Synnemata Production by *Isaria tenuipes* using Colored Cocoon Silkworm, Golden Silk

In-Pyo Hong*, Pil-Don Kang, Kee-Young Kim, Man-Young Lee, Yong-Soo Choi, Nam-Suk Kim, Hye-Kyung Kim, Sung-Hee Nam, and Kwang-Gill Lee

Sericultural & Apicultural Materials Division, National Academy of Agricultural Science (NAAS), Suwon 441-100, Korea

(Received 22 October 2010; Accepted 06 December 2010)

This study was conducted to test the use of colored cocoon silkworm, Golden Silk, as a host for the fruiting body production of *Cordyceps* mushroom. Golden Silk variety had a shorter larval period than Yangwonjam at 25.3°C and 78.5% RH: The average larval period of the Golden Silk was 24 days and 7 hr, and that of the Yangwonjam was 24 days and 15 hr. In the infection test of *I. tenuipes* on silkworm, the larval period of normal silkworms was longer than the silkworms inoculated with *I. tenuipes* in both Golden Silk and Yangwonjam. The pupae survival percentage of Golden Silk was 94.4%, whereas it was 91.9% in Yangwonjam. Golden Silk had a higher pupation rate than Yangwonjam. The pupation rate of normal silkworms was about 5% higher than that of silkworms inoculated with *I. tenuipes*. Infection rate of *I. tenuipes* into larvae of 5th instar newly exuviated silkworm was 93.8% in Golden Silk, and 96.9% in Yangwonjam. Synnemata production of *I. tenuipes* was better in Yangwonjam with an incidence rate of 99.8% than Golden Silk with 98.6%. The average synnemata weight of Golden Silk was 1.12 g and that of Yangwonjam 1.29 g. Golden Silk had a lower synnemata weight than Yangwonjam. But no significant difference was observed in synnemata weight varieties. The synnemata of *I. tenuipes* produced on pupae were white or milky-white in color, and similar in shape and color to wild synnemata collected in Korea.

Key words: *Bombyx mori*, Golden Silk, *Isaria tenuipes*, Spraying, Synnemata

Introduction

Sericulture was one of the most important cottage industries in Korea in 1990's. Many researchers have tried to breed the varieties of silkworm (*Bombyx mori* L.) to improve the quantity and quality of raw silk in 1990s. But after the declination of sericulture industry, sericulture farmers have had an attention on the health foods or dietary supplement such as silk powder and male pupa. These demands have forced the development of the new silkworm varieties suitable for special purposes. Recently new silkworm varieties have been bred for these purposes in Korea: Yangwonjam with a sex-limited larval marking for mass production of male pupae (Kang *et al.*, 2000), Hachojam with non-cocooning silkworm variety for synnemata production of *Cordyceps* mushrooms (Kang *et al.*, 2002), Golden Silk making yellow color cocoon (Kang *et al.*, 2007), and Eolrukmal and Hukpyobeom with peculiar marks in larval stage for an education and experience (Kang *et al.*, 2010a) were bred, respectively.

Biological and pharmaceutical compounds such as cordycepin and N⁶-(2-hydroxyethyl) adenosine were reported in *Cordyceps* mushroom (Furuya *et al.*, 1983; Hsu *et al.*, 2002; Li *et al.*, 2003). Artificial culture techniques have been first developed for the fruiting body production of *Cordyceps* mushroom (Dongchunghacho) using silkworm as a host in Korea (Ha *et al.*, 2005). Many researchers have tried to find silkworm varieties suitable for the fruiting body production of these fungi. The Golden Silk was bred for a deluxe clothes, shroud, wall-paper, and handicraft. Handicraftsmen have used cocoons only as handicraft materials except pupae. Therefore we tested the larval period and pupation rate of Golden Silk, the infection rate and incidence rate of *Isaria tenuipes* at 8 local areas, and finally confirmed the possibility use of Golden Silk as a host for the fruiting body production of *Cordyceps* mushroom.

*To whom the correspondence addressed

Sericultural & Apicultural Materials Division, National Academy of Agricultural Science (NAAS), Seodun-dong, Gwonseon-gu, Suwon-si, Gyeonggi-do, 441-100, Korea
Tel: +82-31-290-8461; Fax: +82-31-290-8503;
E-mail: iphong20@korea.kr

Fig. 1. Larvae of 5th instar newly exuviated silkworm (A), and synnemata formed on Golden Silk pupae (B).

