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Annals of Biological Research, 2015, 6 (9):1-6
(http://scholarsresearchlibrary.com/archive.html)

ISSN 0976-1233
CODEN (USA): ABRNBW

Performances of F_2 and backcross populations in silkworm, *Bombyx mori* under high temperature

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ABSTRACT

The silkworm, Bombyx mori is a domesticated insect sensitive to temperature challenges. In India, high temperature prevails during summer season. Rearing silkworms at such temperature leads to poor silk productivity. Developing a silkworm breed tolerant to high temperature is the most effective measure to perpetuate silk productivity. Choosing the right segregating population in which to initiate selection and successive crosses is one of the most important steps for development of new breeds. In this study, two multivoltine (Nistari and Cambodge) and a bivoltine (CSR2) breed, their F_1s, F_2s and backcross populations without reciprocal crosses were reared at 25 and 36°C. Data on commercially important rearing traits were collected. The rearing traits were significantly ($P < 0.05$) decreased in the breeds, hybrids, F_2s and backcross populations at 36°C. Backcross populations performed better than the F_2s at 36°C. Higher cocoon shell percent was also recorded with backcross populations with CSR2 as a female component. Similarly, the backcross populations with Nistari or Cambodge as a female component showed higher survival. At 36°C, CSR2 x (Nistari x CSR2) was the better performer in terms of silk yield and quality, whereas, Nistari x (Nistari x CSR2) was more stable with least percentage reduction over control in larval weight, cocoon yield / 10,000 larvae by number and weight and cocoon weight, and second lowest percentage reduction in cocoon shell weight and cocoon shell percent. The information generated by this study would be valuable in selecting the cross to initiate breeding for increasing silk productivity at high temperature conditions.

Keywords: Silkworm, Rearing traits, Silk productivity, Survival

INTRODUCTION

The silkworm is a poikilothermic insect whose growth and development is affected by high temperature condition. Silkworm breeds are mainly categorised into uni-, bi- and multivoltine. Multivoltine breeds are habitant of tropical regions and are hardy to high temperature, which produces low quality and quantity of silk. Bivoltine breeds are habitant of temperate regions and are prone to high temperature, which produces high quality and quantity of silk [1]. During the summer season, sericulturists in tropical rural areas like India face unfavourable environmental conditions that directly affect the silk production. Of them, the main culprit in constraining silkworm growth and silk productivity is high temperature. A negative relationship is found between high temperature and silk yield. Rearing thermotolerant silkworm breeds is one of the effective methods to increase silk productivity in summers. Attempts of silkworm breeders to develop a thermotolerant breed that can be employed in the fields were not successful [2], [3], [4], [5] because thermotolerance trait in silkworm is controlled by genetic and environmental factors and therefore it is difficult to develop a thermotolerant breed [3].

Selection is important for successful breeding as it can change the gene frequencies within the population. Another important criterion for successful breeding is choosing right segregating population (F_2 and backcross). The chosen segregating population should have an acceptable value for the targeted trait and adequate genetic variability to permit effective selection. The performance of the segregating population forms a base and provides valuable information for selecting the optimum type of successive cross in the process of breed development [6].

Most of the studies report on the screening of newly evolved silkworm breeds for thermotolerance and rearing performances of the hybrids *viz*., bi x bi and multi x bi [7], [8], [9], double hybrids [3] at high temperature. Furthermore, the influence of different environmental conditions on commercially important reeling parameters like filament length, denier, renditta and raw silk percent in judging the quality of silk has been reported [10], [11]. The aim of this study is to determine the better segregating population to initiate breeding for thermotolerance in silkworms by assessing the rearing traits of F_2s and backcross populations without reciprocal crosses at 25°C and 36°C.

MATERIALS AND METHODS

Silkworm rearing and segregating populations

Two multivoltine breeds, namely Nistari and Cambodge, and one bivoltine breed, namely CSR2 were selected as parents to raise the segregating populations. The performances of Nistari, Cambodge and CSR2 breeds, their F_1s, F_2s and backcross populations without reciprocal crosses were analysed at 25 and 36°C.

