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Multivoltine silkworm lines, *Bombyx mori* L. in the Philippines: New breeds for higher cocoon yield

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Abstract

A multivoltine silkworm breed can produce quality silk seed with considerable resistance to diseases. The study isolated and characterized multivoltine breeds at the Sericulture Research and Development Institute in Northern Philippines, and evaluated their performance on four quantitative characters such as single shell weight (SSW), single cocoon weight (SCW), cocoon shell percentage (CSP) and cocoon yield per box CYB^{-1}) in three rearing seasons (January-February, August-September and October-November). Locally acquired silkworm hybrid cocoons were processed and isolated following the Mass Selection Method. The study was laid out in Randomized Complete Block Design with 3 replications. Data gathered were subjected to Analysis of Variance (ANOVA) and further tested using Least Significant Difference (LSD). Three multivoltine breeds were isolated and characterized DMMMSU 1002, DMMMSU 1003 and DMMMSU 1016. In January-February season, DMMMSU 1002 performed better than the check breed, DMMMSU 1000 in all the economic parameters tested, but only on SSW in August-September; SCW and CYB^{-1} in October-November seasons. DMMMSU 1003 performed better than DMMMSU 1000 in all the parameters evaluated in January-February; for SSW in August-September); and SCW in October-November rearing seasons. DMMMSU 1016 performed better on SSW and CSP (January-February); CYB^{-1} (August-September) and SSW, SCW and CSP in October-November rearing season. Based on these findings, the newly isolated lines can be used as potential parents of superior silkworm hybrids for higher cocoon yield.

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Introduction

Sericulture is a labor intensive agro-based industry which involves mulberry leaf production, silkworm rearing for cocoon production and cocoon processing for silk fabric and other by-products production. It is a potential earner in developing countries like the Philippines where unemployment and poverty problems exist. Sericulture can improve the rural economy because it provides high employment and income generation capability with minimum investment (Hiware, 2001).

The main product of sericulture is silk, rightly called the queen of textiles for its luster, royal appeal, luxury, comfort, elegance, sensuousness, and glamor (Padaki *et al.*, 2015). High demand for silk has been noted in the Asia-Pacific region and projected to have reached USD 16.94 billion in 2021 (Silk Market Reports, 2017). Hence, increasing silk production using various silkworm rearing and breeding technologies is essential. The acquisition of desirable level of cocoon production depends on the successful selection of initial breeding resource. Development of potential hybrid required for the field has become a very difficult task to silkworm breeders. Inspite of continuous efforts for the development of sericulture through various conventional silkworm breeding programs, there is still a demand for productive superior hybrids to fulfill the need of the sericulture industry. In consideration to crop sustainability, development of productive and qualitatively superior cross breeds is necessary.

The role of silkworm breeds in making sericulture a successful occupation through sustainable cocoon yield with superior quality silk is very significant. Silkworm cocoon traits like cocoon weight, cocoon shell weight and cocoon shell ratio have high economic potential, values and heritabilities, and farmer incomes are based on total cocoon weight and hence, cocoon traits are the most important economical traits in sericulture (Mirhosseini *et al.*, 2006; Ghanipoor *et al.*, 2007; Mirhosseini *et al.*, 2010).

The growth and development of silkworm is greatly influenced by environmental conditions. The biological as well as cocoon-related characters are influenced by ambient temperature, rearing seasons, and genetic constitution of silkworm strains. Different seasons affect the performance of silkworms. The seasonal differences in the environmental components considerably affect the genotypic expression in the form of phenotypic output such as cocoon weight, shell weight, and cocoon shell ratio (Hussain *et al.*, 2011). Another study revealed that cocoon yield and economic traits of silkworms were significantly influenced by different rearing seasons (Kumar *et al.*, 2013) period.

Multivoltines were proved to be superior in terms of survival and hardiness. They are also well-adapted to tropical climatic conditions. Thus, maintenance of multivoltine silkworm genetic resources has become very important for meeting the desired objectives of the breeder for immediate or long-term utilization in silkworm breeding programs. However, it is necessary to maintain them in their original form for their rational use in different breeding and other research purposes (Mukarjee *et al.*, 1999; Basavaraja *et al.*, 2003; Rao *et al.*, 2006).

The Sericulture Research and Development Institute, Don Mariano Marcos Memorial State University (DMMMSU-SRDI), Philippines maintains silkworm genetic resources of which only three (3) are multivoltines. The continuing collection, evaluation, maintenance and development of silkworm strains are essential undertakings to acquire new races of silkworm which have good potential for high performance of productivity. This study was undertaken to isolate and characterize multivoltine purelines from silkworm hybrids and evaluate their quantitative performance.

