



New Bivoltine Hybrids of the Silkworm (*Bombyx mori* L.) Performance at Farmer's Site

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/IJECC/2023/v13i113648

Open Peer Review History:

This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: <https://www.sdiarticle5.com/review-history/108879>

Original Research Article

Received: 30/09/2023

Accepted: 05/12/2023

Published: 12/12/2023

ABSTRACT

The rise in atmospheric temperature, due to global warming, offers a great challenge to make a successful cocoon harvest, as it not only affects the progression of the larvae but also leads to outbreak of diseases. Thus, silkworm breeds have potential to thrive well under such harsh fluctuating climatic conditions without sacrificing commercial traits of cocoon and silk are in-need. Towards this, new bivoltine breeds developed employing heat shock technology, for the first time, were used to develop four bivoltine double hybrids (NBH1 x FC1, NBH2 x FC1, FC1 x NBH1, and FC1 x NBH2) and two poly hybrids (PM x NBH1 and PM x NBH2) and subjected to evaluation for their performance at the farmer's site. Notably, all four double hybrids distributed to farmers performed very well with substantial cocoon yield. Among them, NBH1 x FC1 and NBH2 x FC1 were outperformed with their high fecundity (675 and 700 eggs/df) and cocoon yield of 549 and 228 kg, out of 579 and 225 dfls reared by the farmers respectively. On the other hand, 360 and 248 kg

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of cocoons were harvested as against a total of 325 dfls and 250 dfls of PM x NBH1 and PM x NBH2 distributed to farmers. Eventually, all the farmers were delighted to rear these new double hybrids and poly hybrids, as it fetched a good price to sell the cocoons. Thus, we suggest all these new double hybrids and poly hybrids shall be used for large-scale silkworm rearing in the field conquering the climate change threat to produce both qualitatively and quantitatively better cocoons and silk that shall protect the wealth of Seri-farmers and silk reelers

Keywords: Commercial traits; double hybrids; heat shock; poly hybrids.

1. INTRODUCTION

Among silk producing countries, next to China, India is the one that has incredible potential to monopolise the World sericulture industry with the production of international-grade raw silk by promoting bivoltine silkworm rearing. Still, it is a challenging task as temperature and humidity of the rearing environment fluctuate frequently from dawn to dusk and have a direct impact on their physiology, which in turn affects metabolic activity, growth and development [1]. In recent years, it has become nastiest as the mean annual temperature is rising significantly and affecting the success of cocoon production in India's tropical and subtropical climatic zones [2]. As a consequence, maintaining ideal temperature and humidity of 24 to 28°C and 75 to 85% respectively in the silkworm rearing house to achieve better growth and development of larvae is a difficult task for small-scale farmers in the rural sector. To overcome these limitations and achieve successful cocoon production both qualitatively and quantitatively good is the need of the hour.

Realising this, scientists with their concerted efforts have developed a few bivoltine breeds and hybrids, and suggested to rear in place of multi x bivoltine crossbreeds. These multi x bivoltine crossbreeds ruled the commercial market over a long period due to their hardiness thriving well under varied or fluctuating environmental climatic conditions while the cocoon and silk produced remain inferior to bivoltine silkworm breeds. Contrastingly, despite the fact that bivoltine breeds and hybrids could spin better quality cocoons and produces silk than crossbreeds, its performance and success during all the seasons does not reach the assured level [3] due to their poor adaptability to the fluctuating environmental conditions. Thus, farmers are snagging in harvesting an assured cocoon crop throughout the year [4] and aspire seemingly for the breed that could not only thrive well under high temperature but also produce high-quality and quantity of silk [5].

Towards this, employing different conventional and molecular breeding strategies many bivoltine silkworm breeds have evolved, among them a few have performed better throughout the year [6, 7], but have not been popularised much or accepted by the farmers. Thus, it has created a big vacuum, offering ample scope to evolve robust and productive silkworm breeds/hybrids which have greater tolerance to harsh climatic conditions. Basically, thermotolerance of an organism from prokaryotes to eukaryotes under unfavourable situations is influenced by the expression of a set of stress proteins – heat shock proteins (HSPs). This phenomenon was first evaluated by Vasudha et al. [5] by subjecting different instar silkworm larvae of *B. mori* to varied heat shock (HS) treatments and correlated the differential expression of HSPs with biological and commercial traits. Eventually, the concept of heat shock technology coupled with a proteomic-based breeding strategy for the first time has been adopted to evolve new bivoltine breeds (Unpublished).

Silkworm breeding programs in the country emphasise that the silkworm breeds/hybrids developed have to be field-tested under naturally fluctuating environmental conditions for their adaptability to the local environment, season-specific response and better productivity [8]. Hence, the current study was undertaken at farmers' site to evaluate the performance of new bivoltine double hybrids and poly hybrids with a main aim of commercial exploitation.

