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On the Breeding of Bivoltine Double Hybrid of Silkworm *Bombyx mori* (Lepidoptera: Bombycidae) Tolerant to High Temperature and Low Humidity Conditions of the Tropics

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Abstract: In order to introduce bivoltine races in a tropical country like India, it is necessary to have stability in cocoon crop under high temperature environment. Considering the poor performance of productive bivoltine hybrids during summer season, emphasis was given to evolve bivoltine silkworm breeds suitable to tropical conditions for achieving the primary objective of establishing bivoltine sericulture with quality raw silk among sericulturists. One of the main aims of the breeders is to recommend silkworm breeds/hybrids to farmers that are stable under different environmental conditions and minimize the risk of falling below a certain yield level. Silkworm breeds that are reared over a series of environment exhibiting less variation are considered stable. Therefore, it becomes imperative or essential to develop bivoltine breeds/hybrids which can with stand high temperature stress conditions. Keeping these in view an attempt is made to develop bivoltine double hybrid tolerant high temperature and low humidity conditions of the tropics.

Key words: *Bombyx mori* · Temperature tolerant · Bivoltine hybrid

INTRODUCTION

Silkworm breeding aims to achieve superior performances in respect of egg yield, cocoon raw silk yield, cocoon stability and production followed by expansion to new areas besides others. Silkworm breeders continue to strive for an inherent gain in resistance by incorporating resistant genes into the genetic backgrounds of high yielding temperate bivoltines. Besides this, the cocoon crop stability also relies more on improving other production technologies which have to be explored. India enjoys the patronage of second position for the production of silk in the world next only to China. Sericulture in India is practiced predominantly in tropical environmental regions such as Karnataka, Tamil Nadu andhra Pradesh and West Bengal and to a limited extent in temperate environment of Jammu and Kashmir. The existing tropical situation provides scope for exploiting multivoltine x bivoltine hybrid at commercial venture as they are hardy and have tremendous ability to survive and reproduce under varied or fluctuating environmental climatic conditions. But its quality is at low ebb when compared to the existing international standard.

Considering these drawbacks, adoption of bivoltine sericulture became imperative and imminent considering its potentiality even under Indian tropical conditions. Keeping this in view, breeding experiments were initiated at Central Sericultural Research and Training Institute, Mysore to evolve hardy bivoltine silkworm races suited to tropical conditions for achieving the primary objective of establishing bivoltine hybrids as a concept among sericulturists. Accordingly, many productive and qualitatively superior bivoltine hybrids have been developed by utilizing Japanese commercial hybrids as breeding resource material [1]. However, the hot climatic conditions prevailing particularly in summer are not conducive to rear these high yielding bivoltine hybrids throughout the year. It is well established fact that under tropical condition, unlike polyvoltines, bivoltines are more vulnerable to various stresses i.e hot climatic conditions of tropics, poor leaf quality and improper management during summer which are not conducive for bivoltine rearing. In order to select efficiently the breeds with high temperature tolerance, it is important to analyse the impact of high temperature on many silk yielding attributes of silkworm races and their heritability.

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The hot climatic conditions of tropics prevailing particularly in summer are contributing to the poor performance of the bivoltine breeds and the most important aspect is that many quantitative characters such as viability and cocoon traits decline sharply when temperature is higher than 28°C [2]. Therefore, it is very much essential to develop bivoltine breeds/hybrids which can withstand the high temperature stress conditions. Keeping these in view compatible bivoltine hybrids for rearing throughout the year were developed by utilizing Japanese thermo-tolerant hybrids as breeding resource material [3-8] and suggested that any study involving cocoon traits is a trend setter to provide basis to formulate appropriate selection policies for required environments. While studying the performance of robust and productive hybrids under two temperature conditions [5, 9] indicated that the deleterious effect of high temperature was more pronounced in productive hybrids than the robust hybrids.

The continued efforts for the improvement of cocoon characters of domesticated silkworm were aimed at increased quality silk production. The main objective of silkworm rearing is to produce qualitatively and quantitatively superior cocoons, which in turn will have a direct bearing on the raw silk production. Therefore, it becomes imperative or essential to develop bivoltine breeds/hybrids which can with stand high temperature stress conditions. Sericulture, the viable agro-based industry aptly matches the socio-economic backdrop of rural India. One of the main aims of the breeders is to recommend silkworm breeds/hybrids to farmers that are stable under different environmental conditions and minimize the risk of falling below a certain yield level. Silkworm breeds that are reared over a series of environment exhibiting less variation are considered stable. The climatic conditions prevailing in the tropics are most unpredictable and the problems of tropical sericulture are occurrence of aggravated silkworm diseases, unsuitable mulberry leaf for bivoltine silkworms and lack of sustainable silkworm breeds for effective selection of desirable characters. In order to introduce bivoltine races in a tropical country like India, it is necessary to have stability in cocoon crop under high temperature environment. The pre-requisite of summer hybrid is healthiness and adaptability to adverse conditions of high temperature, low food quality, relatively higher economic traits, with potential for increased cocoon production. Therefore, in the present study was undertaken to develop compatible robust bivoltine double hybrid of the silkworm, *Bombyx mori* L. under high temperature and low humidity conditions.

