natural colored cocoon resources and practical improvement of varieties. A natural green cocoon quaternary hybrid combination Jingsong A G 9202 • Jingsong B G 9202 × Haoyue A G 9202 • Haoyue B G 9202, which has the characteristics of good healthiness and easy rearing, stable silk color, good silk quality and high practicality, was developed by recurrent backcross breeding and pedigree separation. It was approved by the Hunan Provincial Crop Variety Approval Commit-

tee in 2016 and designated Xiangcailu No. 1 (XPD 009-2016).

## New Variety Selection

### Parents and generations

The essence of practical genetic transformation of natural colored cocoon resources is to introduce their natural characteristic cocoon control genes into practical varieties, when keeping other traits in practical variety. To this end, four elite pure lines of the national designated control variety Jingsong × Haoyue for spring rearing were selected as the receptor parents<sup>[6]</sup>, into which the natural green cocoon control genes of the basic variety G 9202 were introduced, respectively. At the same time, due to the trend of declining healthiness of Jingsong × Haoyue<sup>[7]</sup>, on the basis of the combining ability test, the white cocoon varieties with good silk quality and strong healthiness, improved 21K and improved 22K bred by Sericultural Research Institute of Hunan Province, were separately selected as the intervening parents of Chinese or Japanese lines<sup>[8]</sup> to improve their healthiness (Fig. 1).

Chinese female parents (receptor parents, recurrent parents): Jingsong A and Jingsong B are two different Chinese lines, introduced from Sericultural Research Institute, Chinese Academy of Agricultural Sciences, bred by intervarietal crossing between two different Chinese varieties<sup>[6]</sup>. They have the characteristics of good healthiness, active mulberry intake, long silk with excellent neatness and good combining ability, but they also have poor moisture resistance and high rate of unfertilized eggs. In spring rearing, they show the larva-pupa rate higher than 91%, the whole cocoon weight of 1.80–2.10 g, the cocoon shell ratio of 24.5%–25.5%, the cocoon filament length of 1300–1400 m, the reelability percentage of 70%–75% and the neatness of 95 points.

Japanese female parents (receptor parents, recurrent parents): Haoyue A and Haoyue B are two different Japanese lines, introduced from Sericultural Research Institute, Chinese Academy of Agricultural Sciences, bred by intervarietal crossing between

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Hong XUE (1977–), male, P. R. China, Senior agronomist, devoted to research sericultural resources and breeding.

\* Corresponding authors. E-mail: jwai718@sina.com; simyh@suda.edu.cn.

two different Japanese varieties<sup>[6]</sup>. It has the advantages of easy rearing , strong body and high cocoon production , but the fineness is slightly poor , and there are non-cocooning larvae and three-molting silkworms. In spring rearing , they show the larva-pupa

rate higher than 92% , the whole cocoon weight of 1.60 – 1.75 g , the cocoon shell ratio of 24.0% – 25.5% , the cocoon filament length of 1 150 – 1 250 m , the reelability percentage of 80% – 85% and the neatness of 95 points.