2. MATERIALS AND METHODS

2.1 Silkworm Breeds

Four new bivoltine double hybrids - NBH1 x FC1, NBH2 x FC1, FC1 x NBH1, FC1 x NBH2, and Two poly hybrids - PM x NBH1 and PM x NBH2, were used in the present study. NBH1 and NBH2 bivoltine single hybrids were newly developed by Prof. H.B. Manjunatha and his research team employing a novel technology "heat shock technology" (HST).

2.2 Silkworm Rearing

The disease free layings of new bivoltine double hybrids - NBH1 x FC1, NBH2 x FC1, FC1 x NBH1 and FC1 x NBH2, and poly hybrids - PM x NBH1 and PM x NBH2 were prepared at Kakaramanahalli Silkworm Seed Organisation (KSSO) [Plate 1A-D] and incubated in incubator with optimum environmental conditions of $25 \pm 1^\circ\text{C}$ temperature and $75 \pm 5\%$ relative humidity until eight day followed by black-boxing to achieve uniform hatching. Newly hatched larvae were brushed with freshly chopped mulberry leaves. Chawki silkworm rearing was carried out at Keeranagere Chawki Rearing Centre [Plate 1E & F] following standard rearing procedure [9]. The larvae out-off second moult were distributed to selected farmers in the Ramanagara area of Karnataka and reared at their site with the resources available.

2.3 Analysis of Biological and Commercial Traits

2.3.1 Fecundity

The fecundity was calculated by counting the number of eggs laid by a single female moth.

2.3.2 Hatching percentage

Total number of eggs hatched was calculated using the formula and expressed in percentage.

Hatching percentage = $\frac{\text{Number of eggs hatched}}{\text{Total number of eggs}} \times 100$

2.3.3 Total yield

The total yield was recorded based on the cocoon market transaction data collected from individual farmers.

3. RESULTS

3.1 Fecundity

Among four bivoltine double hybrids, notably new double hybrid NBH2 x FC1 cross has shown highest fecundity an average of 700 eggs/dfls, while NBH1 x FC1 cross poses 675 eggs/dfls which is substantially higher than that of its reciprocal crosses FC1 x NBH1 and FC1 x NBH2 in which the fecundity rate was 515 and 500/dfls respectively (Fig. 1).

Among the two new poly hybrids, the PM x NBH2 cross has shown the highest fecundity with an average of 530 eggs/dfls, while 500 eggs/dfls was recorded from PM x NBH1 cross (Fig. 1).

3.2 Hatching Percentage

Per cent of hatching was higher in the double hybrids NBH1 x FC1 and NBH2 x FC1 accounting 98 and 97% respectively, while its reciprocal crosses FC1 x NBH1 and FC1 x NBH2 has shown 95 and 96% respectively (Fig. 2).

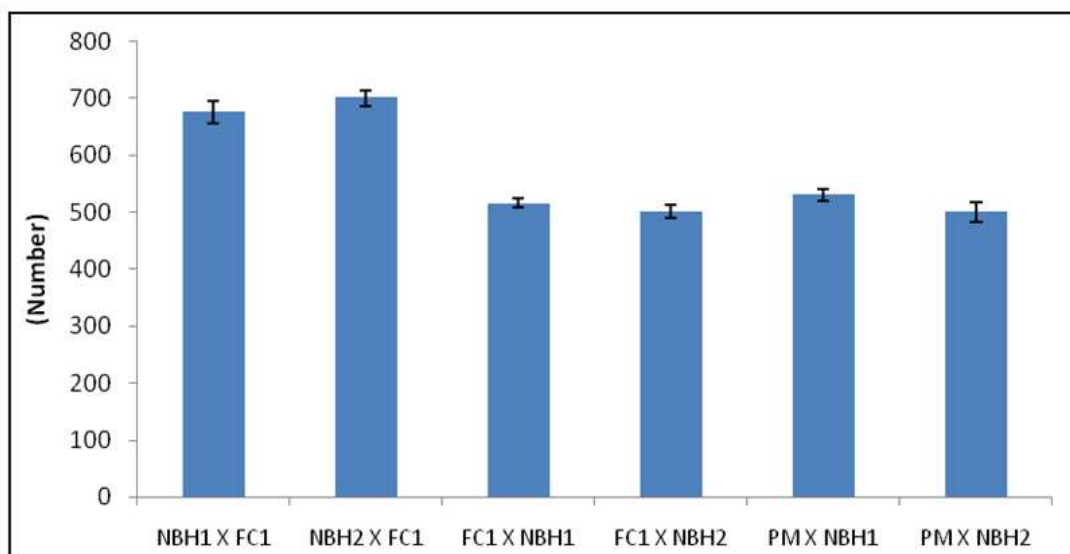


Fig. 1. Comparative analyses on the fecundity of new bivoltine double and poly hybrids

Interestingly, both poly hybrids exhibited a good percentage of egg hatching, wherein 98 and 97% of hatching was recorded from PM x NBH1 being higher than PM x NBH2 eggs respectively (Fig. 2).

