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Development of Bivoltine Double Hybrid of the Silkworm, *Bombyx Mori* L. Tolerant to High Temperature and High Humidity Conditions of the Tropics

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Abstract:
Cocoon crop stability with sustainably good performance is a prerequisite to introduce bivoltine races in a tropical country like India under high temperature and humidity stress. 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. In India, it is not conducive to rear highly productive bivoltine hybrids, especially in summer. Therefore, attempts are being made to develop bivoltine silkworm hybrids tolerant to high temperature situations of the tropics. Attempts made earlier on these lines had resulted in the development of robust bivoltine hybrids viz., CSR18 x CSR19, CSR46 x CSR47 and CSR50 x CSR51. However, these hybrids could not make any impact in Indian sericulture industry. Keeping this in view, attempt is being made here to develop bivoltine double hybrid tolerant to high temperature and high humidity conditions of the tropics. The breeding process as well as the comparative performance of the new hybrid with the already developed double hybrid is also discussed in detail. The study has resulted in the development of bivoltine double hybrid tolerant to high temperature and high humidity conditions of the tropics.

Key words: *Bombyx mori*, High temperature, High humidity, Bivoltine, Double hybrid

1.0 Introduction:
The success of sericulture industry depends upon several factors of which the impact of the environmental factors such as biotic and abiotic factors is of vital importance. Among the abiotic factors, temperature plays a major role on growth and productivity of silkworm, as it is a poikilothermic (cold blooded) insect. It is also known that the late age silkworms prefer relatively lower temperature than young age and fluctuation of temperature during different stages of larval development was found to be more favourable for growth and development of larvae than constant temperature. There is ample literature stating that good quality cocoons are produced within a temperature range of 22-27°C and above these levels makes the cocoon quality poorer (Krishnaswami *et al.,* 1973). However, polyvoltine races reared in tropical countries are known to tolerate slightly higher temperature (Hesieh *et al.,* 1995), which is also true with cross breeds that have been evolved specially for tropical climate.

The effect of temperature higher than 30°C on silkworm larvae was reported earlier by Shirota (1992) and Tazima and Ohnuma (1995) while attempting to synthesise high temperature resistant silkworm races confirmed the genetical nature of thermo-tolerance by selection based on pupation rate of silkworms reared under high temperature conditions in fifth instar. 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 (Datta *et al*., 1997; Datta *et al.,* 2000, Datta *et al.,* 2001; Suresh Kumar *et al.,* 2002,2006: Dandin et al 2006) 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 Suresh Kumar *et al.,* (1999) and Datta *et al.,* 2001) indicated that the deleterious effect of high temperature was more pronounced in productive hybrids than the robust hybrids. Studies on the effect of high temperature of F1 hybrids between polyvoltines and bivoltine (Suresh Kumar *et al.*,2001) indicated that there was maternal effect regarding temperature tolerance as evident from the better performance of those hybrids where the female parent used was more tolerant to high temperature than the male parent. While studying the heterotic effect of CSR bivoltine hybrids under room and high temperature conditions, Suresh Kumar *et al.,* (2000) observed that the expression of hybrid vigour was different in hybrids at different temperature treatments and all the hybrids excelled their parents in many characters with significant positive heterosis over mid parent value and better parent value.

One of the main aims of the breeders is to recommend silkworm breeds/hybrids to the stakeholders that can sustain and produce stable silkworm crops in adverse climatic conditions and the yield should not be below economic threshold level. Moreover, silkworm breeds/hybrids which perform consistently good under adverse climactic conditions are considered as stable. In order to introduce bivoltine races in a tropical country like India, it is necessary to have stability in cocoon crop under high temperature environments. 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. 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. Thus a compatible robust bivoltine hybrid, CSR18 × CSR19 was evolved from a Japanese hybrid, B201 × BCS12 under high temperature (36±1°C) and high humidity (85±5 %) conditions (Datta, *et al.,* 1997; Suresh Kumar *et al.,* 2002) by taking clues from earlier experiments conducted by Japanese experts (Shirota, 1992 and Tazima and Ohnuma, 1995). Though, this hybrid was authorized by Central Silk Board for commercialization, large scale testing in the field is yet to take momentum.

2.0 Materials and Methods:

Silkworm rearing was conducted following the standard method under recommended temperature and RH till 2nd day of 5th instar. Three trials with three replicates were conducted for this study. On the third day of 5th instar, 200 larvae each for two thermal treatments (40 ± 1°C and 50 ± 5 % RH and 40 ± 1°C and 85 ± 5 % RH) were separated from each bed for thermal treatment. The remaining larvae in the tray were treated as control at normal temperature and humidity (25 ± 1°C and 65 ± 5 % RH). For thermal exposure, the larvae of 3rd day of the 5th instar were kept in plastic trays and reared in SERICATRON (Environment chamber with temperature and humidity control) at two different temperatures and humidity. Three replicates were kept in 40 ± 1°C and 50 ± 5 % RH and 40 ± 1°C and 85 ± 5 % RH and were fed with fresh mulberry leaves twice a day. The thermal exposure was given everyday for 6 hours duration. When the larvae started spinning, they were shifted to 25 ± 1°C and 65 ± 5 % RH. Plastic collapsible mountages were used for mounting the ripened larvae. After 48 hours of mounting, when the larvae formed hammock, the mountages were turned upside down. Cocoon harvesting was carried out on the 7th day of spinning. The cocoons were deflossed and the defective ones were sorted out. Assessment was carried out on the subsequent day. The survival rate was calculated as the number of live pupae to the number of larvae treated.