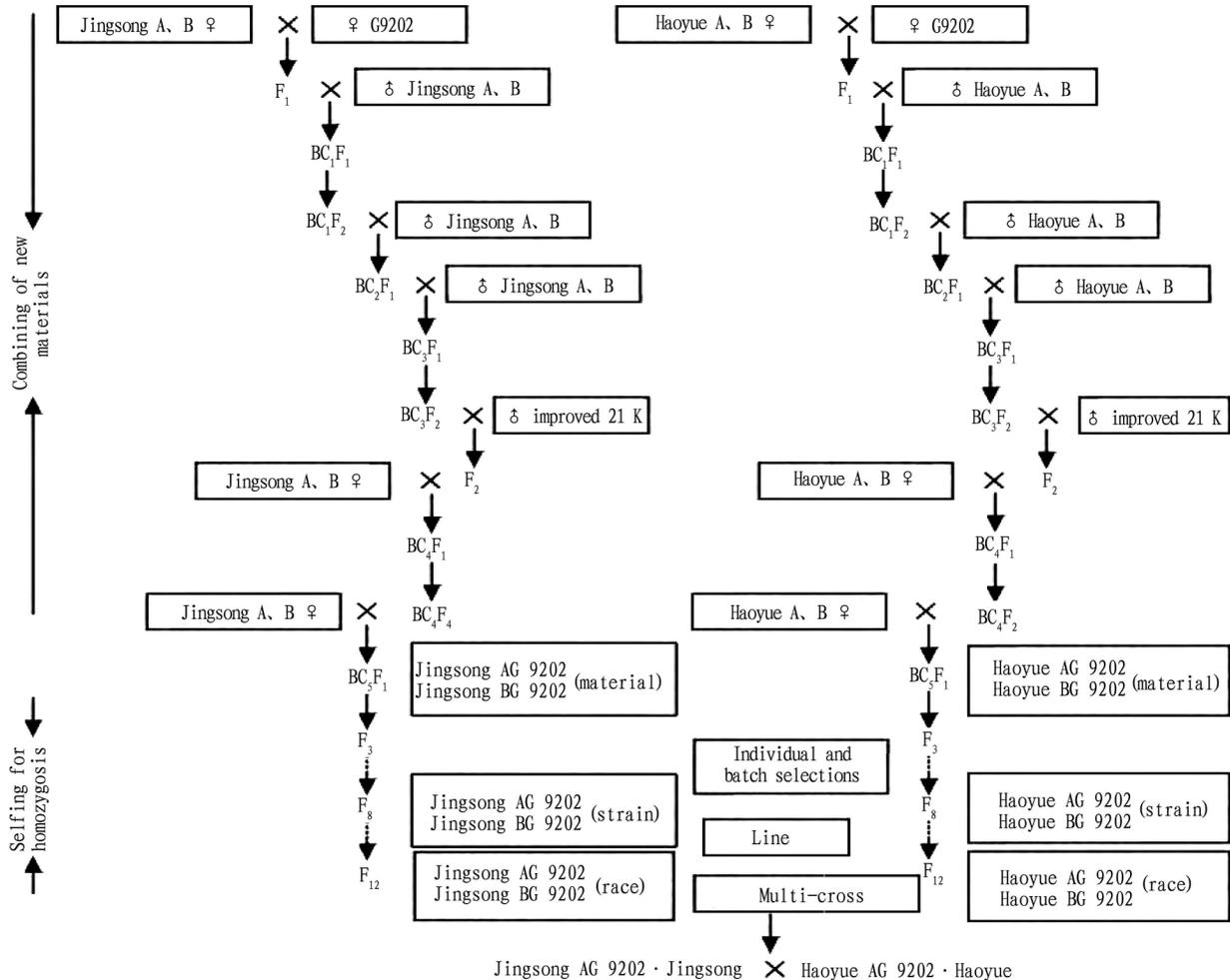


Fig. 1 Pedigree of Xiangcailu No. 1

Male parent ( donor parent , non-recurrent parent ) : G 9202 is a local improved variety provided by Soochow University , and the basic characters are not yet fully stable. It has the characteristics of natural green color of silkworm cocoons , stable color and the same inner and outer color of cocoons , but also has the disadvantages of thin cocoon shell and weak body resulting in uneasy raising. In spring rearing , it shows the larva-pupa rate higher than 87% , the whole cocoon weight of 1.45 – 1.55 g , the cocoon shell ratio of 20.0% – 22.0% , the cocoon filament length of 700 – 820 m , the reelability percentage of 45% – 65% and the neatness of 88 points.

The Chinese intervening male parent ( intervening parent ) : Improved 21K was improved from the variety 7521 for summer and autumn rearing by Sericultural Research Institute of Hunan Province through hybridization during the " Twelfth Five-Year Plan" period , bred by intervarietal crossing between two different chinese parental lines<sup>[8]</sup>. It has the characteristics of sex limited marking , strong body and easy rearing , but the filament length is

short. In spring rearing , it shows the larva-pupa rate higher than 95% , the whole cocoon weight of 1.70 – 1.75 g , the cocoon shell ratio of 24.0% – 25.0% , the cocoon filament length of 1 150 – 1 250 m , the reelability percentage of 80% – 85% and the neatness of 94 points.

The Japanese intervening male parent ( intervening parent ) : Improved 22K was improved from the variety 7522 for summer and autumn rearing by Sericultural Research Institute of Hunan Province through pedigree separation during the " Twelfth Five-Year Plan" period , bred by intervarietal crossing between two different Japanese parental lines<sup>[8]</sup>. It has the advantages of sex limited marking , strong body and easy rearing , good combining ability and excellent neatness , but aspect of laid silkworm eggs is relatively poor. In spring rearing , it shows the larva-pupa rate higher than 94% , the whole cocoon weight of 1.55 – 1.65 g , the cocoon shell ratio of 23.5% – 24.0% , the cocoon filament length of 1 050 – 1 150 m , the reelability percentage of 85% – 90% and the neat-

ness of 96 points.