Silkworm larvae reared from hatching to till spinning at 25°C were considered as control. Treated larvae were reared at 25°C from hatching to 3rd day of 5th instar, which were shifted to a Sericatron chamber (a unit for maintaining uniform temperature and humidity) for treatment at 36°C for 6 hours every day till spinning. During rearing, the silkworms were fed with sufficient mulberry leaves and the rearing trays were cleaned from time to time. The ripened larvae were allowed to spin cocoons on plastic mountages. The experiment was performed in duplicate. Healthy cocoons were harvested and maintained till eclosion of moths. Male moths of CSR2 breed were mated with the females of Nistari and Cambodge separately. Thus the developed F_1 hybrids were selfed to raise F_2 populations and males of F_1 hybrids were crossed to females of either parent separately to develop backcross populations. Disease free eggs so developed were reared and cocoons were harvested as aforesaid. The control and treated larvae of F_2 and backcross populations were also maintained in duplicates. Data on economically important rearing parameters of control and treated silkworm larvae were estimated as follows

Larval weight (g): It is the total weight of 10 larvae on final day of fifth instar.

Cocoon yield / 10,000 larvae by number: It is the total weight of live cocoons expressed in kilogram for unit number of larvae retained after 3rd moult.

$$\frac{\text{Actual no. of cocoons obtained}}{\text{Unit number of larvae retained after third moult (250)}} \times 10000$$

Cocoon yield / 10,000 larvae by weight (kg): It is the weight of cocoons with live pupae recovered out of the weight of larvae retained after 3rd moult.

$$\frac{\text{No. of good cocoons + (No. of double cocoons x 2)}}{\text{Larvae retained after 3}^{\text{rd}}\text{ moult – Uzi infested coccons}} \times 10000$$

Cocoon weight (g): The average weight of 10 male and 10 female cocoons taken randomly on 6th or 7th day after onset of spinning.

$$\frac{\text{Weight of 10 male (g) cocoon + Weight of 10 female cocoon (g)}}{20}$$

Cocoon shell weight (g): The average weight of 10 male and 10 female cocoon shells of same cocoons taken randomly.

$$\frac{\text{Weight of 10 male (g) cocoon shells + Weight of 10 female cocoon shells (g)}}{20}$$

Cocoon shell percent (%): It is the average ratio of 10 cocoon shells each of male and female to the total cocoon weight.

$$\frac{\text{Cocoon shell weight}}{\text{Cocoon weight}} \times 100$$

Statistical analysis

The data on 6 rearing parameters *viz*., larval weight (g), cocoon yield / 10,000 larvae by number, cocoon yield / 10,000 larvae by weight (kg), cocoon weight (g), cocoon shell weight (g) and cocoon shell percent (%) were collected by standard procedure. Significant differences between the means of the control and treated larvae were analysed by applying independent t test (two-sample t test). Percentage change over control in the rearing traits was calculated as follows [1].

$$\text{Percentage change} = \frac{\text{Treated} - \text{Control}}{\text{Control}} \times 100$$

RESULTS

Morphological variations

Morphological traits of the breeds, their F_1s and segregating populations were varying. Segregation pattern of the larval marking and cocoon colour were studied in F_2 and backcross populations. Nistari and Cambodge are multivoltines with slender body, which spun spindle shaped yellow cocoons and CSR2 is a bivoltine characterized by bulky body, which spun oval shaped white cocoons. Nistari is marked whereas Cambodge and CSR2 are plain larvae. All the larvae of F_1 hybrid were marked and spun yellow coloured cocoons. Laval marking pattern and cocoon colour followed phenotypic segregation ratio of 3:1 in F_2 populations and 1:1 in backcross populations suggesting that they are inherited in a monogenic fashion.

Performances of breeds at 36°C

Significant variations were found in 6 rearing traits between control and treated larvae of breeds, hybrids and segregating populations. Data collected on larval weight, cocoon yield / 10,000 larvae by number and weight, cocoon weight, cocoon shell weight and cocoon shell percent of breeds, F_1 hybrids, F_2s and backcross populations at 25 and 36°C are presented in Table 1and 2. Amongst breeds, higher larval weight was recorded with CSR2 (37.79 g) followed by Cambodge (23.56 g) and Nistari (20.8 g) with an average of 27.38 g. Highest cocoon yield / 10,000 larvae by number was observed in Nistari (83%) followed by Cambodge (79.39%) and CSR2 (31.5%) with an average of 64.63%. Highest cocoon yield / 10,000 larvae by weight was noted in Cambodge (8.664 kg) followed by Nistari (6.8 kg) and CSR2 (6.416 kg) with an average of 7.29 kg. Maximum cocoon weight (1.501 g), shell weight (0.28 g) and shell percent (18.64%) were recorded with CSR2, minimum cocoon weight (0.535 g), shell weight (0.128 g) and shell percent (13.39%) were recorded with Nistari. The average cocoon weight, shell weight and shell percent were 1.018 g, 0.182 g and 15.23%, respectively (Table 1and 2).