3.3 Cocoon Yield

Interestingly, all the newly evolved bivoltine double hybrids performed very well in the farmers' field. After cocoon transactions at the cocoon markets, the data was collected from the farmers and pooled for each hybrid. Of the 579 dfls of NBH1 x FC1 hybrid distributed to the farmers, they have traded a total of 549 kg of cocoons in the cocoon market. Whereas in NBH2 x FC1 double hybrid, the total weight of cocoons transacted was 228 kg out of 225 dfls brushed.

Concerning its reciprocal cross, a higher yield of 284 kg cocoons was harvested and transacted out of 250 dfl's of FC1 x NBH2 hybrids brushed,

while it was 332 kg of cocoons harvested from the 425 dfl's of FC1 x NBH1 distributed to the farmers (Table 1).

Notably, farmers who have reared the new poly hybrids harvested an excellent cocoon crop accounting for 360 kg and 248 kg of cocoons from 325 dfls of PM x NBH1 and 250 dfls of the PM x NBH2 poly hybrid respectively (Table 1).

3.4 Price

Among the cocoons of four new bivoltine double hybrids transacted at the cocoon market, the cocoon lot of NBH2 x FC1 fetches an average highest price of Rs.368/kg of cocoons compared to other double hybrid cocoons. The next highest price of Rs.360/kg was bid for the cocoon lot of NBH1 x FC1. The other double hybrid cocoons of FC1 x NBH2 and FC1 x NBH1 also fetch marginally good prices of Rs.358/kg and Rs.350/kg respectively.

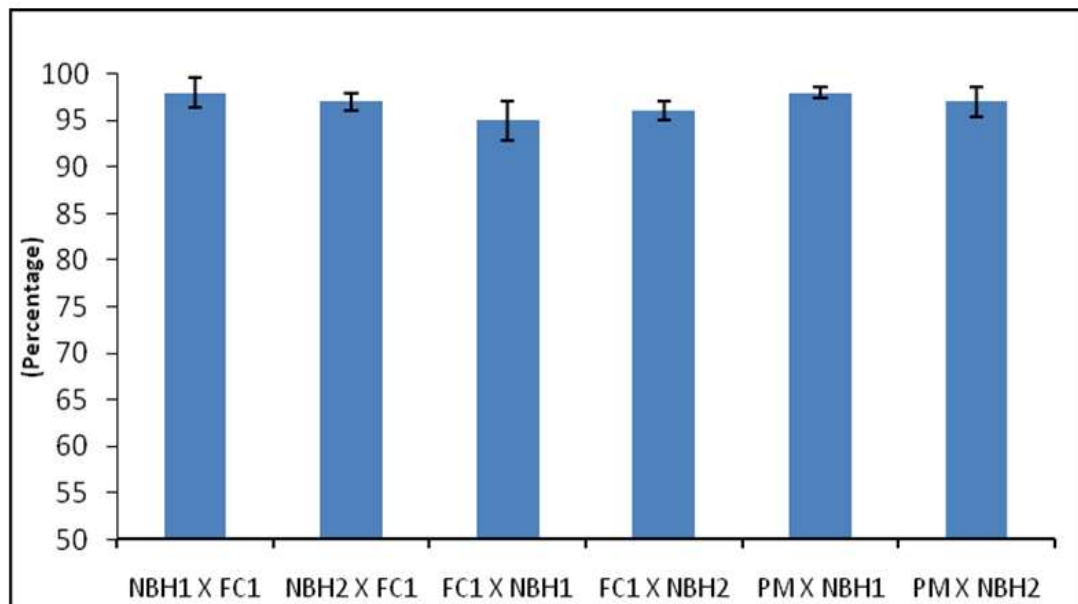


Fig. 2. Comparative analysis on the hatching of eggs of new bivoltine double and poly hybrids

Table 1. Comparative yield and cost analysis of new bivoltine double and poly hybrids

Double/poly hybrids	Dfls brushed (No.)	Total Yield (Kg)	Average Rate (Rupees per kg)
NBH1 x FC1	579	549	360
NBH2 x FC1	225	228	368
FC1 x NBH1	425	332	350
FC1 x NBH2	250	284	358
PM x NBH1	325	360	337
PM x NBH ₂	250	248	297

Cocoons of both two new poly hybrids PM x NBH1 and PM x NBH2 transacted at the cocoon market fetched an average

highest price of Rs.337/kg and Rs.297/kg of cocoons respectively.