Materials and methods

Silkworm hybrid cocoons were collected and subjected to moth emergence, coupling and decoupling, egg laying and mother moth examination until eggs were derived. From each silkworm hybrid, 5-10 disease-free layings (dfls) were reared employing the silkworm rearing procedures. Larval growth and development were monitored whereby runts and diseased silkworms were discarded.

After the fourth molt or at the onset of fifth instar, superior larvae or segregants were selected based on larval makings following the mass selection method. Selected larvae were labeled and coded according to strain. Optimum rearing conditions such as adequate space, quality and good ventilation were provided until maturation. One day before spinning, larval weight was recorded. The matured larvae were collected and mounted.

Cocoon assessment

After cocoon harvesting, pupation rate was determined by shaking each cocoon gently for live pupa sound. Live cocoon number and weight were recorded. Visual selection on cocoon characters (color and shape) followed. The selected cocoons were arranged separately. After selection, general assessment was carried out by taking 50-60 cocoons randomly from each batch. Superior individuals were selected based on the breeder's criteria and individual cocoon assessment using the electronic weighing balance. Final selection of cocoons was done based on quantitative characters such as single shell weight, single cocoon weight and cocoon shell percentage.

Grainage operation

At the grainage, selected cocoons were preserved separately for moth emergence. Selected female moths were made to lay eggs separately in a cellule, examined for Pebrine disease and were tested in successive generations.

Silkworm rearing

From the produced eggs, ten (10) disease-free layings were selected for rearing in the next generation. Standard procedures in egg disinfection, treatment and preservation were undertaken. The procedures were done for the first four generations (G1-G4) following the SOP for recombination of genes.

Characterization and selection

A record was kept for all characters of interest throughout the entire life cycle of a sample population from which the individuals were selected.

For the succeeding generations, cellular rearing for qualitative and quantitative characters was considered for selection.

Visual selection on larvae and cocoon characters were done. Lines showing desired characters were selected from among the lines with uniform individual progeny.

All stages of development and characters of interest throughout the life cycle of a sample population were selected and recorded.

The selection procedure was repeatedly done until desired characters and values of uniformity were stabilized among the batches of each line. Qualitative characters such as larval makings and cocoon characters were stabilized or fixed at the 12th generation.

Performance evaluation

Quantitative performance of the evolved purelines was evaluated in three rearing seasons considering the economic traits such as single shell weight (SSW), single cocoon weight (SCW), cocoon shell percentage (CSP) and cocoon yield per box (CYB^{-1}) which were computed as follows:

Single Shell Weight.

This refers to the average weight of cocoon shell sample and measured in gram. This was derived by dividing the weight of cocoon shell samples over the number of cocoon shells.

Single Cocoon Weight.

This refers to the average weight of 10 randomly taken cocoons measured in gram. SCW was computed by dividing the total weight of cocoon samples over the number of cocoon samples.

Cocoon Shell Percentage. This refers to the trait showing the total shell content available in the cocoon which is an estimate of raw silk yield. It is the average ratio of total cocoon shell weight to the total cocoon weight and assessed in percentage.

Cocoon Yield Box^{-1}

This is the total weight of cocoons expressed in kilogram per unit number of larvae retained at third instar and it is important as directly associated with the economics of cocoon crops. This was calculated by dividing the actual yield weight over the number of larvae at 3rd instar, then dividing the value by 1000 g multiplied to 20,000 (number of larvae reared).

Data analysis

Data gathered were analyzed using the Analysis of Variance (ANOVA) and Least Significant Difference (LSD) for further test of significance among treatment means.

Results and discussion

Isolation and characterization of lines

Pureline development involves the selection of lines from a local/ traditional variety, or some other mixed population which expresses the desired characteristics such as resistance to pest and diseases, early maturity, yield, and others.

Traditional F_1 hybrid cocoons were collected. Selection was done for higher yield based on SSW, SCW, CSP and CYB^{-1} using the norms for P_3 and P_2 for multivoltine race as selection criteria.

Four multivoltine cocoon color segregants were observed and isolated from the silkworm hybrid: peach, green, white and yellow. At the selection and purification stage, at generations five onwards (G5-), three new pure lines were evolved. Their qualitative characters were fixed at generation twelve (G12) and coded as DMMMSU 1002, DMMMSU 1003 and DMMMSU 1016.