To initiate the breeding programme of high temperature (40 ±1°C) and high low humidity (50±5%) and high temperature (40 ±1°C) and high humidity (85±5%), six bivoltine breeds three each of oval viz., CSR18, CSR46 and CSR50 and dumbbell viz., CSR19, CSR47 and CSR51 were found to be temperature tolerant and selected as parental breeds for breeding programme. In oval lines CSR46 and CSR50 are characterized by plain larvae while CSR18 is characterized by marked and plain (sex limited) larvae where female is marked and male is plain similarly in dumbbell lines CSR19 is characterized by sex limited (marked and plain) larvae and CSR47 and CSR51 are characterized by marked larvae.

For the development of breeds through appropriate techniques breeding programme was initiated with an objective to introduce the bivoltine breeds/hybrids for high temperature 40 ± 1°C i.e. and low relative humidity i.e. 85 ± 5°% and high temperature i.e. 40 ± 1°C and high relative humidity i.e. 85 ± 5%. Twelve breeds were developed, six for high temperature and low humidity and six for high temperature and high humidity conditions. Out of six breeds for one temperature there are three each of oval breeds namely viz., HL1, HL3 and HL5 and dumbbell breeds namely viz., HL7, HL10 and HL12 (for high temperature and low RH) were developed. In the case for high temperature and high relative humidity there are also three each of oval breeds i.e. HH1, HH3 and dumbbell breeds i.e. HH8, HH10 and HH12 were introduced.

Cellular rearing was resorted to from F6 onwards to F12 with minimum five replications preceded by half sib/full sib mating for three different temperatures. At the time of brushing, the rich egg layings showing good hatching % were selected from each set of room temperature and two different high temperatures and reared. Owing the thermal effect in successive generations, it was observed that after 5th generation both qualitative and quantitative characters have declined sharply. So the experiment was modified in such a way that the every alternate generation from F6 onwards to F12 both high temperature, lines were brought to room temperature conditions and reared continuously till spinning to recoup the lost vitality under stress conditions.

3.0 Results and Discussion:

3.1 Performance of foundation crosses developed under high temperature (40±1°C) and high humidity (85±5%) conditions:

3.1.1 Pre-cocoon parameters:

The fecundity is the same for both the treatments and it ranged from 547 to 578 with the highest of 578 recorded for HH1 × HH3 and the lowest of 547 recorded for HH1 × HH6.At 40±1°C, the pupation rate ranged from 70.3 to 89.3 % with the highest of 89.3 % recorded for HH1 × HH3 and the lowest of 70.3 % recorded for HH1 × HH6. At 25±1°C, the pupation rate ranged from 92.6 to 94.5 % with the highest of 94.5 % recorded for HH10 × HH12 and the lowest of 92.6 % recorded for HH8 × HH10 . At 40±1°C, the yield/10000 larvae ranged from 16.07 to 16.77 kg with the highest of 16.77 kg recorded for HH3 × HH6 and the lowest of 16.07 kg recorded for HH1 × HH6. At 25±1°C, the yield/10000 larvae ranged from 18.36 to 19.24 kg with the highest of 19.24 kg recorded for HH8 × HH12 and the lowest of 18.36 kg recorded for HH3 × HH6 . At 40±1°C, the cocoon weight ranged from 1.490 to 1.599 g with the highest of 1.599 g recorded for HH10 × HH12 and the lowest of 1.490 g recorded for HH8 × HH10. At 25±1°C, the cocoon weight ranged from 1.707 to 1.845 g with the highest of 1.845 g recorded for HH10 × HH12 and the lowest of 1.707 g recorded for HH8 × HH10. At 40±1°C, the cocoon shell weight ranged from 0.306 to 0.337 g with the highest of 0.337 g recorded for HH3 × HH6 and the lowest of 0.306 g recorded for HH8 × HH10. At 25±1°C, the cocoon shell weight ranged from 0.398 to 0.436 g with the highest of 0.436 g recorded for HH1 × HH3 and the lowest of 0.398 g recorded for HH8 × HH10. At 40±1°C, the cocoon shell percentage ranged from 20.50 to 21.58 % with the highest of 21.58 % recorded for HH1 × HH6 and the lowest of 20.50 % recorded for HH8 × HH10 and HH8 × HH12. At 25±1°C, the cocoon shell percentage ranged from 23.25 to 23.77 % with the highest of 23.77% recorded for HH3 × HH6 and the lowest of 23.25 % recorded for HH8 × HH12 (Table 1).

