Breeding of a Silkworm Variety for Synnemata Production of *Isaria tenuipes*

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(Received June 16, 2010. Accepted July 30, 2010)

This study was conducted out to select a silkworm variety suitable for synnemata production of *Isaria tenuipes*. Four kinds of the mulberry silkworm varieties, *Bombyx mori*, were hybridized using a Japanese parental line and a Chinese parental line, and used to test for synnemata formation in *I. tenuipes*. The larval period of normal silkworms was 22 hr longer than the silkworms inoculated with this fungus. Among the silkworm varieties tested, Hachojam had the shortest larval period with 23.02 days. The non-cocooning silkworm had a shorter larval period than the cocoon producing silkworms. The pupation rate of normal silkworms was about 9% higher than that of silkworms sprayed with *I. tenuipes*. Hachojam had the highest infection rate at 99.8%, but no significant difference was observed for the infection rate by silkworm variety. The production of synnemata was the best in JS171 × CS188 with an incidence rate of 99.3%, followed by Hachojam, and Chugangjam. The synnemata produced from Hachojam were the heaviest and showed white or milky-white in color.

KEYWORDS : *Bombyx mori*, *Isaria tenuipes*, Spraying, Synnemata

There are more than 700 species of entomopathogenic fungi in the world [1, 2]. About 78 species have been collected and identified in Korea based on host type and fruit body shapes [3]. *Isaria tenuipes* (formerly *Paecilomyces tenuipes*) is a common entomogenous fungus found in many mountainous areas of Korea [4]. The synnemata of this fungus is called snowflake Dongchunghacho due to its appearance. It has been reported to have biological and pharmaceutical compounds such as adenosine and N⁶-(2-hydroxyethyl) adenosine [5-7]. Artificial culture techniques have been developed for synnemata production of this fungus [8]. Most farmers have used cereal substrates such as unconverted rice grain to produce the synnemata of entomopathogenic fungi. Many researchers have tried to find alternative substrates (insects) suitable for stromata production of these fungi. There is a well established cottage industry in Korea to produce silkworm powder as a dietary supplement to improve health. We have bred many mulberry silkworm varieties, *Bombyx mori*, for these purposes, but no silkworm varieties have been bred for stromata production of this fungus. Therefore we tested the larval period and pupation rates of the bred silkworm varieties, and infection and incidence of *I. tenuipes* on them, and finally selected the silkworm variety suitable for synnemata production of this fungus.

Materials and Methods

Host insect. Four kinds of silkworm varieties of *B. mori* including a non-cocooning silkworm were used for synne-

mata formation by *I. tenuipes*.

Chugangjam is an F1 hybrid between the Japanese race Jam 147 and the Chinese race Jam 148 produced in 2003. The Japanese race pure line Jam 147 (breeding line JS 143) was crossed between stock lines of P 8503 and 8453 in 1994, and the Chinese pure line Jam 148 was crossed between stock lines of M 8306 and Jam 130 in 1991. A non-cocooning silkworm variety, Hachojam, was bred by single cross method between the Japanese race and the Chinese race. Hachojam is a variety generated by a single cross F1 hybrid between the Japanese race Jam 307 (breeding line D 98) and the Chinese race Jam 126 (breeding line CS 82) in 2002. The Japanese pure line Jam 307 was selected from a stock line of naked pupae, and the Chinese race pure line Jam 126 was a hybrid between breeding lines of CS 24 and jam 118. Furthermore, new silkworm varieties such as JS171 × CS188 and JS173 × CS188 were bred for this experiment. The crossed silkworms were reared with natural mulberry leaves by the guide book of the silkworm rearing of the National Academy of Agricultural Science, Rural Development Administration (RDA), Korea.

Fungal strain. Strain of *I. tenuipes* used in this study was isolated from conidiospores of a specimen collected at Mt. Halla on Cheju Island in July 2003.

