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Breeding of biparental sex-limited larval marking yellow cocoon variety "Hanbyeolnue"

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Abstract

All of silkworm varieties reared in farmhouses are first generation hybrids, for the production of these hybrid silkworms precise and labor-saving sex discrimination is necessary. The new variety "Hanbyeolnue" is biparental sex-limited larval marking yellow cocoon variety which was bred from biparental sex-limited strain of Japanese originated Jam 319 and Chinese originated Jam 320. Productivity test of Hanbyeolnue in 2015 showed high healthiness and short larval period. The Hanbyeolnue was evaluated as an excellent variety which can be utilized for special purpose silkworm such as male pupae or Cordyceps production.

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Introduction

All of commercial silkworm varieties being reared in the farm houses are first generation hybrid (F_1). For the production of commercial varieties, sex discrimination of parent strain is essential. Sex discrimination is conducted by the external characteristic of sexual spot of larvae or pupae with naked eyes and sometimes it is conducted by the weight difference of male and female cocoons. But all of these sex discrimination methods need experienced manpower, have problems such as sex discrimination error and limited time window for sex discrimination. Simpler and more accurate sex discrimination methods having been developed to solve these problems. One of this is using sex-limited inheritance. Sex-limited inheritance is allowing useful marker allele expression only to female larvae by translocation of the allele from a chromosome to that of female sex-determining chromosome (Hasimoto, 1948, Lee *et al.,* 1989), and put to practical use as sex-limited silkworm varieties.

Several biparental sex-limited larval marking varieties such as Yagwonjam(Kang *et al*., 2000) , Hansaengjam(Kang *et al*., 2011) and Dodamnue, and sex-limited biparental yellow cocoon variety Hwangbojam(Kang *et al*., 2013) were developed by NAAS, RDA and being supplied to farmhouses. To increase the sex discrimination efficiency, the biparental sex-limited larval marking yellow cocoon variety was developed to integrate advantage of each variety.

The Hanbyeolnue designated as a recommended silkworm variety was bred from sex-limited larval marking yellow cocoon variety parent and it is superior to biparental sex-limited larval marking varieties and biparental sex-limited yellow cocoon varieties in practical sex discrimination efficiency. This variety can be utilized for producing functional material which can

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be used separately by sex, and is labor-saving variety bred to respond the needs of times which can reduce silkworm egg production effort significantly.

We report briefly the major characteristics and breeding history of the "Hanbyeolnue" world first biparental sex-limited larval marking yellow cocoon variety which enables sex discrimination by larval marking and cocoon color in single variety

Material and Methods

Rearing method and cocoon reeling test

This study was conducted in 2015 following "Sericultural Experiment Guide" published by RDA using "Hansaengjam" and "Hwangbojam" as control varieties at 8 local places of Korea(RDA 2010).

Silkworms were hatched under the conditions of 15~26°C, 75~80% humidity, 16 h light and 8 h darkness of photoperiod, and reared in the spring of 2015.

Silkworms were reared under 25~26°C, 75~85% humidity during 1st~3rd instar covered with wax paper, then reared under 23~24°C temperature, 65~75 humidity in silkworm rearing basket during 4th and 5th instar following "Sericultural Experiment Guide" (RDA, 2010), they were fed three times a day through each instar. Each hybrid variety had 1,500 heads of silkworms to be tested from 2nd day of 4th instar, and rotation mounting frames were used. The economic characteristics of each hybrid were investigated on 8th day of mounting by investigating pupation ratio, cocoon yield of 10,000 head silkworms. Weights of male and female single cocoon, cocoon shell and cocoon shell percentage were measured separately and average was determined. Also characteristics of parent strain, examination of hypoglycemic component of silkworm, Cordyceps productivity examination and reeling test were conducted by "Sericultural Experiment Guide(RDA 2010)".