Materials and Methods

Host insect

Two kinds of silkworm varieties of *B. mori*, Golden Silk and Yangwonjam as control group, were used for synnema formation by *I. tenuipes*. Golden Silk is an F1 hybrid between the Japanese race Jam 311 and the Chinese race Jam 312 in 2006 (Kang *et al.*, 2007). The Japanese race pure line Jam 311 (breeding line JS 169) was crossed between stock lines of Jam125, and Kyunsakjuk and Huka in 2002, and the Chinese pure line Jam 312 (breeding line CS 186) was crossed between stock lines of Jam 304 and Goechuk in 2003. Yangwonjam is an F1 hybrid between the Japanese race Jam 144 and the Chinese race Jam in 1999 (Kang *et al.*, 2000). The Japanese race pure line Jam 144 (breeding line JS 129) was crossed between stock lines of P 8503 and H5R1 in 1993, and the Chinese pure line Jam 144 (breeding line CS 98) was crossed between stock lines of M 8312 and 8190 in 1984. 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 Jeju 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 flask, and each flask was sterilized 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 (Ha *et al.*, 2005). 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 (Fig. 1 A). Each test was sprayed three times at 12-hr intervals (Ha *et al.*, 2005). 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.

Table 1. Larval period of the silkworm varieties bred

Hybrid	25.3°C and 78.5% relative humidity	
Hybrid	Larval period (days. hr)	
Hybrid	Normal silkworm	Inoculated silkworm
Golden Silk	24.07 ± 0.16	24.03 ± 0.18
Yangwonjam	24.15 ± 0.65	24.07 ± 0.16

Table 2. Pupation rate by silkworm variety

Hybrid	Pupation rate (%)	
Hybrid	Normal silkworm	Inoculated silkworm
Golden Silk	94.4 ± 5.73	89.4 ± 7.24
Yangwonjam	91.9 ± 8.54	84.7 ± 9.07

Results and Discussion

Larval period

The average larval period of the Golden Silk was 24 days and 7 hr, and Yangwonjam was 24 days and 15 hr at 25.3°C and 78.5% RH (Table 1). Golden Silk variety had a shorter larval period than Yangwonjam. In the infection test of *I. tenuipes* on silkworm, the Golden Silk sprayed with *I. tenuipes* had 24 days and 3 hr, and the Yangwonjam 24 days and 7 hr in the average larval period. The larval period of normal silkworms was longer than the silkworms inoculated with *I. tenuipes* in both Golden Silk and Yangwonjam. It was assumed that the developmental period was shortened because of the physiological changes in the silkworm from the fungi inoculation (Yun, 2005). Our results agree that Hachojam inoculated with *I. tenuipes* had a shorter larval period than normal silkworms (Kang *et al.*, 2010b), though using different silkworm variety.

Pupation rate

The pupae survival percentage of Golden Silk was 94.4%, whereas it was 91.9% in Yangwonjam (Table 2). Golden Silk had a higher pupation rate than Yangwonjam. 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 normal silkworms was about 5% higher than that of silkworms inoculated with *I. tenuipes*. In silkworms sprayed with *I. tenuipes*, 1786 normal pupae were harvested from 1999 silkworms in Golden Silk (89.4%), and 1693 silkworms from 2000 in

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

Hybrid	No. of silkworm tested	No. of pupae infected	Infection rate (%)
Golden Silk	1803	1691	93.8 ± 2.7
Yangwonjam	1726	1672	96.9 ± 3.1

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

Hybrid	No. of pupae tested	No. of pupae produced	Incidence rate (%)
Golden Silk	1687	1663	98.6 ± 2.0
Yangwonjam	1669	1665	99.8 ± 0.3

Yangwonjam (84.7%). Golden Silk had a higher pupation rate than Yangwonjam.

Infection

After cutting the harvested cocoons, the pupae infected with *I. tenuipes* were counted by hardness. Infection rate of *I. tenuipes* into larvae of 5th instar newly exuviated silkworm was good in both of Golden Silk and Yangwonjam at following three sprays (Table 3). Golden Silk was 93.8%, and Yangwonjam 96.9% in an Infection rate.