MATERIALS AND METHODS

Twenty two silkworm breeds were drawn from the germplasm of Central Sericultural Research and Training Institute, Mysore and screened under high temperature and high humidity conditions (40±1°C and high humidity i.e. 85±5%,) to select the breeding resource materials [10]. Silkworm rearing was conducted following the standard method under recommended temperature and humidity till 2nd day of 5th instar. On the third day of 5th instar 10 replicates of 100 larvae/breed were subjected to temperature treatments for six hours daily i.e., from 10AM to 4PM till spinning. The remaining larvae served as control and were reared at 25±1°C and 65±5% relative humidity. For thermal exposure, the larvae were kept in plastic trays and reared in SERICATRON (Environmental chamber with precise and automatic control facilities for uniform maintenance of temperature and humidity) at 40±1°C and 85±5% and were fed with fresh mulberry leaves twice a day. When the larvae started spinning, they were shifted to 25±1°C and 65±5% relative humidity. Plastic collapsible mountages were used for mounting the ripened larvae. Cocoon harvested was carried out on the 7th day and assessment was carried out on the subsequent day. The pupation rate was calculated as the number of live pupae to the number of larvae reared at 25±1°C and 65±5% relative humidity and 40±1°C and high humidity i.e. 85±5% respectively. Three oval and three dumbbell breeds were developed by utilizing selected bivoltine breeds as breeding resource material and crosses were made as oval x oval and dumbbell x dumbbell. In this breeding programme, repeated back crossing was given in the earlier (F2 to F5) by the respective productive breeds to increase the productivity traits in the resultant breeds. Owing to thermal effect in successive generations, it was observed after 5th generation that both qualitative and quantitative characters have declined. So the normal rearing was conducted every alternate generation to regain the lost vitality. Mass rearings were conducted from F1 to F5, while cellular rearings were conducted from F6 onwards. Further directional selection was employed in the following 5 generations. To obtain stability based on pupation rate and cocoon shape as important selection criteria. During the process of breeding care was also taken to maintain the productivity traits in the resultant breeds [11].

The parentages of the selected breeding lines are as follows

Sl. No.	Breeding lines	Parentage	Breeding Plan
Oval			
1	HL1	CSR18, CSR46, CSR50.	(CSR46 ×CSR18) × CSR50
2	HL3		(CSR50 × CSR46)) × CSR50
3	HL5		(CSR18 × CSR50) × CSR50
Dumbbell			
1	HL8	CSR19, CSR47, CSR51.	(CSR47 × CSR19) × CSR51
2	HL10		(CSR51 × CSR19) × CSR51
3	HL12		(CSR51 × CSR47) × CSR51

The breeding lines were subjected for high temperature (40 ±1°C) and high humidity (85± 5% RH) as well as at room temperature (25 ±1°C and 65±5% RH) treatments. Based on high pupation rate at high temperature and high humidity conditions, three oval lines were continued. Though, the resultant lines were more robust and relatively tolerant to silkworm diseases, the productivity traits were inferior to that of the already developed productive CSR breeds. Therefore, resorted to repeated out crossing with productive breeds to improve the productivity traits. Accordingly, all the three dumbbell lines (HH8, HH10 and HH12) were out crossed with CSR51 to improve the quantitative traits. Similarly, all the oval lines (HL1, HL3 and HL5) were out crossed with CSR50 to improve the quantitative traits Mass rearing with directional selection was resorted up to F5 generations. Based on high pupation rate a minimum of three bathes were selected and resorted to either inbreeding or inter batch crossing. Further, directional selection was employed in the following 5 generations based on pupation rate and cocoon shape as selection criteria. Single cocoon assessment was carried out to select the cocoons for continuation of the progeny by maintaining the qualitative and quantitative characters at high profile. Though, no positive selection response was noticed on survival rate when reared at high temperature followed by directional selection, progenetive lines performed remarkably superior in subsequent generations at room temperature conditions.

The foundation crosses for each temperature treatment were prepared by utilizing new breeds developed at high temperature and low humidity and high temperature and high humidity and reared at both temperature treatments. Based on high pupation rate at treatment level, the foundation crosses were short-listed. By utilizing the short listed foundation crosses, double hybrids were prepared. The double hybrids were reared at both high temperature and low humidity and also at room temperature and the data generated for different metric traits were analyzed statistically.

RESULTS

Performance of Foundation Crosses Developed under High Temperature (40±1°c) and Low Humidity (50±5%) Conditions

Fecundity: The fecundity is the same for both the treatments and it ranged from 553 to 589 with the highest of 589 recorded for HL1 × HL3 and the lowest of 553 recorded for HL1 × HL5. (Table 1).

Pupation Rate: At 40±1°C, the pupation rate ranged from 64.0 to 85.0% with the highest of 85.0% recorded for HL1 × HL3 and the lowest of 64.0% recorded for HL1 × HL12. At 25±1°C, the pupation rate ranged from 92.9 to 93.6% with the highest of 93.6% recorded for HL1 × HL5 and HL10 × HL12 and the lowest of 92.9% recorded for HL1 × HL3. (Table 1).

Yield/10000 Larvae: At 40±1°C, the yield/10000 larvae ranged from 16.84 to 17.45 kg with the highest of 17.45 kg recorded for HL10 × HL12 and the lowest of 16.84 kg recorded for HL1 × HL10. At 25±1°C, the yield/10000 larvae ranged from 18.67 to 19.28 kg with the highest of 19.28 kg recorded for HL1 × HL5 and the lowest of 18.67 kg recorded for HL7 × HL10 (Table).

Cocoon Weight: At 40±1°C, the cocoon weight ranged from 1.533 to 1.607 g with the highest of 1.607 g recorded for HL1 × HL5 and the lowest of 1.533 g recorded for HL7 × HL10. At 25±1°C, the cocoon weight ranged from 1.756 to 1.857 g with the highest of 1.857 g recorded for HL7 × HL10 and HL10 × HL12 and the lowest of 1.756 g recorded for HL7 × HL12 (Table 1).

Cocoon Shell Weight: At 40±1°C, the cocoon shell weight ranged from 0.315 to 0.341 g with the highest of 0.341 g recorded for HL1 × HL5 and the lowest of 0.315 g recorded for HL7 × HL12. At 25±1°C, the cocoon shell weight ranged from 0.393 to 0.424 g with the highest of 0.424 g recorded for HL1 × HL3 and the lowest of 0.393 g recorded for HL7 × HL12 (Table 1).

Cocoon Shell Percentage: At 40±1°C, the cocoon shell percentage ranged from 20.27 to 21.37% with the highest of 21.37% recorded for HL1 × HL3 and the lowest of 20.27% recorded for HL7 × HL12. At 25±1°C, the cocoon shell percentage ranged from 22.29 to 23.81% with the highest of 23.81% recorded for HL1 × HL3 and the lowest of 22.29% recorded for HL7 × HL10 (Table 1).