### Breeding process

In the spring of 2010, the four elite pure races of Jingsong  $\times$  Haoyue were used as the female parents which were hybridized with the natural green cocoon race G9202 producing natural green cocoons, and the hybrid progeny materials were backcrossed with their respective female parents (recurrent parents), followed by selfing. In the selfing offspring, the groups showing the cocoon colors that meet or are close to the target-focused were selected to participate in the subculture, during which they were backcrossed with the recurrent parents  $\delta$  twice in succession. Then, a new round of selfing and 2 times of backcrossing were carried out, of which the first backcrossing was changed to hybridization with the intervening parent improved 21K or improved 22K, and the second backcrossing was performed with the respective recurrent parents  $\varphi$ . The next was the last time of selfing and one time of backcrossing, of which the backcrossing also used the respective recurrent parents  $\varphi$ , until the autumn of 2012 the four new materials

for the combination were obtained. After three generations of mixed batches rearing, the single batch-rearing was started, mating within the same bath and giving priority to interbatch selection, and until the fourth generations of single batch rearing, crossing among different batches. From the  $F_8$  generation, good lines of each race were selected to combine in 2-way or 4-way-cross, following check test to select excellent combinations in laboratory. In the autumn of 2014 and the spring of 2015, the combination with best comprehensive economic characters, Jingsong A G 9202  $\cdot$  Jingsong B G 9202  $\times$  Haoyue A G 9202  $\cdot$  Haoyue B G 9202 participated in and passed the identification and production tests in the rural identification areas of silkworm varieties in Hunan Province. In 2015, the color fastness test of characteristic raw silk was carried out, and the result was in line with the national standards for the color fastness of textiles. In the spring of 2016, it was evaluated and approved by the expert group of Hunan Provincial Crop Variety Approval Committee and designated Xiangcailu No. 1. The breeding process is shown in Table 1.

**Table 1** Breeding of natural green cocoon variety Xiangcailu No. 1 (rearing once in spring, summer, autumn and late autumn, respectively, in one year)

Generation	Season	Breeding process and method	Rearing form	Material
Female parent	Spring of 2010	Introduced from Sericultural Research Institute, Chinese Academy of Agricultural Sciences in 2006	Single batch rearing	Jingsong A B, Haoyue A B
Male parent	Spring of 2010	Provided by Soochow University in 2009	Single batch rearing	G 9202
$F_1$ , $BC_1F_1$	Summer of 2010 – autumn of 2010	Hybridizing the male and female parents in spring, rearing $F_1$ and backcrossing the recurrent parent $\delta$ in summer	Mixed batches rearing	Jinglu A B, Haolu A B
$BC_1F_2$ , $BC_2F_1$ , $BC_3F_1$	Late autumn of 2010 – summer of 2011	Selfing and back-crossing with the recurrent parent $\delta$ twice	Mixed batches rearing	Jinglu A B, Haolu A B
$BC_3F_2$ , $F_2$ , $BC_4F_1$	Autumn of 2011 – spring of 2012	Due to the female complete linkage in an autosome of the female silkworms <sup>[9]</sup> , selfing, then hybridizing with the intervening parent $\delta$ , and finally backcrossing the recurrent parent $\varphi$	Mixed batches rearing	Jinglu A B, Haolu A B
$BC_4F_2$ , $BC_5F_1$ , $F_3$ – $F_4$	Summer of 2012 – spring of 2013	Selfing, then backcrossing with the recurrent parent $\varphi$ and finally selfing in succession	Mixed batches rearing	Jinglu A B, Haolu A B
$F_5$ – $F_7$	Summer of 2013–late autumn of 2013	Mainly based on interbatch selection, partially based on selection individuals. Reeling good cocoons in alive from excellent batch with the characteristics of high larva-pupa rate and thick cocoon shell <sup>[10]</sup> , and constructing good lines by excellent $\times$ excellent mating	Single batch rearing, mating in the same batch	Jingsong A G 9202, Jingsong B G 9202, Haoyue A G 9202, Haoyue B G 9202
$F_8$ – $F_{12}$	Spring of 2014 – spring of 2015	Mainly based on interbatch selection, partially based on selection individuals. Combining with good lines based on multiple hybrids, continuously carrying out $F_1$ hybrid laboratory tests and silkworm eggs production trials, and participating in the identification tests in the rural identification areas of Hunan Province and the large-scale trials in rural areas in the late autumn of 2014 and the spring of 2015.	Single batch rearing, mating in different batches	Jingsong A G 9202, Jingsong B G 9202, Haoyue A G 9202, Haoyue B G 9202
	Autumn of 2015	Color fastness test of characteristic raw silk (satisfying the standards) in National Silk and Garment Quality Supervision and Testing Center.		
	Spring of 2016	On-site evaluation and variety approval by the expert group of Hunan Provincial Variety Approval Committee.		