3.1.2 Post-Cocoon Parameters:

At 40±1°C, the highest reelability was recorded for HH1 × HH3 and HH10 × HH12 (84.67 %) and the lowest of 83.67 % was recorded for HH3 × HH6 and HH8 × HH10. At 25±1°C, the highest reelability was recorded for HH3 × HHH6 (87.0 %) and the lowest of 85.0 % was recorded for HH1 × HHH6. At 40±1°C, the longest filament length was recorded for HH8 × HH12 (1058 m) and the shortest of 955 m was recorded for HH1 × HH6. At 25±1°C, the longest filament length was recorded for HH8 × HH12 (1095 m) and the shortest of 997 m was recorded for HH8 × HH10. At 40±1°C, the highest renditta was recorded for HH8 × HH12 (6.65) and the lowest of 6.34 was recorded for HH3 × HH6. At 25±1°C, the highest renditta was recorded for HH8 × HH12 (5.45) and the lowest of 5.30 was recorded for HH1 × HH3.At 40±1°C, the highest raw silk percentage was recorded for HH3 × HH6 (15.77 %) and the lowest of 15.03 % was recorded for HH8 × HH12. At 25±1°C, the highest raw silk percentage was recorded for HH1 × HH3 (18.88 %) and the lowest of 18.35 % was recorded for HH8 × HH12. At 40±1°C, the thickest filament size was recorded for HH10 × HH12 (2.76 d) and the thinnest of 2.56 d was recorded for HH8 × HH12. At 25±1°C, the thickest filament size was recorded for HH8 × HH12 (2.87 d) and the thinnest of 2.83 d was recorded for HH3 × HHH6. At 40±1°C, the highest neatness was recorded for HH1 × HH3 (91.0 p) and the lowest of 90.0 p was recorded for HH1 × HH6. At 25±1°C, the highest neatness was recorded for HH1 × HH3 and HH3 × HH6 (93.0 p) and the lowest of 92.0 p was recorded for HH10 × HH12 (Table 2).

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

	40+1ºc and 85+5%RH						25+1ºc 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 %
HH1XHH3	578	89.3(71.0)	16.63	1.570	0.334	21.27(27.46)	93.4(75.2)	19.20	1.840	0.436	23.72(29.14)
HH1XHH6	547	70.3(57.1)	16.07	1.554	0.335	21.58(27.68)	92.7(74.4)	18.81	1.764	0.414	23.44(28.96)
HH3XHH6	561	80.0(63.5)	16.77	1.570	0.337	21.46(27.45)	93.6(75.5)	18.36	1.830	0.435	23.77(29.18)
HH8XHH10	569	73.7(59.5)	16.14	1.490	0.306	20.5(26.99)	92.6(74.0)	18.44	1.707	0.398	23.35(28.89)
HH8XHH12	553	88.7(70.6)	16.60	1.522	0.312	20.5(26.91)	93.1(74.9)	19.24	1.835	0.427	23.25(28.83)
HH10XHH12	569	86.3(68.6)	16.62	1.599	0.329	20.6(26.90)	94.5(76.6)	18.10	1.845	0.430	23.31(28.87)

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

	40+1ºc and 85+5%RH						25+1ºc 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)
HH1XHH3	84.67(66.95)	1034	6.38	15.68(23.32)	2.73	91.00(72.54)	86.00(68.03)	1070	5.30	18.88(25.75)	2.86	93.00(74.66)
HH1XHH6	83.67(66.17)	955	6.35	15.74(23.38)	2.65	90.00(71.57)	85.00(67.21)	1012	5.41	18.49(25.47)	2.84	92.33(73.93)
HH3XHH6	84.33(66.69)	1002	6.34	15.77(23.40)	2.61	90.67(72.22)	87.00(68.87)	1033	5.38	18.61(25.56)	2.83	93.00(74.66)
HH8XHH10	83.67(66.17)	964	6.60	15.15(22.90)	2.74	90.33(71.89)	86.33(68.31)	997	5.37	18.65(25.58)	2.85	92.33(73.93)
HH8XHH12	84.33(66.69)	1058	6.65	15.03(22.81)	2.56	90.33(71.89)	86.67(68.59)	1095	5.45	18.35(25.37)	2.87	92.67(74.30)
HH10XHH12	84.67(66.95)	957	6.64	15.07(22.84)	2.76	90.67(72.22)	85.33(67.49)	1057	5.41	18.50(25.48)	2.84	92.00(73.57)

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

	40+1ºc and 85+5%RH						25+1ºc 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 %
(HH1XHH3)X(HH8XHH12)	688	86.33(68.3)	17.44	1.628	0.364	22.38(28.24)	94.8(76.8)	19.44	1.910	0.452	23.67(29.12)
(HH1XHH3)X(HH10XHH12)	664	72.7(58.5)	16.92	1.585	0.340	21.46(27.59)	93.1(74.8)	18.92	1.870	0.440	23.51(29.00)
(HH3XHH6)X(HH8XHH12)	641	76.3(61.1)	16.47	1.598	0.345	21.61(27.69)	93.2(74.9)	19.13	1.864	0.438	23.50(28.99)
(HH3XHH6X(HH10XHH12)	649	71.0(57.4)	16.96	1.551	0.346	22.30(28.18)	94.5(76.4)	19.29	1.841	0.429	23.2828.85)