Reelability: At 40±1°C, the highest reelability was recorded for HL1 × HL3 and Hl10 × HL12 (84.33%) and the lowest of 82.67% was recorded for HL3 × HL5 and HL7 × HL10. At 25±1°C, the highest reelability was recorded for HL1 × HL3 and HL10 × HL12 (86.67%) and the lowest of 85.67% was recorded for HL7 × HL12 (Table 2).

Filament Length: At 40±1°C, the longest filament length was recorded for HL1 × HL3 (1058 m) and the shortest of 1002 m was recorded for HL7 × HL12. At 25±1°C, the longest filament length was recorded for HL10 × HL12 (1082 m) and the shortest of 998 m was recorded for HL7 × HL12 (Table 2).

Renditta: At 40±1°C, the highest renditta was recorded for HL7 × HL12 (6.43) and the lowest of 6.12 was recorded for HL1 × HL3. At 25±1°C, the highest renditta was recorded for HL10 × HL12 (5.73) and the lowest of 5.39 was recorded for HL1 × HL3 (Table 2).

Raw Silk Percentage: At 40±1°C, the highest raw silk percentage was recorded for HL1 × HL3 (16.35%) and the lowest of 15.56% was recorded for HL7 × HL12. At 25±1°C, the highest raw silk percentage was recorded for HL1 × HL3 (18.55%) and the lowest of 17.45% was recorded for HL10 × HL12 (Table 2).

Filament Size: At 40±1°C, the thickest filament size was recorded for HL1 × HL3 (2.78 d) and the thinnest of 2.47 d was recorded for HL7 × HL10. At 25±1°C, the thickest filament size was recorded for HL1 × HL5 (2.94 d) and the thinnest of 2.79 d was recorded for HL7 × HL10 (Table 2).

Neatness: At 40±1°C, the highest neatness was recorded for HL1 × HL3 (91.33 p) and the lowest of 90.0 p was recorded for HL1 × HL5. At 25±1°C, the highest neatness was recorded for HL7 × HL10 and HL10 × Hl12 (93.0 p) and the lowest of 92.33 p was recorded for HL1 × HL5 (Table 2).

Performance of Double Hybrids Developed under High Temperature (40±1°c) and Low Humidity (50±5%) Conditions

Fecundity: The fecundity is the same for both the treatments and it ranged from 649 to 660 with the highest of 660 recorded for (HL1 × HL3) × (HL10 x HL12) and the lowest of 649 recorded for (HL1 × HL5) × (HL10 × HL12) (Table 3).

Pupation Rate: At 40±1°C, the pupation rate ranged from 71.7 to 88.7% with the highest of 88.7% recorded for (HL1 × HL3) × (HL10 × HL12) and the lowest of 71.7% recorded for (HL1 × HL5) × (HL10 × HL12) and (HL3 × HL5) × (HL10 × HL12). At 25±1°C, the pupation rate ranged from 93.3 to 94.4% with the highest of 94.4% recorded for (HL1 × HL3) × (HL10 × HL12) and the lowest of 93.3% recorded for (HL3 × HL5) × (HL10 × HL12) (Table 3).

Yield/10000 Larvae: At 40±1°C, the yield/10000 larvae ranged from 17.06 to 17.53 kg with the highest of 17.53 kg recorded for (HL1 × HL3) × (HL10 × HL12) and the lowest of 17.06 kg recorded for (HL1 × HL5) × (HL10 × HL12). At 25±1°C, the yield/10000 larvae ranged from 19.19 to 19.50 kg with the highest of 19.50 kg recorded for (HL1 × HL3) × (HL10 × HL12) and the lowest of 19.19 kg recorded for (HL3 × HL5) × (HL10 × HL12) (Table 3).

Cocoon Weight: At 40±1°C, the cocoon weight ranged from 1.535 to 1.586 g with the highest of 1.586 g recorded for (HL3 × HL5) × (HL10 × HL12) and the lowest of 1.535 g recorded for (HL1 × HL5) × (HL10 × HL12). At 25±1°C, the cocoon weight ranged from 1.826 to 1.882 g with the highest of 1.882 g recorded for (HL1 × HL3) × (HL10 × HL12) and the lowest of 1.826 g recorded for (HL1 × HL5) × (HL10 × HL12) (Table 3).

Cocoon Shell Weight: At 40±1°C, the cocoon shell weight ranged from 0.332 to 0.351 g with the highest of 0.351 g recorded for (HL1 × HL3) × (HL10 × HL12) and the lowest of 0.332 g recorded for (HL1 × HL5) × (HL10 × HL12). At 25±1°C, the cocoon shell weight ranged from 0.429 to 0.458 g with the highest of 0.458 g recorded for (HL1 × HL3) × (HL10 × HL12) and the lowest of 0.429 g recorded for (HL3 × HL5) × (HL10 × HL12) (Table 3).

Cocoon Shell Percentage: At 40±1°C, the cocoon shell percentage ranged from 21.23 to 22.24% with the highest of 22.24% recorded for (HL1 × HL3) × (HL10 × HL12) and the lowest of 21.23% recorded for (HL3 × HL5) × (HL10 × HL12). At 25±1°C, the cocoon shell percentage ranged from 23.22 to 24.35% with the highest of 24.35% recorded for (HL1 × HL3) × (HL10 × HL12) and the lowest of 23.22% recorded for (HL3 × HL5) × (HL10 × HL12) (Table 3).

Reelability: At 40±1°C, the highest reelability was recorded for (HL1 × HL3) × (HL10 × HL12) (86.0%) and the lowest of 82.0% was recorded for (HL1 × HL5) × (HL10 × HL12). At 25±1°C, the highest reelability was recorded for (HL1 × HL3) × (HL10 × HL12) (87.33%) and the lowest of 86.0% was recorded for (HL1 × HL5) × (HL10 x HL12) (Table 4).