## Identification of the $F_1$ Hybrid

### Laboratory comparison test

In the spring of 2014 – 2016, with the national designated control variety Jingsong  $\times$  Haoyue as the control, the larva-pupa rate

was slightly higher than that of the control, and the whole cocoon weight (2.07 g), cocoon crop per 10 000 silkworms (4.97 kg), cocoon filament length (1 252.2 m) and non-broken filament length (1 005.1 m) were all similar to those of the control. The neatness

was 94.9 points, which was 0.8 point higher than that of the control. It shows that the new variety for special purposes is strong to rear easily, with high yield, excellent silk quality and high practical level. The test results of various indexes are shown in Table 2.

**Table 2 Laboratory identification results of Xiangcailu No. 1 (spring of 2014 – 2016)**

Variety	Season	Achievements						Silk quality					
		Percentage of dead worm cocoons // %	Larva-pupa rate %	Whole cocoon weight g	Cocoon shell weight g	Cocoon shell ratio %	Cocoon crop per 10 000 larvae // kg	Cocoon shell weight per 10 000 larvae // kg	Cocoon filament length m	Non-broken filament length m	Reelability %	Denier D	Neatness points
Xiangcailu No. 1	Spring of 2014	1.02	97.74	2.06	0.490	23.79	20.63	4.91	1 248.0	972.6	77.90	2.617	95.0
	Spring of 2015	1.66	95.90	2.03	0.494	24.33	19.76	4.81	1 211.7	973.4	80.30	3.033	94.5
	Spring of 2016	2.13	95.78	2.12	0.522	24.62	21.09	5.19	1 296.8	1 069.2	82.45	2.860	95.3
	Mean	1.60	96.47	2.07	0.502	24.25	20.49	4.97	1 252.2	1 005.1	80.22	2.837	94.9
Jingsong × Haoyue	Spring of 2014	0.95	97.99	2.08	0.505	24.28	20.55	4.99	1 271.0	950.8	74.80	2.807	92.5
	Spring of 2015	1.81	94.90	2.07	0.510	24.64	19.71	4.86	1 275.1	1 043.3	81.80	2.888	94.5
	Spring of 2016	2.41	95.03	2.15	0.541	25.17	21.22	5.34	1 337.5	1 068.1	79.86	2.932	95.2
	Mean	1.72	95.97	2.10	0.519	24.70	20.49	5.06	1 294.5	1 020.7	78.82	2.876	94.1
	Difference	-0.12	+0.50	-0.03	-0.017	-0.45	0	-0.09	-42.3	-15.6	+1.4	-0.039	+0.8

**Rural trials and large-scale rearing**

In the late autumn of 2014 and the spring of 2015, multipoint comparative tests of new varieties were carried out in different cities and counties such as Jinshi, Xiangtan, Xiangxiang, Yuanling and Yueyang. In the late autumn, the cocoon output per box was 38.4 kg, which was 1.31% higher than that of the control variety Jingsong × Haoyue. In the spring of 2015, the cocoon output per box reached 39.9 kg, which was 1.79% higher than that of the control variety (39.2 kg). Meanwhile, in order to compare and identify the yield stability and high yielding property of new silkworm varieties, the rural comparative trials of new varieties were organized in the key sericultural villages of the above five sericultural cities and counties. Among them, in the late autumn of 2014, a total of 40 boxes of the new silkworm variety Xiangcailu No. 1 and the control were reared at four bases, respectively, and the new variety showed the cocoon output per box of 37.05 kg, 1.47% higher than that of the control (36.63 kg). In the spring of 2015, a total of 54 boxes of Xiangcailu No. 1 and the control were reared at five bases, and the new variety exhibited the cocoon output per box of 37.62 kg, which was slightly higher than that of the control (37.58 kg).