Examination of hypoglycemic agent content in silkworm

For the quantification of hypoglycemic agent, 1-Deoxynojirimycin (DNJ) was extracted from 0.1g of dried sample. Sample was intensely stirred 2 times for 15 s in the 10mL 0.05 M HCL solution and diluted with 100 mL water. After adding "FMOC99-Fluorenyl Methyl Oxy Carbonyl, quantification was conducted following "Quality control guidance for DNJ quantification"(Kim *et al*., 2003).

Examination of Cordyceps productivity

To test Cordyceps productivity of Hanbyeolnue, 3 repetition of 250 heads were designated on 2nd day of 4th instar, and Cordyceps was inoculated 3 times with 12 h interval after more than 90% of silkworm became 5th instar. Cultivation and maintenance of Cordyceps was conducted following "Sericultural Experiment Guide (RDA, 2010).

Results and Discussion

Breeding history

The "Hanbyelnue" was crossbred from sex-limited larval marking yellow cocoon strain possessed by NAAS RDA using breeding technique focusing improvement of useful characteristics. These parent strains were succeeded and selected 3 times every year, they were reared from 1 egg batch until the generation for combination test, and selected by characteristics of larva and cocoon.

This variety is 1st generation hybrid of Japanese and Chinese bi-voltine strain, Japanese originated Jam319(JS205) is sex-limited larval marking yellow strain from crossbreeding of MY 802 and M9806 in 2010, and Chinese originated sex-limited larval marking yellow cocoon strain Jam 320 (CS218) is from crossbreeding of MY802 and M9806 in 2010. Combining ability test of these strains were conducted through spring and autumn rearing of 2014, investigation of original strain characteristics,

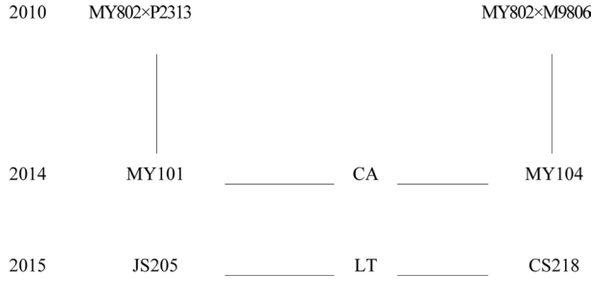

Fig. 1. The Pedigree of Hanbyeolnue, the F_1 hybrid between JS205 × CS218.
CA : Combining ability test, LT : Local adaptability test

Table 1. The important economic characteristics of Hanbyeolnue from the combining ability test in spring, 2014

Variety	Pupation percentage (%)	Cocoon yields from 10,000 3rd molted larvae (kg)	Single cocoon weight (g)	Cocoon shell weight (cg)	Cocoon shell percentage (%)	Filament length (m)	Reel-ability (%)	Raw silk percentage (%)
Hansaengjam	95.2	19.1	2.27	55.9	24.6	1,555	78	20.74
Hwangbojam	95.3	18.3	2.14	54.3	25.4	1,607	79	20.82
Hanbyeolnue	96.7	19.1	2.09	41.6	19.9	1,507	85	18.02

Table 2. Rearing results of Hanbyeolnue through the local adaptability test performed at 8 places in spring 2015

Variety	Useful hatchability (%)	Larval period (d.h)	Pupation percentage (%)	Best cocoon rate (%)	Double cocoon rate (%)
Hansaengjam	95	25.04	94.1	94.1	0.7
Hwangbojam	96	25.03	94.3	94.2	1.2
Hanbyeolnue	96	24.03	94.9	94.7	1.9

Variety	Cocoon yield per 10,000 3rd molted larvae (kg)	No. of cocoons per liter (ea)	Single cocoon weight (g)	Cocoon shell weight (cg)	Cocoon shell percentage (%)
Hansaengjam	21.4	56	2.32	57.3	24.7
Hwangbojam	21.2	52	2.29	56.2	24.6
Hanbyeolnue	20.6	60	2.22	48.4	21.8

DNJ content, Cordyceps productivity were finished in 2011 spring rearing, collaborated productivity test with 7 local sericultural experiment station proved the superiority as special purpose variety, and designated the 1st biparental sex-limited larval marking yellow cocoon variety named as "Hanbyeolnue" through Sericulture Promotion Board.