Filament Length: At 40±1°C, the longest filament length was recorded for (HL1 × HL3) × (HL10 × HL12) (948 m) and the shortest of 909 m was recorded for (HL1 × HL5) × (HL10 × HL12). At 25±1°C, the longest filament length was recorded for (HL1 × HL3) × (HL10 × HL12) (1133 m) and the shortest of 1067 m was recorded for (HL3 × HL5) × (HL10 × HL12) (Table 4).

Renditta: At 40±1°C, the highest renditta was recorded for (HL3 × HL5) × (HL10 × HL12) (6.16) and the lowest of 5.90 was recorded for (HL1 × HL3) × (HL10 × HL12). At 25±1°C, the highest renditta was recorded for (HL3 × HL5) × (HL10 × HL12) (5.47) and the lowest of 5.24 was recorded for (HL1 × HL3) × (HL10 × HL12) and (HL1 × HL5) × (HL10 × HL12) (Table 4).

Raw Silk Percentage: At 40±1°C, the highest raw silk percentage was recorded for (HL1 × HL3) × (HL10 × HL12) (16.95%) and the lowest of 16.28% was recorded for (HL3 × HL5) × (HL10 × HL12). At 25±1°C, the highest raw silk percentage was recorded for (HL1 × HL3) × (HL10 × HL12) and (HL1 × HL5) × (HL10 × HL12) (19.11%) and the lowest of 18.28% was recorded for (HL3 × HL5) × (HL10 × HL12) (Table 4).

Filament Size: At 40±1°C, the thickest filament size was recorded for (HL1 × HL3) × (HL10 × HL12) (2.54 d) and the thinnest of 2.45 d was recorded for (HL1 × HL5) × (HL10 × HL12). At 25±1°C, the thickest filament size was recorded for (HL1 × HL3) × (HL10 × HL12) (2.96 d) and the thinnest of 2.84 d was recorded for (HL1 × HL5) × (HL10 × HL12) (Table 4).

Neatness: At 40±1°C, the highest neatness was recorded for (HL1 × HL3) × (HL10 × HL12) (90.67 p) and the lowest of 89.67 p was recorded for (HL1 × HL5) × (HL10 × HL12) and (HL3 × HL5) × (HL10 × HL12). At 25±1°C, the highest neatness was recorded for (HL1 × HL3) × (HL10 × HL12) (94.0 p) and the lowest of 92.33 p was recorded for (HL1 × HL5) × (HL10 × HL12) (Table 4).

Evaluation Index: In the evaluation index method, the foundation crosses with high evaluation index at 40±1°C and 50±5% RH, were selected. The overall evaluation indices for pupation rate, yield/10000 larvae, cocoon weight, shell weight, cocoon shell percentage, reelability percentage, filament length, raw silk percentage and neatness ranged from 40.6 to 60.4 with the highest of 60.4 recorded in HL1 × HL3 and the lowest of 40.6 recorded in HL7 × HL12. However, evaluation indices for renditta and filament size are to be considered for least value and ranged from 43.8 to 55.63 with the least of 43.8 recorded for HL1 × HL5 and the highest of 55.63-recorded HL10 × HL12. (Table 5). Similarly, at 25 ±1°C and 65 ±5%RH, the overall indices values for all the characters other than renditta and filament size ranged from 41.5 to 54.3 with the highest of 54.3 for HL10 × HL12 and the lowest of 41.5 for HL7 × HL12 were recorded and the overall evaluation indices of renditta and denier ranged from 43.6 to 57.0 with the lowest of 43.6 for HL3 × HL5 and the highest of 57.0 in the hybrid (HL7 × HL12). (Table 6).

In the evaluation index method, the double hybrid with high evaluation index at 40±1°C and 50±5% RH, were selected. The overall evaluation indices for pupation rate, yield/10000 larvae, cocoon weight, shell weight, cocoon shell percentage, reelability percentage, filament length, raw silk percentage and neatness ranged from 43.6 to 60.2 with the highest of 60.2 recorded in (HL1 × HL3) × (HL10 × HL12) and the lowest of 43.6 recorded in (HL1 × HL5) × (HL10 × HL12). However, evaluation indices for renditta and filament size are to be considered for least value and ranged from 45.7 to 54.4 with the least of 45.7 recorded for (HL1 × HL5) × (HL10 × HL12) and the highest of 54.4 recorded for (HL3 x HL5) x (HL10 x HL12). (Table 7). Similarly, at 25 ±1°C and 65 ±5%RH, the overall indices values for all the characters other than renditta and filament size ranged from 42.9 to 59.4 with the highest of 59.4 for (HL1 × HL3) × (HL10 × HL12) and the lowest of 42.9 for (HL3 x HL5) x (HL10 x HL12) were recorded and the overall evaluation indices of renditta and denier ranged from 43.9 to 53.3 with the lowest of 43.9 for (HL1 × HL5) × (HL10 × HL12) and the highest of 53.3 in the hybrid (HL3 × HL5) × (HL10 × HL12). (Table 8).

Heterosis and Heterobeltiosis: Heterosis (hybrid vigour over mid parent value) and heterobeltiosis (hybrid vigour over better parent value) were estimated for both rearing and reeling characters for double hybrids at 40±1°C and 50±5% RH, 40±1°C and 85±5% RH and 25±1°C and 60±5% RH.