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

Hybrid	40±1ºc and 85+5%RH						25±1ºc and65+5%RH					
	Reelability %	Filament length(m)	Renditta	Raw silk %	Filament size(d)	Neatness (p)	Reelability %	Filament length(m)	Renditta	Raw silk %	Filament size(d)	Neatness (p)
(HH1XHH3)X(HH8XHH12)	85.00	944	6.07	16.47	2.45	91.00	87.00	1088	5.39	18.55	2.96	93.67
(HH1XHH3)X(HH10XHH12)	84.33	924	6.40	15.63	2.37	90.67	86.33	1027	5.36	18.65	2.95	92.33
(HH3XHH6)X(HH8XHH12)	84.00	912	6.36	15.75	2.88	90.33	86.00	1027	5.40	18.52	2.87	92.33
(HH3XHH6X(HH10XHH12)	83.67	905	6.13	16.31	2.54	90.00	86.33	1044	5.41	18.50	2.88	92.33

Table 5: Evaluation index of the foundation crosses at 40±1ºc and 85±5%RH

HYBRID	Fecundity	Pupation rate (%)	Yield/ 10000 larvae(kg)	Cocoon Wt.(g)	Shell Wt.(g)	Cocoon Shell%	Reelability %	Filament Length	Raw silk %	Neatness (p)	Evaluation Index	Renditta	Filament size(d)	Evaluation Index
HH1XHH3	63.3	59.9	55.4	54.9	56.4	55.6	59.8	58.9	57.5	64.3	58.1	42.3	57.1	49.7
HH1XHH6	36.1	36.3	36.3	50.8	57.2	61.7	37.8	40.9	59.4	35.7	46.2	40.7	47.1	43.9
HH3XHH6	48.5	48.3	60.4	54.9	58.7	59.5	52.4	51.6	60.2	54.8	55.6	39.9	41.7	40.8
HH8XHH10	55.0	40.4	38.5	34.4	35.2	41.2	37.8	42.8	42.8	45.2	39.8	56.9	57.9	57.4
HH8XHH12	41.4	59.0	54.5	42.6	39.8	40.2	52.4	64.4	39.6	45.2	48.6	60.4	35.8	48.1
HH10XHH12	55.6	56.1	54.9	62.4	52.7	41.7	59.8	41.3	40.5	54.8	51.6	59.8	60.4	60.1

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

HYBRID	Pupation rate (%)	Yield/ 10000.0 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
HH1XHH3	51.3	60.9	56.5	58.6	61.2	49.3	57.2	66.7	61.0	58.1	33.1	58.4	45.7
HH1XHH6	41.0	52.6	42.9	43.7	48.6	36.3	41.2	44.9	44.5	44.0	54.9	42.4	48.7
HH3XHH6	54.1	42.9	54.7	57.9	63.5	62.2	47.0	51.7	61.0	55.0	48.5	40.0	44.3
HH8XHH10	39.6	44.7	32.7	32.8	44.3	53.6	37.3	53.8	44.5	42.6	46.6	52.2	49.4
HH8XHH12	47.1	61.6	55.6	52.5	39.9	57.9	63.8	37.4	52.8	52.1	62.6	64.5	63.5
HH10XHH12	66.8	37.3	57.4	54.5	42.5	40.6	53.5	45.6	36.2	48.3	54.3	42.4	48.4

Table 7: Evaluation index of the doublehybrids at 40±1ºc and 85±5%RH

HYBRID	Fecundity	Pupation rate (%)	Yield/ 10000.0 larvae(kg)	Cocoon Wt.(g)	Shell Wt.(g)	Cocoon Shell%	Reelability %	Filament Length	Raw silk %	Neatness (p)	Evaluation Index	Renditta	Filament size(d)	Evaluation Index
(HH1XHH3)X(HH8XHH12)	63.2	64.2	62.4	61.8	64.5	59.4	63.2	63.2	60.5	61.6	62.3	39.6	45.1	42.4
(HH1XHH3)X(HH10XHH12)	51.9	44.3	49.4	48.3	41.7	39.9	51.5	51.8	40.1	53.9	46.8	59.8	41.5	50.7
(HH3XHH6)X(HH8XHH12)	40.5	49.6	37.9	52.4	46.4	43.0	45.6	44.5	42.9	46.1	45.4	57.2	64.3	60.8
(HH3XHH6X(HH10XHH12)	44.4	41.9	50.3	37.6	47.4	57.7	39.8	40.4	56.5	38.4	45.5	43.4	49.1	46.2

Table 8: Evaluation index of the doublehybrids at 25±1ºc and 65±5%RH

HYBRID	Pupation rate (%)	Yield/ 10000.0 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
(HH1XHH3)X(HH8XHH12)	60.3	61.0	63.5	62.9	61.3	63.9	64.4	48.7	65.0	61.2	51.2	59.7	55.4
(HH1XHH3)X(HH10XHH12)	40.9	37.6	49.6	50.3	51.1	48.0	43.1	64.4	45.0	47.8	35.5	57.5	46.5
(HH3XHH6)X(HH8XHH12)	42.0	47.2	47.5	48.2	50.7	40.1	43.4	44.9	45.0	45.4	55.9	40.3	48.1
(HH3XHH6X(HH10XHH12)	56.9	54.2	39.5	38.6	36.9	48.0	49.0	42.0	45.0	45.6	57.4	42.5	50.0