**Table 3 Color fastness detection analysis of natural green silk**

No.	Detection items	Unit	Detection result
1	Color fastness to soaping	Changed	Grade 4 – 5
		Stained	Grade 4 – 5
2	Color fastness to dry rubbing	Grade	4 – 5
3	Color fastness to wet rubbing	Grade	4 – 5
4	Color fastness to light	Grade	4

The data is provided by National Silk and Garment Quality Supervision and Testing Center. 4 to 5 means that half grade is supplemented between grade 4 and grade 5.

**Identification of colored silk fastness**

Color fastness refers to the resistance of the color of a textile to various effects during processing and use. We sent the natural colored silk samples produced by this variety to National Silk and

Garment Quality Supervision and Testing Center. The natural colored filament produced by this variety has the color fastness to light of grade 4, and the color fastness to dry rubbing, wet rubbing fastness and soaping all of grade 4 – 5, respectively. The test results show that the natural colored silk produced by this variety has stable color and can meet the national standards of for color fastness of textiles<sup>[11-14]</sup>. The results are shown in Table 3.

**Main Traits and Characteristics**

**Characters of original varieties**

Jingsong A G 9202 • Jingsong B G 9202: Chinese strain, bivoltine, F<sub>1</sub> hybrid for crossing, four-molting. The hibernating eggs have a gray color in the yellow-green background and the egg shell is pale yellow. The newly hatched larvae are dark brown, and the newly hatched larvae and young silkworms are characterized with tendency to density and phototaxis; Each grown silkworm has a strong bluish white body with pale marking, and has the characteristics of uniform molting and fast food taking; and the silkworms get mature uniformly, urinate more, and like cocooning in the upper layer of the frame, which is in the shape of a short ellipsoid, medium to thin in size of wrinkle, in green color. The moths are white, appear in concentrated form, and have a good mating performance. A moth spawns about 500 eggs. The hatching period of silkworm eggs is about 10 d, the silkworm rearing season is 24 d, and the pupal period is 15 d. When mating with Haoyue A G 9202 • Haoyue B G 9202, the eggs should be taken out from the storage 2 d later than that of the race for of reciprocal cross, and mounting should be late 1d.

Haoyue A G 9202 • Haoyue B G 9202: Japanese strain, bivoltine, F<sub>1</sub> hybrid for crossing, four-molting. The hibernating eggs are maroon, and the egg shell is white. The newly hatched larvae are black brown, and the newly hatched larvae and young silkworms are dispersive; each grown silkworm has a bluish white body slightly thin and long with normal marking, and has the characteristics of slowly moulting and slightly slow food taking. The newly molted silkworms of 5<sup>th</sup> instar show brown secretion at the caudal

portion. They mount uniformly, and the cocoons are in the shape of slightly narrow waist, medium to thin in size of wrinkle, in green color. The moths are white, appear uniformly, and have a good mating performance. A moth spawns about 450 eggs. The hatching period of silkworm egg is about 10 d, the silkworm rearing season is 25 d, and the pupal period is 16 d. When mating with Jingsong A G 9202 • Jingsong B G 9202, the eggs should be taken out from the storage 2 d earlier than that of the race for reciprocal cross, and mounting should be brought forward 1 d.

### Characters of F<sub>1</sub> hybrid

This hybrid is a natural green cocoon silkworm variety for spring rearing. It has the characteristics of stable and consistent raw silk color, good healthiness and easy rearing, high cocoon production and excellent silk quality. It is suitable for spring promotion in the Yangtze River basin including Hunan Province. When using Jingsong A G 9202 • Jingsong B G 9202 as the female parent, the hibernating eggs of the hybrid is gray in the yellow-green background; the egg shell is light yellow; and the number of eggs per 1 g is about 1 600, and the number of newly hatched larvae per 1 g is about 2 200. When using Haoyue A G 9202 • Haoyue B G 9202 as the female parent, the hibernating eggs is brown red, and the egg shell is white; and the number of eggs per 1 g is about 1 700, and the number of newly hatched larvae per 1 g is about 2 300. The eggs hatch uniformly, and the newly hatched larvae are dark brown and dispersive. During the stage of young larvae, silkworms are phototaxis, molt uniformly and exuviating evenly, have the characteristics of fast moulting, and attention should be paid to dispersing and spacing timely the silkworms. The larvae eat mulberry leaves lively, and the grown silkworms eat fast and strongly, have no habit of treading on leaves, so they can be reared easily. The grown silkworms are bluish white with light normal marking, stout and strong, but the moisture resistance is slightly poor during the 5th instar and the mounting, when attention should be paid to ventilation and drying. The silkworms mature uniformly. The mature silkworms are light beige red, and like cocooning in the upper layer of the frame, cocoon grain is large and even, green, medium to thin in size of wrinkle. In spring, the hybrid exhibits the cocoon layer ratio of 24.0% – 24.5%, the cocoon filament length of 1 250 – 1 350 m, the non-broken filament length of 950 – 1 100 m, moderate denier, and excellent neatness.