Table 1: Mean performance of foundation crosses for rearing at two temperature conditions

	40±1°C and 50+5%RH						25±1°C and65+5%RH				
HYBRID	Fecundity (No.)	Pupation rate (%)	Yield/10,000 larvae (kg)	Cocoon Wt. (g)	Shell Wt. (g)	Cocoon Shell (%)	Pupation rate (%)	Yield/10,000 larvae (kg)	Cocoon Wt. (g)	Shell Wt. (g)	Cocoon Shell (%)
HL1XHL3A	589	85.0 (67.3)	17.40	1.570	0.336	21.37 (27.53)	92.9 (74.6)	19.07	1.779	0.424	23.81 (29.21)
HL1XHL5A	553	76.3 (60.9)	17.19	1.607	0.341	21.24 (27.45)	93.6(75.4)	19.28	1.762	0.412	23.41 28.94)
HL3XHL5A	561	74.7 (59.8)	17.23	1.548	0.329	21.28 (27.47)	93.4 (75.1)	19.10	1.781	0.417	23.39 (28.93)
HL7XHL10A	560	67.0 (54.9)	16.84	1.533	0.316	20.64 (27.02)	93.5 (75.2)	18.67	1.857	0.414	22.29 (28.17)
HL7XHL12A	574	64.0 (53.1)	17.30	1.553	0.315	20.27 (26.76)	93.5 (75.3)	18.83	1.756	0.393	22.39 (28.25)
HL10XHL12A	584	75.7 (60.5)	17.45	1.575	0.325	20.64 (27.02)	93.6 (75.4)	19.09	1.857	0.415	22.37 (28.23)

Table 2: Mean performance of foundation crosses for reeling at two temperature conditions

	40±1°C and 50±5%RH						25±1°C and65±5%RH					
Hybrid	Reelability (%)	Filament length (m)	Renditta	Raw silk (%)	Filament size (d)	Neatness (p)	Reelability (%)	Filament length (m)	Renditta	Raw silk (%)	Filament size (d)	Neatness (p)
HL1XHL3	84.33 (66.69)	1058	6.12	16.35 (23.85)	2.78	91.33 (72.89)	86.67 (68.59)	1052	5.39	18.55 (25.51)	2.87	92.67 (74.30)
HL1XHL5	84.00 (66.42)	1031	6.13	16.31 (23.82)	2.58	90.00 (71.57)	86.33 (68.31)	1049	5.40	18.53 (25.50)	2.94	92.33 (73.93)
HL3XHL5	82.67 (65.40)	1019	6.14	16.30 (23.81)	2.65	90.33 (71.89)	86.00 (68.03)	1047	5.41	18.49 (25.46)	2.85	92.00 (73.57)
HL7XHL10	82.67 (65.41)	1017	6.34	15.76 (23.39)	2.47	90.33 (71.89)	86.33 (68.32)	1022	5.70	17.56 (24.78)	2.79	93.00 (74.68)
HL7XHL12	84.00 (66.43)	1002	6.43	15.56 (23.24)	2.57	90.67 (72.22)	85.67 (67.76)	998	5.64	17.73 (24.90)	2.91	92.67 (974.30)
HL10XHL12	84.33 (66.73)	1048	6.36	15.73 (23.36)	2.65	90.33 (71.89)	86.67 (68.59)	1082	5.73	17.45 (24.69)	2.86	93.0 (74.68)

Table 3: Mean performance of double hybrids for rearing at two temperature conditions

	40±1°C and 50+5%RH						25±1°C and65+5%RH			
HYBRID	Fecundity (No.)	Pupation rate (%)	Yield/10,000 larvae (kg)	Cocoon Wt. (g)	Shell Wt. (g)	Cocoon Shell (%)	Pupation rate (%)	Yield/ 10,000 larvae (kg)	Cocoon Wt. (g)	Shell Cocoon Wt. (g) Shell (%)
(HL1XHL3)X(HL10XHL12)	660	88.7 (70.4)	17.53	1.577	0.351	22.24 (28.14)	94.4 (76.4)	19.50	1.882 0.458	24.35 (29.56)
(HL1XHL5)X(HL10XHL12)	649	71.7 (57.8)	17.06	1.535	0.332	21.65 (27.73)	94.8 (76.8)	19.26	1.826	0.437 23.92 (29.27)
(HL3XHL5)X(HL10XHL12)	653	71.7 (57.8)	17.22	1.586	0.337	21.23 (27.43)	93.3 (75.1)	19.19	1.846	0.429 23.22 (28.81)

Table 4: Mean performance of hybrids for reeling at two temperature conditions

	40±1°C and 50+5%RH						25±1°C and65+5%RH					
Hybrid	Reelability (%)	Filament length (m)	Renditta	Raw silk (%)	Filament size (d)	Neatness (p)	Reelability (%)	Filament length (m)	Renditta	Raw silk (%)	Filament size (d)	Neatness (p)
(HL1XHL3)X(HL10XHL12)	86.00 (68.03)	948	5.90	16.95 (24.31)	2.54	90.67 (72.22)	87.33 (69.16)	1133	5.24	19.11(25.92)	2.96	94.00 (75.85)
(HL1XHL5)X(HL10XHL12)	82.00 (64.90)	909	6.05	16.53 (23.99)	2.45	89.67 (71.25)	86.00 (68.03)	1069	5.24	19.11 (25.32)	2.84	92.33 (73.93)
(HL3XHL5)X(HL10XHL12)	83.33 (65.91)	910	6.16	16.28 (23.79)	2.49	89.67 (71.25)	86.33 (68.32)	1067	5.47	18.28 (25.32)	2.85	92.67 (74.30)

Table 5: Evaluation index of the foundation crosses at 40±1°C and 50±5%RH

HYBRID	Fecundity	Pupation rate (%)	Yield/10000 larvae (kg)	Cocoon Wt. (g)	Shell Wt .(g)	Cocoon Shell (%)	Reelability (%)	Filament length (m)	Raw silk (%)	Neatness (p)	Evaluation Index	Renditta	Filament size (d)	Evaluation Index
HL1XHL3	63.0	65.0	57.5	52.2	58.6	60.3	58.5	63.9	59.8	68.1	60.4	40.3	65.9	53.1
HL1XHL5	38.2	53.4	48.0	66.5	63.3	57.4	54.2	50.7	58.6	39.1	54.6	41.3	46.2	43.8
HL3XHL5	43.7	51.2	49.8	43.7	51.9	58.3	37.3	45.0	58.5	46.4	49.1	41.5	52.9	47.2
HL7XHL10	43.0	40.9	31.8	37.9	39.5	44.0	37.3	44.2	43.3	46.4	40.6	56.6	36.3	46.4
HL7XHL12	52.4	36.9	53.1	45.6	38.6	35.9	54.2	37.2	37.6	53.6	43.6	62.5	45.2	53.9
HL10XHL12	59.8	52.5	59.8	54.1	48.1	44.1	58.5	59.1	42.3	46.4	51.7	57.8	53.5	55.6