Plate 1. Preparation of disease free layings and distribution of chawki worms to farmers. (A. De-pairing of moths, B. Egg laying, C. Loose eggs, D. Oviposition for the laying of poly hybrid layings, E. Chawki worms, F. Distribution of chawki worms to farmers)

4. DISCUSSION

With the recent advances in technology, globally, scientists through their concerted efforts generate new silkworm (*Bombyx mori*) breeds regularly to replace the older lines [10]. As a consequence, the multi x bivoltine crossbreeds the major contributor of commercial cocoon production has been trying to be replaced by bivoltine pure and hybrids, because the silk produced from crossbreed cocoons comparatively inferior to bivoltine cocoons. Towards this, more emphasis has been given to promote bivoltine rearing in the field with the hope that this concept can meet the goal of producing international-grade silk so that a sizable import of raw silk from other countries shall be minimised, China in particular. Towards this, intensive efforts have been made to develop more productive bivoltine breeds that have the potential to produce good-quality silk. However, hot climatic conditions prevailing in India has not been favourable to rear these productive bivoltine breeds/hybrids [3] as a result it did not reach the anticipated target of the Seri-Stakeholders [11]. The main constraint for this cause is that, the bivoltine breeds are more susceptible to high temperature coupled with high and low humidity and display poor performance, while many quantitative traits like viability and cocoon traits sharply decline when temperature is higher than 28°C [12]. Thus, bivoltine breeds that could produce steady harvests and high quality cocoons and silk despite unfavourable weather conditions, are necessary to address this bottleneck issue. In light of this disparity, a few novel bivoltine breeds are developed employing heat shock technology for the first time and utilised as potent parents for generation of bivoltine double hybrids and poly hybrids, which are presently undergoing on-site assessment.

Notably, among the bivoltine breeds that exist in the sericulture industry, the highest fecundity an average of 700 and 675 eggs/df was recorded, for the first time, from the newly evolved NBH2 and NBH1 respectively, while the other ruling bivoltine breed has the reproductive potential with the number of eggs laid by a moth of CSR2 [3] and FC1 was 610 and 515 (current data). As fecundity is determined by the genotype of the maternal parent and the environmental conditions which the breed experienced is referred to as one of the fitness components reflecting productivity of the newly evolved NBH1

and NBH2 breeds. Thus performance of these breeds/hybrids at field has significance because; fecundity is one of the most important commercial traits of quality seed, cocoon crop harvest and promote sericulture industry.

Despite, the new NBH1 and NBH2 based double hybrids have been exposed to diverse environmental conditions, qualitative and quantitative features of mulberry leaves, and silkworm rearing management at different farmers level, they not only confer superiority with regard to tolerance to adverse conditions but also exhibit consistency in the expression of productivity with an average cocoon yield of more than 90 kg per 100 dfls, whereas an average estimated cocoon yield for other bivoltine breeds is 60 kg/100 dfls [13] and 66.95 to 78.99 kg/100 dfls [3]. Interestingly, for the bivoltine double hybrid cocoons of NBH produced by the farmers, they bid the highest price in the cocoon market for which all the farmers were happy. Thus, NBH based double hybrids shall be promising silkworm hybrids for large-scale production of cocoons and silk to elevate not only the economic status of the Seri-farmers but also stabilise the sericulture industry in India, Karnataka in particular.

Accordingly, PM x NB4D2 was the dominant crossbreed in South India, for a considerable amount of time before it PM x CSR2 took its position on the recommendation of one of the authors [14]. Despite, its reputation as a resilient hybrid that can survive in a variety of climates, growers experience crop loss due to its intolerance of extreme weather. In addition, it has a reputation for having a low percentage of cocoon shell and a lesser output of raw silk that is of inferior quality. Therefore, it called for the replacement of PM x CSR2 with a superior cross breed that would produce cocoon and silk both qualitatively and quantitatively better [15]. In response, recently developed poly hybrids - PM x NBH1 and PM x NBH2 showed great promise in the field yielding superior cocoons of 360 and 248 kg from 325 and 250 dfls respectively and fetching higher prices than the dominant cross breed PM x CSR2 in the market.

5. CONCLUSION

Farmers embraced new bivoltine double and poly hybrids that performed incredibly well in the field and in which one of the parents (NBH1 and NBH2) evolved employing heat shock technology. In light of the newly evolved double

hybrids' and poly hybrids' overall performance as well as their competitive pricing and market value, we suggest growing these hybrids to combat the threat posed by climate change and produce cocoons on a big scale that are both more qualitatively and quantitatively superior.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:

The peer review history for this paper can be accessed here:

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