Based on qualitative characterization of the different lines on egg shell color, egg shape, cocoon color, cocoon shape, larval markings and moth emergence, it was observed that all lines have grayish white egg shell color, elliptical egg shape and have 20-22 days larval duration. At the larval stage, all lines exhibited milky white body color. DMMMSU 1002 and DMMMSU 1003 have plain larvae while DMMMSU 1016 have double dark marked larvae. They differ in cocoon color and cocoon shape. DMMMSU 1002 has mint green cocoon color and oval cocoon shape while DMMMSU 1003 has white and peanut shaped cocoon. On the other hand, DMMMSU 1016, has peach cocoon color and oval cocoon shape (Table 1).

Table 1. Morphological characters of the evolved multivoltine silkworm lines.

Morphological Characters	Multivoltine Purelines		
	DMMMSU 1002	DMMMSU 1003	DMMMSU 1 016
EGG Shell			
A. Color	Grayish white	Grayish white	Grayish white
B. Shape	Elliptical	Elliptical	Elliptical
LARVAE			
A. Duration (days)	20-22	20-22	20-22
B. Marking	Plain	Plain	Double dark mark
C. Body color	Milky white	Milky White	Milky White
COCOON			
A. Color	Mint Green	White	Peach
B. Shape	Oval	Peanut	Oval
MOTH			
A. Eye color	Black	Black	Black
B. Egg pattern	Donut-shaped	Donut- shaped	Donut-shaped
C. Egg Laying	Uniform	Uniform	Uniform

The different multivoltine lines have similar moth characters, black eye color, clockwise egg laying direction and pattern.

Performance of newly-evolved multivoltine purelines based on SSW, SCW, CSP and CYB^{-1} in different rearing seasons

According to Gangwar, 2011, as cited by Licay, 2013, rearing performance of different silkworm strains is dependent on environmental conditions like temperature, humidity and quality of mulberry leaves. Temperature plays a vital role in influencing the life process of organisms viz., ingestion, digestion, absorption, assimilation, elimination and thereby growth of silkworms. Thus, in order to achieve high and quality yields from mulberry silkworms, it is important to provide a favorable ecological environment for them (Jumagulov *et al.*, 2021).

The standard temperature and relative humidity requirements for the normal growth and development of silkworms ranged from 23°C- 27°C and 70-90%, respectively (Table 2).

Any deviation from the standard requirements would affect the growth and development of silkworms. Hence, the performance of the silkworm is also affected.

Table 2. Standard temperature and relative humidity requirements for normal growth and development of silkworm.

Environmental Factors	I Instar	II Instar	III Instar	IV Instar	V Instar
Temperature (°C)	27	27	26	24-25	23-24
Humidity (%)	85-90	85-90	80	75	70

Source: DMMMSU-SRDI Technoguide on Silkworm Rearing, Vol. 3 No. 1, March 2016

Single Shell Weight (SSW)

Single shell weight of the evolved multivoltine purelines was significantly heavier than the Check, DMMMSU 1000 in January-February rearing season. In August-September, evolved lines DMMMSU 1002 and DMMMSU 1003 significantly produced heavier cocoon shells compared to DMMMSU 1000 (0.25 g), with 0.31 g and 0.29 g, respectively. In October-November rearing, DMMMSU 1016 significantly produced heavier single shell of 0.31g than the DMMMSU 1000 with 0.25 g (Table 3).

Table 3. Mean performance of the evolved multivoltine purelines with DMMMSU 1000 (Check) on SSW in different rearing seasons.

Treatment	Rearing Seasons		
	Jan.-Feb.	Aug.-Sept.	Oct.-Nov.
DMMMSU 1002	0.32 a	0.31 a	0.24 c
DMMMSU 1003	0.31 a	0.29 a	0.27 b
DMMMSU 1016	0.31 a	0.25 b	0.31 a
DMMMSU 1000 (Check)	0.20 b	0.25 b	0.25 bc

Means with the same letter in a column are not significantly different at 0.5%, LSD.

Single Cocoon Weight (SCW)

The mean performance of the evolved multivoltine pureline was assessed based on SCW in different rearing seasons (Table 4). Compared to DMMMSU 1000, only DMMMSU 1002 (1.52g) and DMMMSU 1003 (1.40g) produced significantly heavier cocoon in January-February rearing season, than DMMMSU 1000 (1.18g). While in October-November, all evolved lines performed better than DMMMSU 1000. The single cocoon weight produced by the lines in August-September rearing season ranged from 1.35-1.54g.

Table 4. Mean performance of the evolved multivoltine purelines with DMMMSU1000 (Check) on SCW in different rearing seasons.