Table 6: Evaluation index of the foundation crosses at 25±1°C and 65±5%RH

HYBRID	Pupation rate (%)	Yield/10000 larvae(kg)	Cocoon Wt.(g)	Shell Wt.(g)	Cocoon Shell%	Reelability (%)	Filament length(m)	Raw silk (%)	Neatness (p)	Evaluation Index	Renditta	Filament size(d)	Evaluation Index
HL1XHL3	30.7	53.0	45.7	61.1	63.0	60.0	53.6	59.5	51.4	53.1	40.5	49.5	45.0
HL1XHL5	58.4	62.5	42.1	49.5	56.9	51.4	52.6	59.2	42.9	52.8	40.9	63.8	52.4
HL3XHL5	48.3	54.2	46.2	54.3	56.8	42.9	51.8	58.3	34.3	49.7	41.7	45.6	43.6
HL7XHL10	53.3	34.7	62.6	51.4	40.2	51.4	43.2	40.7	60.0	48.6	59.3	35.0	47.2
HL7XHL12	53.3	41.8	40.8	31.3	41.8	34.3	34.7	43.8	51.4	41.5	56.0	57.9	57.0
HL10XHL12	55.9	53.8	62.6	52.4	41.4	60.0	64.1	38.5	60.0	54.3	61.6	48.1	54.9

Table 7: Evaluation index of the double hybrids at 40±1°C and 50±5%RH

HYBRID	Fecundity	Pupation rate (%)	Yield/10000 Larvae (kh)	Cocoon Wt.(g)	Shell Wt. (g)	Cocoon Shell%	Reelability (%)	Filament length (m)	Raw silk (%)	Neatness (p)	Evaluation Index	Renditta	Filament size(d)	Evaluation Index
(HL1XHL3)X(HL10XHL12)	61.0	61.5	60.9	54.0	61.2	60.5	60.9	61.5	60.7	61.5	60.3	39.6	60.4	50.0
(HL1XHL5)X(HL10XHL12)	41.5	44.2	41.2	38.6	41.9	48.9	41.3	43.9	48.3	44.2	43.6	50.9	40.4	45.7
(HL3XHL5)X(HL10XHL12)	47.4	44.2	47.9	57.3	47.0	40.6	47.8	44.6	40.9	44.2	46.1	59.5	49.2	54.4

Table 8: Evaluation index of the double hybrids at 25±1°C and 65±5%RH

HYBRID	Pupation rate (%)	Yield/10000 larvae(kg)	Cocoon Wt.(g)	Shell Wt. (g)	Cocoon Shell%	Reelability (%)	Filament length(m)	Raw silk (%)	Neatness (p)	Evaluation Index	Renditta	Filament size(d)	Evaluation Index
(HL1XHL3)X(HL10XHL12)	52.5	61.3	60.8	61.1	59.1	61.2	61.5	55.8	61.3	59.4	44.2	61.5	52.8
(HL1XHL5)X(HL10XHL12)	58.5	46.4	41.1	47.1	51.6	42.0	44.5	55.8	42.4	47.7	44.3	43.5	43.9
(HL3XHL5)X(HL10XHL12)	39.0	42.3	48.1	41.8	39.3	46.8	44.0	38.5	46.2	42.9	61.5	45.0	53.3

Table 9: Heterosis of rearing and reeling characters in hybrids at 40±1°C and 50±5%RH

Hybrid	Fecundity (No.)	Pupation rate (%)	Yield/10000 larvae(kg)	Cocoon Wt.(g)	Shell Wt.(g)	Cocoon Shell%	Reelability %	Filament length(m)	Renditta	Raw silk %	Filament size(d)	Neatness (p)
(HL1XHL3) X (HL10XHL12)	3.81**	4.10**	3.03**	1.19**	2.96**	1.70**	1.43**	2.07**	-2.36	2.61**	0.03	0.47**
(HL1XHL5) X (HL10XHL12)	3.74**	1.03ns	1.86**	0.52ns	1.57*	1.03ns	0.26ns	1.33**	-2.05	2.20**	-1.35	0.14ns
(HL3XHL5) X (HL10XHL12)	3.92**	0.65ns	2.29**	1.31**	1.97**	0.63ns	0.61**	1.26**	-1.72	1.88*	-0.43	0.14ns

Table 10: Heterobeltiosis of rearing and reeling characters in hybrids at 40±1°C and 50±5%RH

Hybrid	Fecundity (No.)	Pupation rate (%)	Yield/10000 Larvae (kg)	Cocoon Wt.(g)	Shell Wt.(g)	Cocoon Shell%	Reelability %	Filament length(m)	Renditta	Raw silk %	Filament size(d)	Neatness (p)
(HL1XHL3) X (HL10XHL12)	13.40**	8.62**	8.22**	4.51**	10.73**	5.63*	5.31**	5.72**	-11.22	8.34*	-1.55	1.87**
(HL1XHL5) X (HL10XHL12)	14.26**	1.83ns	5.25*	1.86ns	5.60ns	3.29ns	0.82ns	4.031	-11.22	5.22ns	-6.00	0.37ns
(HL3XHL5) X (HL10XHL12)	14.24**	1.42ns	6.28**	5.08**	6.50*	1.16ns	2.46**	3.57ns	-9.69	4.31ns	-3.36	0.37ns

* and ** Denote significant difference at 5% and 1%
ns Denote non significant

Table 11: Heterosis of rearing and reeling characters in hybrids at 25±1°C and 65±5%RH

Hybrid	Pupation rate (%)	Yield/10000 larvae(kg)	Cocoon Wt.(g)	Shell Wt.(g)	Cocoon Shell%	Reelability %	Filament length(m)	Renditta	Raw silk %	Filament size(d)	Neatness (p)
(HL1XHL3) X (HL10XHL12)	0.34ns	0.91**	1.16**	2.71**	1.49**	0.24ns	2.20**	-1.29	1.37**	0.66**	0.36**
(HL1XHL5) X (HL10XHL12)	0.66**	1.00**	0.38*	1.40**	1.01**	-0.10ns	0.64ns	-1.42	1.53**	-0.22	-0.04
(HL3XHL5) X (HL10XHL12)	0.05ns	1.06**	0.69**	1.00**	0.31ns	0.05ns	0.68ns	-0.09	0.08ns	0.28	0.00ns