* Sex-limited larval marking : sex discrimination of male and female by larval marking(♀: marking, ♂ : plain)

* Sex-limited yellow cocoon : sex discrimination of male and female by cocoon color(♀ : yellow, ♂ : white)

Combining ability test

The methods used to select crossbreed combination in silkworm breeding are Diallele cross and Top cross for analysis of quantitative characteristics, and Top cross method is extensively used currently as it was recognized as effort efficient and improving analysis results(Sohn and Hong, 1986; Harada, 1961).

Table 1 shows the combining ability test results of Hanbyeolnue which was crossbred of Chinese strain and Japanese strain by 3 x 3 top cross, and Hansaengjam and Hwangbojam was compared as control. Hanbyeolnue showed 96.7% of pupation ratio, 2.09 g of single cocoon weight, 19.9% of cocoon shell percentage, 1,507m of filament length and 85% of reelability. This variety showed higher pupation ratio than control, but its single cocoon shell weight is lower than control results lower silk yield.

Table 2 shows the rearing results conducted by RDA and 7 local sericultural experiment station to examine the productivity and local adaptation ability of Hanbyeolnue (Jam 319 Jam 320) during spring rearing season in 2015. The hatchability of Hanbyeolnue was 96%, it is 1% higher than that of control Hanseangjam (Jam 153x Jam 154), also it is higher than recommended variety designation standard of 90%, and its larval period was 23 h shorter than control.

Table 3. Cocoon reeling results of Hanbyeolnue through the local adaptability test performed at 8 places in spring 2015

Variety	Filament length (m)	Filament weight(cg)	Filament size (d)	Reelability (%)	Raw silk percent (%)	Raw silk yield (kg)
Hansaengjam	1,409	49.1	3.15	77	20.89	4.46
Hwangbojam	1,376	46.5	3.04	76	19.80	4.20
Hanbyeolnue	1,254	40.4	2.90	79	18.02	3.71

※ Raw silk yield was calculated from multiplication between cocoon yield per 10,000 3rd molted larvae and raw silk percent.

The pupation percentage and best cocoon rate were similar with control varieties,. Because of Hanbyeolnue's short larval period, its 20.6 kg of 10,000 cocoon yield was 0.8 kg less than that of control 21.4 kg.

Cocoon reeling test

Table 3 shows reeling test results of Hanbyeolnue. The filament length of Hanbyelnue is 1,254 m which is 155 m shorter than control Hansaengjam, and filament weight 40.4 cg was 8.7 cg less than 49.1 cg of control. Its reelability was 2% higher than control. The raw silk yield of 10,000 cocoon was 3.71 kg which is 0.75 kg less than control, and its filament size was 2.90 denier.

Examination of DNJ content and Cordyceps productivity

The DNJ(1-Deoxynojirimycin) content of Hanbyeolnue on 5th instar 3rd day was 3.23mg/g which is more than 2.97mg/g of control Hansaengjam (Table 4). Hanbyeolnue showed 94.2% of pupation rate, 90.7% of incidence ratio, and more fruit body which are better than control. The cultivation period was 17 d (Table 5).

Table 4. Examination of hypoglycemic agent content(DNJ) spring 2015 (unit : mg/g)

Variety	Hansaengjam	Hwangbojam	Hanbyeolnue
DNJ content	2.97	2.57	3.23

Major characteristics of parent strain

The major characteristics of Jam 319 and Jam 320, the parent strain of Hanbyeolnue were compared with Jam 153 and Jam 154, the parent strain of control Hansaengjam is shown in Table 6. Japanese originated Jam 319 has sex-limited larval marking (♀:mark, ♂:plain), sex-limited cocoon color(♀:yellow, ♂: white) and peanut shaped cocoon. It showed better results in pupation percentage (88.8%), raw silk yield(11.6kg) and oviposition (562 eggs/batch) than those of control.