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 (Fig. 1 B). Synnema production of *I. tenuipes* was better in Yangwonjam with an incidence rate of 99.8% than Golden Silk with 98.6% (Table 4). But no significant difference was observed in synnemata formation among the silkworm varieties.

Synnemata characteristics

The average synnema weight of Golden Silk was 1.12 g, and Yangwonjam was 1.29 g. Golden Silk had a lower synnemata weight than Yangwonjam. But no significant difference was observed in synnemata weight among the silkworm varieties. The number of synnemata with over 3 cm long ranged from 13 to 16 (mean = 13.3) on a pupa. Twelve synnemata were produced from a pupa of Golden Silk, and 14 from Yangwonjam. The colour of these Synnemata produced in this experiment was white or milky white (Fig. 1B). The synnemata of *I. tenuipes* produced on pupae were similar in shape and color to wild synnemata collected in Korea (Sung, 1996).

In conclusion, these findings suggest that the pupae of Golden Silk can be used as a host for the fruiting body

Table 5. Characteristics of synnemata by silkworm variety

Hybrid	Synnema weight (g)	No. of synnemata
Golden Silk	1.12 ± 0.3	12 ± 3.9
Yangwonjam	1.29 ± 0.3	14 ± 5.9

production of *Cordyceps* mushroom, and also its cocoon as handicraft materials.

Acknowledgements

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

References

- Furuya T, Hirotani M, Matsuzawa M (1983) N⁶-(2-hydroxy-ethyl) adenosine, a biologically active compound from cultured mycelia of *Cordyceps* and *Isaria* species. *Phytochemistry* 22, 2509-2512.
- Ha NG, Kim SY, Kang JH, Kang PD, Sung GB and Hong IP (2005) Biological activities and cultural characteristics of an Entomogenous fungus, *Paecilomyces tenuipes* (Peck) Samson. *Korean J Seric Sci.* 47, 12-17.
- Hsu TH, Shiao LH, Hsieh CH and Chang DM (2002) A comparison of the chemical composition and bioactive ingredients of the Chinese medicinal mushroom *DongChongXiaCao*, its counterfeit and mimic, and fermented mycelium of *Cordyceps sinensis*. *Food Chemistry* 78, 463-469.
- Kang PD, Kim KM, Sohn BH, Woo SO, Ryu KS (2000) Breeding of Yangwonjam, a both parent sex-limited larval marking variety suitable for spring and autumn rearing season. *Korean J. Seric. Sci.* 42, 24-27.
- Kang PD, Sohn BH, Lee SU, Hong SJ (2002) Breeding of a new non-cocooning silkworm variety, Hachojam, suitable for autumn rearing season. *Int. J. Indust. Entomol.* 4, 77-81.
- Kang PD, Lee SU, Jung IY, Sohn BH, Kim YS, Kim KY, Kim MJ, Hong IP, Lee KG, Park KY (2007) Breeding of new silkworm Variety Golden Silk, a Yellow cocoon color for spring rearing season. *Korean J. Seric. Sci.* 49, 14-17.
- Kang PD, Jung IY, Kim KY, Kim MJ, Sohn BH, Lee KG (2010a) Breeding of two new silkworm Varieties with peculiar larval mark, Eolrukmal and Hukpyobeom. *Int. J. Indust. Entomol.* 20, 115-116.
- Kang PD, Sung GB, Kim KY, Kim MJ, Hong IP, Ha NG (2010b) Breeding of a silkworm Variety for synnemata production of *Isaria tenuipes*. *Kor. J Mycol.* 38, 180-183.
- Li SP, Zhao KJ, Ji ZN, Song ZH, Dong TTX, Lo CK, Cheung JKH, Zhu SQ, Tsim KWK (2003) A polysaccharide isolated from *Cordyceps sinensis*, a traditional Chinese medicine, protects PC12 cells against hydrogen peroxide-induced injury. *Life Science* 73, 2503-1513.
- Sung JM (1996) *The Insects-born fungus of Korea in color*, pp. 62-72, Kyohak Publishing Co Ltd., Seoul.
- Yun JS (2005) Effect of *Cordyceps*, *Paecilomyces* sp., *Cordyceps pruinosa* and *Paecilomyces japonica*, on the development of domestic silkworm, *Bombyx mori*. *Entomol Res.* 35, 199-205.