Table 12: Heterobeltiosis of rearing and reeling characters in hybrids at 25±1°C and 65±5%RH

Hybrid	Pupation rate (%)	Yield/10000 Larvae (kg)	Cocoon Wt. (g)	Shell Wt .(g)	Cocoon Shell (%)	Reelability (%)	Filament length (m)	Renditta	Raw silk (%)	Filament size (d)	Neatness (p)
(HL1XHL3) X (HL10XHL12)	1.06ns	3.14ns	3.83**	8.53**	4.55**	0.38ns	8.16**	-6.73	3.76**	-1.55	1.44**
(HL1XHL5) X (HL10XHL12)	2.01ns	2.18ns	0.68ns	3.31**	2.62ns	-0.77	0.88ns	-6.68	4.99**	-4.16	-0.35
(HL3XHL5) X (HL10XHL12)	-0.38	1.78ns	2.05**	2.06ns	0.02ns	-0.38	1.61ns	-2.56	-1.89	-0.69	-0.35

* and ** Denote significant difference at 5% and 1%
ns Denote non significant

Table 13: Comparative performance of (HL1x HL3) x (HL10xHL12) at 40±1°C and 50±5% RH (mean of 5 trials)

ybrid	Fecundity (No.)	Pupation rate (%)	Yield/10000 larvae (kg)	Cocoon Wt .(g)	Shell Wt .(g)	Cocoon Shell (%)	Reelability (%)	Filament length (m)	Renditta (%)	Raw silk size(d)	Filament (p)	Neatness
(HL1XHL3) X (HL10XHL12) A	673.7	87.7	17.4	1.662	0.371	22.3	84.0	941.0	5.84	17.1	2.43	91.0
(CSR2XCSR27) x (CSR6XCSR26)	648.0	27.2	8.4	1.522	0.321	21.1	82.0	899.0	6.78	14.8	2.72	90.0
Control Percent improvement over control hybrids												
(HL1XHL3) X (HL10XHL12) A Vs. (CSR2XCSR27) x (CSR6XCSR26)	4.0	222.3	105.5	9.198	15.680	6.0	2.4	4.7	-13.9	16.2	-10.7	1.1

Table 14: Comparative performance of (HL1x HL3) x (HL10xHL12) at 25±1°C and 65±5% RH (mean of 5 trials)

Hybrid	Pupation rate (%)	Yield/10000 larvae(kg)	Cocoon Wt.(g)	Shell Wt.(g)	Cocoon Shell%	Reelability (%)	Filament length(m)	Renditta	Raw silk (%)	Filament size (d)	Neatness (p)
(HL1XHL3) X (HL10XHL12)	93.7	18.5	1.824	0.431	23.6	87.0	1081.0	5.33	18.7	2.77	92.0
(CSR2XCSR27) x (CSR6XCSR26)	94.2	18.6	1.826	0.418	22.9	87.0	1090.0	5.30	18.9	2.85	92.0
Control Percent improvement over control hybrids											
(HL1XHL3) X (HL10XHL12) Vs (CSR2XCSR27) x (CSR6XCSR26)	-0.5	-0.3	-0.088	2.990	3.1	0.0	-0.8	0.6	-0.6	-2.8	0.0

Table 15: Cocoon volume in double hybrids of HL combinations

Sl. No.	Hybrid	Cocoons/litre	Standard Deviation
	At 25±1°C and 65±5% RH.		
1	(HL1XHL3) X (HL10XHL12)	77	5.4
2	(HL1XHL5) X(HL10XHL12)	88	7.9
3	(HL3XHL5) X (HL10XHL12)	92	9.5

Table 16: Cocoon size uniformity in double hybrids of HL combinations

Sl. No.	Hybrid	Cocoon length (cm)	Cocoon width (cm)	Cocoon Length/ width ratio	CV%
1	(HL1XHL3) X(HL10XHL12)	3.23±0.11	1.87±0.07	172.76±6.22	3.60
2	(HL1XHL5) X(HL10XHL12)	3.09±0.15	1.80±0.07	171.67±8.56	4.99
3	(HL3XHL5) X(HL10XHL12)	2.96±0.17	1.81±0.09	164.36±13.07	7.95

Double Hybrids Developed under High Temperature (40 ±1°C) and Low Humidity (50±5%): At 40±1°C and 50±5% RH, (HL1 × HL3) × (HL10 × HL12) manifested maximum hybrid vigour over mid parent value as well as better parent value for all the characters including renditta and filament size (d). (Table 9 and 10). Similarly, at 25±1°C and 60±5% RH, (HL1 × HL5) × (HL10 × HL12) was found maximum hybrid vigour over mid parent value for all the characters including renditta and filament size (d). However, for heterobeltiosis (HL1 × HL3) × (HL10 × HL12) was found maximum hybrid vigour for all the characters including renditta and filament size (d). (Table 11 and 12).

Percent Improvement of Selected Hybrid: Based on the consistency for pupation rate at 40±1°C and 50±5% RH, the the double hybrid, (HL1 × HL3) × (HL10 × HL12) was selected. Further, the selected double hybrid was compared with the respective control hybrid viz., (CSR2 × CSR27) × (CSR6 × CSR26). The percent improvement of 4.0% in fecundity, 222.3 (pupation rate), 105.5(yield/10000 larvae), 9.2% (cocoon weight), 15.7% (shell weight), 6.0% (cocoon shell percentage), 2.4% (reelability), 4.7% (filament length), 16.2% (raw silk percentage) and 1.1% (neatness) and decrement for renditta (-13.9%) and filament size (-10.7%) was recorded in (HL1 × HL3) × (HL10 × HL12) over the control (CSR2 × CSR27) × (CSR6 × CSR26) at 40 ±1°C and 50 ±5%RH. (Table 13). However, at 25 ±1°C and 65 ±5%RH, improvement was noticed in shell weight (2.99%), cocoon shell percentage (3.1%) and renditta (0.6%) and decrement for pupation rate (-0.5%), yield/10000 larvae (-0.3%), cocoon weight (-0.1%), filament size (-2.8%) and raw silk percentage (-0.6%) and there was no improvement for reelability and neatness. (Table 14).