Table 1: Rearing performances of breeds, hybrids, F_2s and backcross populations at 25 and 36°C

Breed/hybrid	Larval wt. (g)		Cocoon yield / 10000 larvae by number		Cocoon yield / 10000 larvae by wt. (kg)	
	25°C	36°C	25°C	36°C	25°C	36°C
CSR2	41.62^{NS}	37.79^{NS}	93.13**	31.50**	17.620**	6.410**
Nistari	23.25**	20.8**	95.60^{NS}	83.00^{NS}	11.360*	6.800*
Cambodge	26.51^{NS}	23.57^{NS}	93.40**	79.40**	11.641*	8.664*
Nistari x CSR2-F_1	46.40**	37.35**	93.14^{NS}	87.76^{NS}	16.593^{NS}	14.813^{NS}
Cambodge x CSR2-F_1	44.60*	34.42*	93.45^{NS}	83.00^{NS}	15.153^{NS}	13.486^{NS}
Nistari x CSR2-F_2	31.80*	27.93*	92.23**	64.04**	9.484^{NS}	7.253^{NS}
Cambodge x CSR2 -F_2	31.32*	26.26*	81.22**	75.15**	8.918^{NS}	8.433^{NS}
Nistari x (Nistari x CSR2)	34.84*	30.86*	91.70^{NS}	86.00^{NS}	11.750^{NS}	11.309^{NS}
Cambodge x (Cambodge x CSR2)	29.36*	25.41*	91.20^{NS}	83.51^{NS}	10.660^{NS}	10.222^{NS}
CSR2 x (Nistari x CSR2)	37.51^{NS}	32.70^{NS}	90.53^{NS}	82.55^{NS}	10.785*	9.359*
CSR2 x (Cambodge x CSR2)	37.71**	31.25**	90.35^{NS}	82.25^{NS}	10.326*	8.705*

* *Significant difference at $P < 0.05$*
** *Significant difference at $P < 0.01$*
^{NS} *No significant difference*

Performances of F_1 hybrids at 36°C

Among hybrids, Nistari x CSR2 showed highest larval weight (37.35 g), cocoon yield / 10,000 larvae by number (87.79%) and weight (14.81 kg), cocoon weight (1.495 g), cocoon shell weight (0.274 g) and cocoon shell percent (18.32%). Average larval weight, cocoon yield / 10,000 larvae by number and weight, cocoon weight, cocoon shell weight and cocoon shell percent for hybrids were 38.88 g, 85.38%, 14.15 kg, 1.418 g, 0.258 g and 18.24%, respectively (Table 1and 2).

Table 2: Cocoon trait performances of breeds, hybrids, F_2s and backcross populations at 25 and 36°C

Breed/hybrid	Cocoon wt. (g)		Cocoon shell wt. (g)		Cocoon shell percent (%)	
	25°C	36°C	25°C	36°C	25°C	36°C
CSR2	1.793^{*}	1.502^{*}	0.403^{**}	0.280^{**}	22.48^{**}	18.65^{**}
Nistari	1.205^{NS}	0.535^{NS}	0.165^{NS}	0.128^{NS}	13.70^{NS}	13.39^{NS}
Cambodge	1.216^{NS}	1.019^{NS}	0.173^{*}	0.139^{*}	14.23^{NS}	13.65^{NS}
Nistari x CSR2-F_1	1.610^{NS}	1.495^{NS}	0.319^{NS}	0.274^{NS}	19.82^{NS}	18.33^{NS}
Cambodge x CSR2-F_1	1.551^{NS}	1.342^{NS}	0.297^{*}	0.244^{*}	19.12^{NS}	18.15^{NS}
Nistari x CSR2-F_2	1.224^{NS}	1.201^{NS}	0.215^{*}	0.200^{*}	17.57^{*}	16.66^{*}
Cambodge x CSR2 -F_2	1.148^{NS}	1.130^{NS}	0.195^{NS}	0.179^{NS}	17.07^{**}	15.28^{**}
Nistari x (Nistari x CSR2)	1.300^{NS}	1.282^{NS}	0.241^{NS}	0.224^{NS}	18.50^{NS}	17.44^{NS}
Cambodge x (Cambodge x CSR2)	1.460^{*}	1.346^{*}	0.249^{NS}	0.214^{NS}	17.05^{NS}	15.87^{NS}
CSR2 x (Nistari x CSR2)	1.713^{NS}	1.546^{NS}	0.342^{**}	0.282^{**}	19.97^{*}	18.24^{*}
CSR2 x (Cambodge x CSR2)	1.433^{NS}	1.333^{NS}	0.281^{*}	0.237^{*}	19.64^{NS}	17.75^{NS}