Table 9: Heterosis of rearing and reeling characters in hybrids at 40±1ºc and 85±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)
(HH1XHH3) X (HH8XHH12)	4.87**	6.44**	2.20**	1.90**	3.83**	1.79**	0.76**	1.29*	-1.67	1.78**	-2.35	0.52ns
(HH1XHH3) X (HH10XHH12)	4.26**	1.60ns	2.02**	1.13**	1.88**	0.73ns	0.45**	0.98ns	-0.57	0.57ns	-3.65	0.33ns
(HH3XHH6) X (HH8XHH12)	2.76**	3.17ns	0.72ns	1.38**	2.34**	0.94*	0.41*	0.22ns	-1.21	1.28**	0.64**	0.72ns
(HH3XHH6) X (HH10XHH12)	3.50**	1.11ns	2.12**	0.54ns	2.41**	1.82**	0.20ns	0.25ns	-2.18	2.39**	-2.93	0.55ns

Table 10: Heterobeltiosis of rearing and reeling characters in hybrids at 40±1ºc and 85±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)
(HH1XHH3) X (HH8XHH12)	18.03**	19.08**	8.16**	6.90ns	14.48**	5.86**	2.41**	3.64ns	-8.74	4.77**	-10.67	1.51ns
(HH1XHH3) X (HH10XHH12)	15.00**	1.56ns	6.19**	4.16ns	6.25**	1.41ns	1.61*	3.32ns	-4.69	-0.22	-17.50	0.37ns
(HH3XHH6) X (HH8XHH12)	9.76**	8.80ns	2.16ns	5.23ns	8.72**	3.31ns	1.22ns	-0.88ns	-5.50	4.38*	0.00	0.00ns
(HH3XHH6) X (HH10XHH12)	11.59**	-2.17	6.72**	1.97ns	8.96**	6.40**	0.00ns	-0.54ns	-9.56	8.56**	-11.70	0.39ns

* and ** Denote significant difference at 5% and 1% ; ns Dénote non significant différence

Table 11: Heterosis of rearing and reeling characters in hybrids at 25+1ºc 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)
(HH1XHH3) X (HH8XHH12)	0.59*	1.50**	1.50**	2.28**	0.73**	0.19ns	1.92**	-0.97	1.02**	0.64**	0.36**
(HH1XHH3) X (HH10XHH12)	0.27ns	0.84*	0.74ns	1.65**	0.88**	0.10ns	0.42ns	-1.26	1.32**	0.64**	0.14ns
(HH3XHH6) X (HH8XHH12)	0.24ns	1.09**	1.03**	1.58**	0.52**	-0.14	0.62ns	-0.95	1.00**	-0.07	-0.05
(HH3XHH6) X (HH10XHH12)	0.74**	1.32**	0.52ns	1.14**	0.60**	0.05ns	1.06ns	-1.08	1.13**	0.10	0.09

Table 12: Heterobeltiosis of rearing and reeling characters in hybrids at 25+1ºc 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)
(HH1XHH3) X (HH8XHH12)	1.28ns	5.63**	4.29*	6.95**	2.55**	0.39ns	5.76*	-4.32	3.59**	1.25	1.08*
(HH1XHH3) X (HH10XHH12)	0.24ns	2.85ns	1.93ns	4.86**	1.93*	0.00ns	0.22ns	-6.05	4.16**	1.61	0.36ns
(HH3XHH6) X (HH8XHH12)	0.07ns	3.78*	1.87ns	4.18*	1.67*	-0.77	-0.12	-4.79	2.93*	-1.71	-0.36
(HH3XHH6) X (HH10XHH12)	1.94ns	4.56**	-0.14	3.09ns	0.71ns	-0.38	2.74ns	-5.29	3.49**	-0.69	0.00ns

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

Table 13. Comparative performance of (HH1x HH3) x (HH8xHH12) at 40+1ºC and 85+5% RH (mean of 5 trials)

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)
(HH1XHH3) X (HH8XHH12)	679.3	89.3	16.4	1.660	0.370	22.3	84.0	890.0	6.37	15.7	2.23	91.0
(CSR2XCSR27) x (CSR6XCSR26) Control	648.0	25.7	8.4	1.501	0.321	21.4	80.0	832.0	6.84	14.6	2.84	90.0
Percent improvement over control hybrids (HH1XHH3) X (HH8XHH12) Vs. (CSR2XCSR27) x (CSR6XCSR26)	4.8	247.6	93.7	10.571	15.161	4.2	5.0	7.0	-6.9	7.4	-19.5	1.1