Cocoon Size Uniformity: Cocoon volume of double hybrids of HL combinations revealed highest cocoon number /litre 92 cocoons in (HL3 × HL5) × (HL10 × HL12) with standard deviation of 9.5 and lowest cocoon number in (HL1 × HL3) × (HL10 × HL12) ie., 77/ liter with 5.4 standard deviation. (Table 15). Cocoon size uniformity of double hybrids were studied by measuring cocoon length and cocoon width of 100 cocoons picked randomly form each hybrid combination to know the cocoon uniformity and coefficient of variance was also calculated The cocoon size uniformity among HL combinations of double hybrids exhibited better uniformity of 172.76 cocoon length width ratio in (HL1 × HL3) × (HL10 × HL12) with less standard deviation (6.22) as well as coefficient of variance (3.60) (Table 16).

DISCUSSION

Silkworm breed which are reared over a series of environments exhibiting less variation are considered stable. One of the objectives of the breeder is to recommend stable breeds to the farmers for rearing under different environmental conditions. Effect of high temperature and low humidity in terms of cocoon crop depends on several factors that operate within and outside the body of the silkworm. In the present study, it was observed that apart from the temperature, humidity also influences the productivity pattern in the silkworm and is in agreement with [12, 13]. [14] observed that the cocoon yield/10000 larvae, cocoon weight, cocoon shell weight and cocoon shell percentage were also low in the high temperature treated batches when compared to the batches reared under optimum rearing conditions which corroborates the findings of the present study. [15] reported the deleterious effect of high temperature and high humidity on quantitative traits of parents, foundation crosses, single and double hybrids of bivoltine silkworm breeds of *Bombyx mori* L.

Improvement of breeds necessarily means selection of desirable genes in appropriate combinations, which contribute to the overall genetic worth of the population. With respect to the economic value, the focus should be on all the genes affecting the traits thereby contributing to the viability and productivity. Therefore, selection of hybrid combinations emphasize the need to organize the genetic material in a way that help to improve the manifestation of commercially important traits [16]. This can be achieved precisely by adopting a strategy by setting up a common index giving adequate weightage to all the component traits manifested among an array of hybrids. The rationale for judging the utility of multiple trait evaluation index in the present study is based on major metric traits., which are considered to be economically important. However, asymmetry is found in most of the traits as evidenced by indices of the individual component traits that the index value obtained individually for each trait can fail but the overall index values help in adjudicating the performance of the hybrids derived. In view of this, all the major traits have been considered together to obtain the aggregate index value, since exclusion of any one trait can result in negative situation.

Geneticists and breeders of all the sericultural countries have experienced the influence of environment during the process of breeding. [2] studied the silkworm viability and cocoon weight for 19 generations at two different temperature and humidity. He observed that the lines selected at high temperature and humidity perform better than the lines selected at normal temperature and humidity. The effect of high temperature more than 30°C on silkworm larvae was reported earlier by [17, 18]. [19, 20] used survival rate of silkworms as a main yardstick character for evaluating thermo-tolerance. [21] conducted a series of experiments and concluded that the resistance to high temperature is a heritable character and it may be possible to breed silkworm races tolerant high temperature. [22, 23] while attempting to synthesise high temperature resistant silkworm races confirmed the genetically heritable nature of thermo-tolerance by selection based on pupation rate of silkworm reared under high temperature conditions during 5^{th} instar. Recently, [3, 5, 6] have evolved compatible robust hybrid CSR18 × CSR19 for rearing throughout the year by utilizing Japanese thermo-tolerant hybrids as breeding resource materials. Though, the introduction of CSR18 × CSR19 in the field during summer months had considerable impact, the productivity level and returns realized does not match to that of other productive CSR hybrids. Therefore, the acceptance level of this hybrid with the farmers was not up to the expected level because of the low productivity traits. This has necessitated in the development of a temperature tolerant hybrid with better productivity traits than CSR18 × CSR19. Considering the gravity of the situation and also to cope up with the challenge, though, it was a difficult task to break the negative correlation associated with survival and productivity traits, attempts on this line had resulted in the development of CSR46 × CSR47 [15], a temperature tolerant bivoltine hybrid with better productivity traits than CSR18 × CSR19. It was also reported that any study involving temperature as one of the environmental factors and viability followed by cocoon traits is a trend setter to provide basis to formulate appropriate selection methods for required environments.

In India, for the last two decades, the introduction of the productive bivoltine CSR hybrids had resulted in a phenomenal increase in yield and income for the farmers and increased output of quality raw silk. However, these productive bivoltine hybrids could make much impact only to the progressive farmers who could provide high input and managerial skills which were essential to realize the maximum potential of these hybrids. Therefore, in spite of the quality linked productivity merit, these hybrids lacked the genetic plasticity to buffer against the adverse conditions of the tropics such as fluctuating temperature and humidity, inferior nutritional status of the leaf and high germ load. Therefore, it became highly imperative to develop silkworm breeds tolerant to diseases and adverse climatic conditions of the tropics which can consistently realize stable cocoon crops with quality. Attempts in this direction had resulted in the development of the double hybrid, (HL1 × HL3) × (HL10 × HL12) which are relatively tolerant to silkworm diseases and high temperature situations than the existing hybrids. Since these bivoltine hybrids have the genetic plasticity to buffer against the adverse climatic conditions to ensure sustainability in cocoon crops and relative tolerance to various diseases and inherent genetic potential to produce good quality raw silk than the existing bivoltine hybrids, it can be reared throughout the year.

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