Inoculum preparation. The conidiospores of *I. tenuipes* were obtained from unconverted rice grain medium. The medium was made by mixing 150 g of unconverted rice grain, 15 g of silkworm pupae powder, and 80~100 mL of distilled water. The mixture was poured into a 500 mL

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flask, and each flask was autoclaved at 121°C for 20 min and inoculated with mycelial discs (5 mm) of *I. tenuipes* from the growing margins on potato dextrose agar medium. The inoculated media were cultured at 25°C for 30 days and shaken at three-day intervals to produce mass conidia [9]. The conidiospores formed on unconverted rice grain media were harvested using a sterile inoculation loop and added with 0.2 mL of 0.02% Tween 20 solution to uniformly disperse the spore suspension.

Inoculation. The concentration of the spore suspension was adjusted with sterilized distilled water to 10^8 spores/mL, and the number of spores was counted with a hemacytometer (Superior, Marienfeld, Germany). The spore suspension added with starch syrup for an easy attachment on the silkworm was inoculated on each larvae of 5th instar newly exuviated silkworm using the spraying method [9]. Each test was sprayed three times at 12-hr

intervals (Fig. 1). The inoculated silkworms were reared with natural mulberry leaves until mounting of the silkworm, as directed by the silkworm rearing guidebook of the National Academy of Agricultural Science, RDA, Korea.

Induction of endosclerotium and synnemata. The cocoons were harvested at 11 days after mounting from the cocoon frames. The silkworm pupae were picked out from the cocoons after cutting the tops with a knife the following day. The infected pupae were placed on a wet cotton cloth at 1-cm square distance in transparent plastic containers. Each container with the inoculated pupae was kept in a growth room controlled at 20~22°C and a relative humidity (RH) of about 95% under a dark condition to induce synnemata formation. The containers were supplied with water periodically to prevent excessive drying.

Results and Discussion

Larval period. The average larval period of a normal silkworm used in this study was 24 days and 18 hr, and that of the silkworms sprayed with *I. tenuipes* was 23 days and 20 hr at 25.4°C and 81% RH (Table 1). The larval period is the time from the stage of a newly hatched silkworm to the mounting of the mature silkworm larva after the fifth instar. The larval period of normal silkworms was longer than the silkworms inoculated with *I. tenuipes*. It was assumed that the developmental period was shortened because of the physiological changes in the silkworm from the fungi inoculation [10]. Hachojam was the shortest with 23.02 days, followed by Chugangjam and JS173 × CS188 with 24.00 days, and JS171 × CS188 with 24.04 days. Non-cocooning silkworm had a shorter larval period than cocoon producing silkworms. Hachojam had the shortest larval period among the tested silkworms.

Table 1. Larval period of the silkworm varieties bred

Hybrid	25.4°C and 81% relative humidity	
	Larval period (days. hr)	
	Normal silkworm	Inoculated silkworm
Chugangjam	24.22	24.00
Hachojam	24.03	23.02
JS171 × CS188	24.22	24.04
JS173 × CS188	25.00	24.00

Table 2. Pupation rate by silkworm variety

Hybrid	Pupation rate (%)	
	Normal silkworm	Inoculated silkworm
Chugangjam	88.0	86.2
Hachojam	82.9	66.9
JS171 × CS188	79.9	76.8
JS173 × CS188	93.3	77.9

Fig. 1. Larvae of 5th instar newly exuviated silkworm, and the spraying of inoculum onto silkworms. A, Silkworm larvae of 5th instar; B, Spraying inoculum.

Pupation rate. The pupae survival percentage of normal silkworm cocoons ranged from 79.9 to 93.3% with an average of 86.0%, whereas it ranged from 66.9 to 86.2% with an average of 77.0% in the silkworms sprayed with *I. tenuipes* (Table 2). The pupation rate of normal silkworms was about 9% higher than that of silkworms inoculated with *I. tenuipes*. In silkworms sprayed with *I. tenuipes*, 629 normal pupae were harvested from 750 silkworms in Chugangjam (86.2%), 502 silkworms in Hachojam (66.9%), 559 silkworms in JS171 × CS188 (76.8%), and 573 silkworms in JS173 × CS188 (78.0%). Silkworms spin cocoons around themselves and become pupae within the cocoon. But some of the silkworms died within the cocoon or missed from the cocoon frame during the rearing period due to various reasons. The pupation rate was calculated with the number of healthy pupae within cocoons that were made after mounting of the mature larvae. The pupation rate of healthy silkworms was higher than the silkworms sprayed with *I. tenuipes*. Chugangjam had the highest survival percentage of healthy pupae among the silkworms tested.