Table 14: Comparative performance of (HH1x HH3) x (HH8xHH12) at 25±1ºC 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)
(HH1XHH3) X (HH8XHH12)	93.5	18.4	1.812	0.417	23.0	87.0	1111.0	5.31	18.8	2.88	92.0
(CSR2XCSR27) x (CSR6XCSR26) Control	94.2	18.6	1.826	0.418	22.9	87.0	1090.0	5.30	18.9	2.85	92.0
Percent improvement over control hybrids (HH1XHH3) X (HH8XHH12) Vs. (CSR2XCSR27) x (CSR6XCSR26)	-0.7	-0.8	-0.785	-0.239	0.6	0.0	1.9	0.2	-0.2	1.1	0.0

Table 15: Cocoon volume and uniformity in double hybrids of HH combinations

Hybrid	Cocoons Per litre	SD	Cocoon length (cm)	Cocoon width (cm)	length/ width ratio	CV%
(HH1XHH3) X (HH8XHH12)	79	5.4	3.28 ±0.10	1.84 ±0.08	178.43 ±7.78	4.36
(HH1XHH3) X (HH10XHH12)	89	9.5	3.21 ±0.12	1.91 ±0.10	168.49 ±9.67	5.74
(HH3XHH6) X (HH8XHH12)	92	8.7	3.11 ±0.11	1.93 ±0.07	161.40 ±8.37	5.19
(HH3XHH6 X (HH10XHH12)	90	8.4	3.13 ±0.12	1.90 ±0.08	165.23 ±8.45	5.11

3.2 Performance of double hybrids developed under high temperature (40 ±1°C) and high humidity (85±5%) conditions:

3.2.1 Pre-Cocoon Parameters:

The fecundity is the same for both the treatments and it ranged from 641 to 688 with the highest of 688 recorded for (HH1 × HH3) × (HH8 x HH12) and the lowest of 641 recorded for (HH3 × HH6) × (HH8 × HH12). At 40±1°C, the pupation rate ranged from 71.0 to 86.33 % with the highest of 86.33 % recorded for (HH1 × HH3) × (HH8 × HH12) and the lowest of 71.0 % recorded for (HH3 × HH6) × (HH10 × HH12). At 25±1°C, the pupation rate ranged from 93.1 to 94.8 % with the highest of 94.8 % recorded for (HH1 × HH3) × (HH8 × HH12) and the lowest of 93.1 % recorded for (HH1 × HH3) × HH10 × HH12). At 40±1°C, the yield/10000 larvae ranged from 16.47 to 17.44 kg with the highest of 17.44 kg recorded for (HH1 × HH3) × (HH8 × HH12) and the lowest of 16.47 kg recorded for (HH3 × HH6) × (HH8 x HH12). At 25±1°C, the yield/10000 larvae ranged from 18.92 to 19.44 kg with the highest of 19.44 kg recorded for (HH1 × HH3) × (HH8 × HH12) and the lowest of 18.92 kg recorded for (HH1 × HH3) x HH10 × HH12). At 40±1°C, the cocoon weight ranged from 1.551 to 1.628 g with the highest of 1.628 g recorded for (HH1 × HH3) × (HH8 × HH12) and the lowest of 1.551 g recorded for (HH3 × HH6) × (HH10 × HH12). At 25±1°C, the cocoon weight ranged from 1.841 to 1.910 g with the highest of 1.910 g recorded for (HH1 × HH3) × (HH8 × HH12) and the lowest of 1.841 g recorded for (HH3 × HH6) × (HH10 × HH12). At 40±1°C, the cocoon shell weight ranged from 0.340 to 0.364 g with the highest of 0.364 g recorded for (HH1 × HH3) × (HH8 × HH12) and the lowest of 0.340 g recorded for (HH1 × HH3) × (HH10 × HH12). At 25±1°C, the cocoon shell weight ranged from 0.429 to 0.452 g with the highest of 0.452 g recorded for (HH1 × HH3) × (HH8 × HH12) and the lowest of 0.429 g recorded for (HH3 × HH6) × (HH10 × HH12). At 40±1°C, the cocoon shell percentage ranged from 21.46 to 22.38 % with the highest of 22.38 % recorded for (HH1 × HH3) × (HH8 × HH12) and the lowest of 21.46 % recorded for (HH1 × HH3) × (HH10 × HH12). At 25±1°C, the cocoon shell percentage ranged from 23.28 to 23.67 % with the highest of 23.67% recorded for (HH1 × HH3) × (HH8 × HH12) and the lowest of 23.28 % recorded for (HH3 × HH6) × (HH10 × HH12) (Table 3).