Chinese originated Jam 320 is sex-limited larval marking yellow cocoon strain similar with Jam 319 with oval shaped cocoon. It showed 84.8% of pupation percentage, 12.5 kg of raw silk yield, and 1.63 g of single cocoon weight. Its oviposition 562eggs/batch was more than that of control.

Summary

The commercial silkworm variety supplied to farmhouses is a hybrid, so correct and labor saving sex discrimination

Table 5. Cordyceps productivity test

Variety	Pupation rate (%)	Infection rate (%)	Living weight (g)	Incidence rate of fruit body			No. of a fruit body (ea)	No. of cultivation days (d)
				No. of Implantation (ea)	No. of Incidence (ea)	incidence rate (ea)		
Hansaengjam	90.6	89.5	1.51	206	201	98	91	17
Hwangbojam	90.2	92.1	1.42	208	203	98	88	17
Hanbyeolnue	94.2	90.7	1.49	213	208	98	88	17

* Result of 250 heads in 2015 spring

Table 6. The major commercial characteristics of the parents of Hanbyeolnue

Variety	Useful hatchability (%)	Larval period (days.hrs)	Pupation rate (%)	Cocoon yield per 10,000 3rd molted larvae (kg)	Single cocoon weight (g)	Cocoon shell weight (cg)	Cocoon shell percentage (%)
Japanese races							
Jam153	92	25.22	34.4	5.2	1.57	35.3	22.5
Jam317	87	25.10	52.4	6.5	1.29	29.0	22.5
Jam319	96	25.03	88.3	11.6	1.53	29.2	19.1
Chinese races							
Jam154	95	25.22	80.6	12.9	1.62	40.7	25.1
Jam318	89	25.22	72.3	11.3	1.67	38.2	22.9
Jam320	89	25.02	84.8	12.5	1.63	34.7	21.3

Variety	Percentage of moth emergence (%)	Duration from incubation to moth emergence (days)	No. of eggs per batch (ea)*	Percentage of moth laid normal eggs (%)	Laval Marking**	Cocoon color**	Cocoon shape (%)
Japanese races							
Jam153	98	57	515	92	A	white	peanut
Jam317	87	57	432	75	mark	B	
Jam319	99	54	562	100	A	B	
Chinese races							
Jam154	97	56	444	89	A	white	elliptical
Jam318	96	56	488	100	white	B	
Jam320	100	53	484	94	A	B	

*Average of eggs/batch from 10 moths
** A: Mark(♀:mark, ♂:plain), B: Color : (♀:yellow, ♂:white)

is necessary. To meet these needs, the "Hanbyeolnue" was developed in 2015, its sex discrimination is possible with marking at larval stage and with cocoon color after mounting.

The major characteristics of Hanbyeolnue are as below.

1. The Japanese originated parent strain Jam 319 is sex-limited larval marking –yellow cocoon which is crossbreed of MY802 and P2313 in 2010, and the Chinese originated parent strain Jam 320 is also sex-limited larval marking – yellow cocoon which is crossbreed of MY802 and M9806 in 2010.

2. The productivity test in 2015

Pupation percentage of Hanbyeolnue is 94.9% and means its larvae are strong and healthy. Single cocoon weigh was 2.22 g and 10,000 cocoon silk yield was 20.6 kg those were both 4% less than control Hansaengjam. Reeling test showed its filament length was 144 m shorter than that of control 1,409 m and its filament weight 40.4cg was 18% less than control.

3. The egg production ability of parent strain was higher than control, Japanese originated strain Jam 319 showed 562 eggs/batch which is 5% more than control Jam 153, and Chinese originated strain Jam 320 showed 484 eggs/batch which is also 5% higher than control Jam 154.

Fig. 2. Characteristics of Hanbyeolnue
(larvae :♀-marking, ♂-plain; cocoon: ♀-yellow, ♂-white)

4. The Hanbyeolnue is evaluated as superior variety with good practical characteristics considering high pupation percentage and shorter larval period even though its 10,000 head raw silk yield is less than control. The peculiarity of this variety is easy sex discrimination of larvae and cocoon which can save 70% of labor and it is suitable for special purpose variety for male pupae or Cordyceps production.

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