Treatment	Rearing Seasons		
	Jan.-Feb.	Aug.-Sept.	Oct.-Nov.
DMMMSU 1002	1.52 a	1.35	1.50 a
DMMMSU 1003	1.40 ab	1.51	1.48 a
DMMMSU 1016	1.22 c	1.54	1.50 a
DMMMSU 1000 (Check)	1.31 bc	1.45	1.19 b

Means with the same letter in a column are not significantly different at 0.5%, LSD.

The performance of the new multivoltine lines on yield-related characters particularly single cocoon weight surpassed the international norms for P_3 and P_2 silkworm breed stocks which is 1.2 g and not less than 1.0 g, respectively (Economic and Social Commission for Asia and the Pacific, 1993).

Cocoon Shell Percentage (CSP)

The mean performances of the evolved multivoltine lines were evaluated in terms of Cocoon Shell Percentage in three distinct rearing seasons (Table5). All lines significantly registered higher CSP than the Check, DMMMSU 1000 in January-February rearing season. However, in October-November, only DMMMSU 1016 (19.87%) performed comparably with the Check, DMMMSU 1000 (20.08%). On the other hand, CSP in August-September rearing season ranged from 18.90-20.75%. Silk ratio (CSP) above 13% is considered excellent based on the standards for multivoltine purelines.

Table 5. Mean performance of the evolved multivoltine purelines with DMMMSU 1000 (Check) on CSP in different rearing seasons.

Treatment	Rearing Seasons		
	Jan.-Feb.	Aug.-Sept.	Oct.-Nov.
DMMMSU 1002	21.33 a	20.18	17.82 b
DMMMSU 1003	20.67 a	20.69	17.69 b
DMMMSU 1016	20.33 a	20.75	19.87 a
DMMMSU 1000 (Check)	17.00 b	18.90	20.08 a

Means with the same letter in a column are not significantly different at 0.5%, LSD.

Cocoon Yield Box^{-1} (CYB^{-1})

The mean performances of the evolved multivoltine lines were evaluated in terms of Cocoon Yield Box^{-1} in three rearing seasons (Table 6).

Cocoon yield per box was affected by rearing seasons in all the lines. However, it was noted that DMMMSU 1002 (17.67kg) and DMMMSU 1003 (17.40kg) still performed comparably with DMMMSU 1000 (17.46kg). On the other hand, DMMMSU 1016 produced 9.55kg. but still comparable to DMMMSU 1000 (13.69kg). In October-November, DMMMSU 1002 significantly produced the highest CYB^{-1} with 17.64kg.

Table 6. Mean performance of the evolved multivoltine purelines with DMMMSU 1000 (Check) on CYPB in different rearing seasons.

Treatment	Rearing Seasons		
	Jan.-Feb.	Aug.-Sept.	Oct.-Nov.
DMMMSU 1002	17.67 a	7.12 b	17.64 a
DMMMSU 1003	17.40 a	9.30 b	7.74 b
DMMMSU 1016	5.32 b	9.55 ab	11.42 b
DMMMSU 1000 (Check)	17.46 a	13.69 a	11.71 b

Means with the same letter in a column are not significantly different at 0.5%, LSD.

The above result is supported by Basavaraj *et al.* (2005) that cocoon productivity depends on the survival of silkworms to spin and genotype of the silkworm breed. Further, Hussain *et al.* (2011) concluded that mean performance of inbred silkworm lines varies significantly when exposed to various conditions of temperature and humidity. Environmental conditions during larval rearing is linked to seed cocoon production.

Conclusions

Three new multivoltine purelines were isolated coded as DMMMSU 1002, DMMMSU 1003 and DMMMSU 1016. They were characterized based on egg, larvae cocoon and moth characters where three multivoltine cocoon color segregants were observed and isolated (peach, green and white). All lines similarly exhibited grayish white egg shell, elliptical egg and have 20-22 days larval duration. All larvae have milky white body color. DMMMSU 1002 and DMMMSU 1003 larvae are plain while DMMMSU 1016 have double dark marks. They differ in cocoon color and cocoon shape. DMMMSU 1002 cocoons are mint green and oval; DMMMSU 1003 cocoons are white and peanut-shaped; while DMMMSU 1016 cocoons are peach and oval. The new lines have similar moth characters, black eye color, clockwise egg laying direction and pattern.

Based on quantitative characters, DMMMSU 1002 and DMMMSU 1003 performed better than DMMMSU 1000 (Check) in all the economic parameters in January-February rearing season. Moreover, both are also better based on SSW during August-September rearing season. The new lines performed better particularly on SCW in October-November rearing season. However, in terms of SSW and CSP, DMMMSU 1016 performed better during the season. On the other hand, DMMMSU 1002 was better on CYB. Overall, the newly-evolved multivoltine lines can be reared in January-February to attain better cocoon performance.

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