3.2.2 Post-Cocoon Parameters:

At 40±1°C, the highest reelability was recorded for (HH1 × HH3) × (HH8 × HH12) (85.0 %) and the lowest of 83.67 % was recorded for (HH3 × HH6) × (HH10 × HH12). At 25±1°C, the highest reelability was recorded for (HH1 × HH3) × (HH8 × HH12) (87.0 %) and the lowest of 86.0 % was recorded for (HH3 × HH6) × (HH8 × HH12). At 40±1°C, the longest filament length was recorded for (HH1 × HH3) × (HH8 × HH12) (944 m) and the shortest of 905 m was recorded for (HH3 × HH6) × (HH10 × HH12). At 25±1°C, the longest filament length was recorded for (HH1 × HH3) × (HH8 × HH12) (1088 m) and the shortest of 1027 m was recorded for (HH1 × HH3) × (HH10 × HH12) and (HH3 × HH6) × (HH8 × HH12). At 40±1°C, the highest renditta was recorded for (HH1 × HH3) × (HH10 × HH12) (6.40) and the lowest of 6.07 was recorded for (HH1 × HH3) × (HH8 × HH12). At 25±1°C, the highest renditta was recorded for (HH3 × HH6) × (HH10 × HH12) (5.41) and the lowest of 5.36 was recorded for (HH1 × HH3) × (HH10 × HH12). At 40±1°C, the highest raw silk percentage was recorded for (HH1 × HH3) × (HH8 × HH12) (16.47 %) and the lowest of 15.63 % was recorded for (HH1 × HH3) × (HH10 × HH12). At 25±1°C, the highest raw silk percentage was recorded for (HH1 × HH3) × (HH10 × HH12) (18.65 %) and the lowest of 18.50 % was recorded for (HH3 × HH6) × (HH10 × HH12). At 40±1°C, the thickest filament size was recorded for (HH3 × HH6) × (HH8 × HH12) (2.88 d) and the thinnest of 2.37 d was recorded for (HH1 × HH3) × (HH10 × HH12). At 25±1°C, the thickest filament size was recorded for (HH1 × HH3) × (HH8 × HH12) (2.96 d) and the thinnest of 2.87 d was recorded for (HH3 × HH6) × (HH8 × HH12) . At 40±1°C, the highest neatness was recorded for (HH1 × HH3) × (HH8 × HH12) (91.0 p) and the lowest of 90.0 p (HH3 × HH6) × (HH10 × HH12) was recorded for (HH3 × HH6) × (HH10 × HH12). At 25±1°C, the highest neatness was recorded for (HH1 × HH3) × (HH8 × HH12) (93.67 p) and the lowest of 92.33 p was recorded for (HH1 × HH3) × (HH10 × HH12), (HH3 × HH6) × (HH8 × HH12) and (HH3 × HH6) × (HH10 × HH12) (Table 4).

3.3 Evaluation Index:

In the evaluation index method, the foundation crosses with high evaluation index at 40±1ºC and 85±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 39.8 to 58.1 with the highest of 58.1 recorded in HH1 × HH3 and the lowest of 39.8 recorded in HH8 × HH10. However, evaluation indices for renditta and filament size are to be considered for least value and ranged from 40.8 to 60.1 with the least of 40.8 recorded for HH3 × HH6 and the highest of 60.1 recorded for HH10 × HH12. (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 42.6 to 58.1 with the highest 58.1 HH1 × HH3 and the lowest 42.6 HH8 × HH10 were recorded and the overall evaluation indices of renditta and denier ranged from 44.3 to 63.6 with the lowest 44.3 for HH3 × HH6 and the highest 63.6 in the hybrid (HH8 × HH12). (Table 6).

In the evaluation index method, the double hybrid with high evaluation index at 40±1ºC and 85±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 45.4 to 62.3 with the highest of 62.3 recorded in (HH1 × HH3) x (HH8 × HH12) and the lowest of 45.4 recorded in (HH3 × HH6) × (HH8 × HH12). However, evaluation indices for renditta and filament size are to be considered for least value and ranged from 42.4 to 60.8 with the lowest of 42.4 recorded for (HH1 × HH3) × (HH8 × HH12) and the highest of 60.8 recorded for (HH3 × HH6) × (HH8 × HH12). (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 45.4to 61.2 with the highest of 61.2 for (HH1 × HH3) × (HH8 × HH12) and the lowest of 45.4 for (HH3 × HH6) × (HH8 × HH12) were recorded and the overall evaluation indices of renditta and denier ranged from 46.51 to 55.4 with the lowest of 46.51 for (HH1 × HH3) × (HH10 × HH12) and the highest of 55.4 in the hybrid (HH1 × HH3) × (HH8 × HH12). (Table 8).

3.4 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 s double hybrids at, 40±1ºC and 85±5% RH and 25±1ºC and 60±5% RH. At 40±1ºC and 85±5% RH, (HH1 × HH3) × (HH8 × HH12) 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±1ºC and 60±5% RH, (HH1 × HH3) × (HH8 × HH12) was found maximum hybrid vigour over mid parent value for all the characters including renditta and filament size (d). On the other hand (HH1 × HH3) × (HH8 × HH12) was also found maximum hybrid vigour over better parent value for all the characters including renditta. (Table 11 and 12).