### Major Points in Rearing of the F<sub>1</sub> Hybrid

The newly hatched larvae have strong phototaxis, meanwhile being dispersive. The illumination for hatching eggs should be postponed appropriately on the day of collecting the larvae, and the preparation for larvae collection should be done in advance.

The phototaxis and tendency to density during the young silkworm period is more obvious, so it is necessary to disperse and space the silkworms frequently. In the stage of grown silkworm period, the silkworms eat a large amount of mulberry leaves and excrete many excretions, and the silkworm rearing beds are moisturized easily, so it is necessary to strengthen ventilation and dusting powder for dry.

The silkworms mature uniformly with a large amount of mois-

ture, the density on the frames should be well controlled. Meanwhile, a good ventilation condition should be given to keep a dry cocooning condition.

Instant acid treatment is carried out at a temperature of 46 °C, in the hydrochloric acid with a specific gravity of 1.075, 5 minutes for the Chinese lines, 5.5 minutes for the Japanese lines. As to acid treatment after chilling, it is carried out at a temperature of 47.8 °C, in the hydrochloric acid with a specific gravity 1.092, 6 minutes for the Chinese lines, 6.5 minutes for the Japanese lines.

### Conclusions and Discussion

Xiangcailu No. 1 is a natural green cocoon silkworm variety bred by Sericultural Research Institute of Hunan Province collaborated with Soochow University, which has the characteristics of good healthiness and easy rearing, stable silk color, good silk quality and high practicality. The main blood line of this variety is the national designated control variety Jingsong × Haoyue for spring rearing, which was subjected to intervening crossing with medium silk amount race improved 21K or improved 22K which is strong to rear easily, thus ensuring the introduction of the natural green cocoon control gene into the basic materials when integrating the characteristics of high yield, good quality and strong body of multiple parental materials. A new combination was obtained through pedigree breeding and multiple cross, and the test and identification results show that it has high practicability and stability, and has achieved the goal of improving the basic variety with natural green cocoon. In the process of hybridization of new materials, five times of backcrossing and one time of intervening crossing were completed by the recurrent backcrossing method, so that theoretical ratios of genetic components of the practical parents in these new materials were close to 99.22%  $[1 - (1 - 1/2)^7]$ . The first three times of backcrossing and one time of intervening crossing all use the male individuals, and the latter two times of backcrossing used the female individuals, mainly to take full advantage of complete linkage in female silkworm individuals, and meiotic autosomal chiasmata and crossing over in male silkworm individuals, accelerate genetic recombination and further expand the chances of obtaining excellent breeding materials. The cocoon color of the natural green cocoon variety G 9202 is controlled by two pairs of complementary genes Ga and Gb<sup>[15]</sup>, which are segregated rapidly during the subculture. To this end, we have established a recurrent backcrossing scheme with two times of backcrossing and one time of selfing<sup>[10]</sup>, and in each generation of subculture individuals, it is necessary to select good individual descendants that meet or approach the target-focused color. In particular, the selfing in each cycle is to re-aggregate the segregated cocoon color control genes in the target-focused individuals, which speeds up the breeding process compared to the traditional method of one time of backcrossing followed by one time of selfing.

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developed with strong rhizomorph and more branches; and the dry weight of mycelium can reach  $(0.2967 \pm 0.0280)$  g. The overall growth status was better than that on natural synthetic media such as wheat bran and PDA alone. However, after adding sawdust to wheat bran and PDA, the growth rate and dry weight of *A. mellea* were significantly improved, and the number of mycorrhizal branches increased slightly as well, which might be because that lignin and hemicellulose in sawdust can promote the growth of *A. mellea* mycelia, which is consistent with the results of Shi *et al.*<sup>[10]</sup>.

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