A

B

Fig. 2. Silkworm pupae infected with *Isaria tenuipes* and synnemata formed on Hachojam silkworms. A, Pupae infected with *I. tenuipes*; B, Synnemata formed on pupae.

Infection rate. After cutting the harvested cocoons, the pupae infected with *I. tenuipes* were counted by hardness (Fig. 2). Infection of *I. tenuipes* into larvae of 5th instar newly exuviated silkworm was excellent in Hachojam with 99.8% (n = 502), followed by Chugangjam with 98.6% (n = 629), JS173 × CS188 with 98.3% (n = 573), and JS171 × CS188 with 97.6% (n = 559) at following three spray. But, no significant difference was observed in the infection rates among the silkworm varieties (Table 3).

Synnema formation. Synnemata were induced from infected pupae in the growth room with a temperature of 18–20°C and RH of about 95% under a dark condition. The synnemata of *I. tenuipes* were produced from almost all pupae tested (Table 4). Synnemata production of was the best in JS171 × CS188 with an incidence rate of 99.3% (n = 555), followed by Hachojam with 99.0% (n = 497), Chugangjam with 98.9% (n = 622) and JS173 × CS188 with 97.9% (n = 561). But no significant difference was observed in synnemata formation among the silkworm varieties.

Synnemata characteristics. The synnemata weight ranged from 1.33 to 1.71 g with an average of 1.60 g (Table 5). The Hachojam produced the heaviest synnemata with 1.71 g, followed by JS173 × CS188 with 1.61 g, JS171 × CS188 with 1.59 g and Chugangjam with 1.49 g.

The number of synnemata with over 3 cm long ranged

Table 3. Infection rate of *Isaria tenuipes* by silkworm variety

Hybrid	No. of silkworm tested	No. of pupae infected	Infection rate (%)
Chugangjam	638	629	98.6 ± 0.6
Hachojam	503	502	99.8 ± 0.6
JS171 × CS188	573	559	97.6 ± 0.6
JS173 × CS188	583	573	98.3 ± 1.2

Table 4. Synnemata formation of *Isaria tenuipes* by silkworm variety

Hybrid	No. of pupae tested	No. of pupae produced	Incidence rate (%)
Chugangjam	629	622	98.9 ± 0.6
Hachojam	502	497	99.0 ± 0.6
JS171 × CS188	559	555	99.3 ± 1.0
JS173 × CS188	573	561	98.3 ± 2.3

Table 5. Characteristics of synnemata by silkworm variety

Hybrid	Synnema weight (g)	No. of synnemata
Chugangjam	1.49 ± 0.0	13 ± 0.9
Hachojam	1.71 ± 0.1	16 ± 2.5
JS171 × CS188	1.59 ± 0.1	13 ± 0.6
JS173 × CS188	1.61 ± 0.0	15 ± 1.8

from 13 to 16 (mean = 14.3) on a pupa. Sixteen synnemata were produced from a pupa of Hachojam, 13 from Chugangjam and JS171 × CS188, and 15 from JS173 × CS188. Synnemata produced in this experiment were white or milky-white in color (Fig. 2). The synnemata of *I. tenuipes* produced on pupae were similar in shape and color to wild synnemata collected in Korea [10].

In conclusion, these findings suggest that the non-cocooning silkworm, Hachojam which was originally bred for autumn rearing in 2001, can be used as a host for the synnemata production of *I. tenuipes* due to high pupation rate, heavier pupa weight, and labor savings than the other varieties tested.

Acknowledgements

This research was supported by a grant from the Korean Rural Development Administration (Agenda Program, PJ0067792010).

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