3.5 Percent Improvement of Selected Hybrids:

Based on the consistency for pupation rate at 40±1ºC and 85±5% RH, the double hybrid viz., (HH1 × HH3) × (HH8 × HH12) was selected. Further the selected hybrid was compared with the control hybrid viz., (CSR2 × CSR27) × (CSR6 × CSR26). Maximum improvement for fecundity (4.8%), pupation rate (247.7%), yield/10000 larvae (93.7%), cocoon weight (10.6%), shell weight (15.2%), cocoon shell percentage (4.2%), reelability (5.0%), filament length (7.0%), raw silk percentage (7.4%) and neatness (1.1%) and decrement for renditta (-6.9%) and filament size (-19.5%) at 40±1ºC and 85±5% RH was noticed in (HH1 × HH3) × (HH8 × HH12) over the control (CSR2 × CSR27) × (CSR6 × CSR26). (Table 13). However, at 25 ±1°C and 65 ±5%RH, the said hybrid expressed improvement for cocoon shell percentage (0.6%), filament length (1.9%) renditta (0.2%) and filament size (1.1%) and decrement for pupation rate (-0.7%), yield/10000 larvae (-0.8%), cocoon weight (-0.8%) shell weight (-0.2%) and raw silk percentage (-0.2%) and there was no improvement for reelability and neatness. (Table 14).

3.6 Cocoon Uniformity:

Cocoon volume of double hybrids of HH combinations revealed highest cocoon number /litre 92 cocoons in (HH3 × HH6) × (HH8 × HH12) with standard deviation of 8.7 and lowest cocoon number in (HH1 × HH3) × (HH18 × HH12) 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 . cocoon size uniformity among HH combinations in double hybrids exhibited better uniformity of 178.43 cocoon length width ratio in (HH1 × HH3) × (HH18 × HH12) with lesser standard deviation (7.18) and lesser coefficient of variance (4.36). (Table 15)

In the present breeding programme, which envisages evolving of new hardy bivoltines, the aim was to develop more resistant bivoltines that can give rise to stable cocoon crops with better viability, even though productivity is low compared to the existing productive bivoltine breeds that are currently used in the field.. In addition, during later generations of inbreeding, selection was applied to select desired genotypes to improve the traits of commercial importance like viability and productivity as suggested by Mano (1993 and 1994) to improve the yield of bivoltines. Development of robust but productive silkworm breed is an important component in the development of sericulture. Systematic silkworm breeding approach in designing and executing experimental protocol coupled with appropriate selection procedure for desirable traits in hybrids have enabled Japanese and Chinese silkworm breeders to develop sustainable breeds to achieve remarkable traits of economic value (Mano *et al*., 1991). The prime objective of the breeder is to isolate robust silkworm breeds with reasonable levels of productivity and to improve post-cocoon parameters related to silkworm breeding (Raju,1990; Nagaraju,1990; Maribashetty, 1991; Kalpana, 1992; Nirmal Kumar, 1995 and Basavaraja,1996).

Thermal acclimatization and stress have been studied during different developmental stages of insects (larvae, pupae and adults) as well as different physiological conditions. Surviving a thermal stress depends in part upon the individual organism's stress history. Brief exposure to a mild stress can protect an organism against a normally lethal stress. Brief exposure to a mild temperature than the optimum temperature induces tolerance (Thermo-tolerance) in insects. Once thermo-tolerance has been induced, it may persist for days (Carretero *et al.,* 1991; Yocum and Denlinger, 1992). Some stresses will even increase an organism's sensitivity to a future stress. Survival of high temperature stress appears to be energy dependent in insects and the loss should be compensated by nutrition.

During the course of breeding varied responses for the traits analyzed from F1~F5 can be attributed to the transgressive segregation of large number of genes along with the influence of environmental effects. Systematic evaluation of major contributing traits of the population raised from F5 onwards made it possible to concentrate on each one of the character in order to follow high intensity of selection for the phenotypic target traits. During the breeding process, emphasis was laid for important aspects such as the number of generations required for segregating populations to regain from the loss of inbreeding and to excel the level of initial generations. The results obtained in the breeding experiments indicate that several important issues need to be addressed for interpretation. The selection response for various traits need to be focused first followed by the results obtained. The polygenic nature of the traits in question and the role of different intensities of selection in changing the mean expression of such characters have been demonstrated in plants and animals by several workers (Dickinson and Hazel,1944: Bell *et al.,* 1955; Robertson, 1956; Clayton *et al.,* 1957; Falconer,1981). Selection can not make new genes but it will alter the gene frequency in the existing population. It is an essential adjunct of the other systems as a means of improvement.

4. Conclusion:

The silkworm breeds developed for tropical conditions in India have to remain adapted to both seasonal and local conditions of that area for ensuring stable cocoon production under the high temperature coupled with other associated biotic/abiotic vagaries. In India, although the mulberry leaves are available throughout the year including the summer months, the rearing of bivoltines during summer is, however, very difficult with frequent crop losses. Since, the rearing of bivoltines in summer months becomes very difficult, the concept of production of bivoltine throughout the year to produce quality silk becomes jeopardised. Further, the adverse climatic condition during summer is not the same across the country. Some locations have high temperature coupled with high humidity and in some high temperature with low humidity prevails besides, poor leaf quality at times. As a matter of fact, the summer breeds are having significant importance in increasing cocoon production through rearing of bivoltine hybrids in tropical areas. Therefore, attempts made to develop bivoltine hybrids tolerant to high temperature and high humidity conditions had resulted in the development of new bivoltine double hybrid to suit the above conditions.

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