* *Significant difference at P < 0.05*
** *Significant difference at P < 0.01*
^{NS} *No significant difference*

Performances of F_2s and backcross populations at 36°C

The larval weight was ranging between 25.41 g for Cambodge x (Cambodge x CSR2) and 32.695 g for CSR2 x (Nistari x CSR2) with an average of 29.06 g. Highest cocoon yield / 10,000 larvae by number (86%) and weight (11.309 kg) were recorded in Nistari x (Nistari x CSR2) and lowest cocoon yield / 10,000 larvae by number (64.04%) and weight (7.253 kg) were recorded in Nistari x CSR2-F_2. Backcross population CSR2 x (Nistari x CSR2) showed highest cocoon weight (1.546 g), cocoon shell weight (0.282 g), cocoon shell percent (18.24%) and Cambodge x CSR2 -F_2 showed lowest cocoon weight (1.13 g), cocoon shell weight (0.179 g) and cocoon shell percent (15.28%) with an average of 1.306 g, 0.222 g and 16.87%, respectively (Table 1and 2).

Table 3: Percentage reduction in rearing traits of breeds, hybrids, F_2s and backcross populations in treated larvae over control

Breed / hybrid	Larval wt. (g)	Cocoon yield / 10,000 larvae by number	Cocoon yield / 10,000 larvae by wt. (kg)	Cocoon wt. (g)	Cocoon shell wt. (g)	Cocoon shell percent (%)
CSR2	-9.20	-66.17	-63.59	-16.23	-30.52	-17.06
Nistari	-10.56	-13.18	-40.14	-55.62	-22.42	-2.25
Cambodge	-11.11	-14.99	-25.57	-16.21	-19.65	-4.10
Nistari x CSR2-F_1	-19.51	-5.78	-10.73	-7.11	-14.11	-7.54
Cambodge x CSR2-F_1	-22.83	-11.18	-11.00	-13.51	-17.88	-5.07
Nistari x CSR2-F_2	-12.19	-30.57	-23.52	-1.92	-6.98	-5.16
Cambodge x CSR2 -F_2	-16.17	-7.47	-5.44	-1.53	-8.21	-10.52
Nistari x (Nistari x CSR2)	-11.43	-6.22	-3.75	-1.42	-7.07	-5.76
Cambodge x (Cambodge x CSR2)	-13.46	-9.81	-4.10	-7.84	-14.26	-6.95
CSR2 x (Nistari x CSR2)	-12.82	-8.82	-13.22	-9.72	-17.54	-8.67
CSR2 x (Cambodge x CSR2)	-17.14	-7.58	-15.70	-7.03	-15.98	-9.61

Percentage change in treated larvae over control of breeds

The negative sign in the percent change in treated larvae indicates a reduction over control and the value near to zero suggests a better performance of the breed / hybrid. Of the breeds, the highest reduction in larval weight was found in Cambodge (-11.11%) followed by Nistari (-10.56%) and CSR2 (-9.2%). Highest reduction in cocoon yield / 10,000 larvae by number was demonstrated in CSR2 (-66.17%) followed by Cambodge (-14.99%) and Nistari (-13.18%). Highest reduction in cocoon yield / 10,000 larvae by weight was found in CSR2 (-63.59%) followed by Nistari (-40.14%) and Cambodge (-25.57%). Highest and lowest reduction in cocoon weight was found in Nistari (-55.65%) and Cambodge (-16.21%), respectively. CSR2 showed highest reduction in cocoon shell weight (-30.52%) and cocoon shell percent (-17.06%). Lowest reductions in cocoon shell weight and cocoon shell percent of -19.65% and -2.25% were found in Cambodge and Nistari, respectively (Table 3).

Percentage change in treated larvae over control of F_1 hybrids

Treated larvae of Nistari x CSR2 hybrid showed lowest reduction over control in larval weight (-19.51%), cocoon yield / 10,000 larvae by number (-5.78%) and weight (-10.73%), cocoon weight (-7.11%) and cocoon shell weight (-14.11%). Lowest reduction in cocoon shell percent (-5.07%) was found in Cambodge x CSR2 hybrid (Table 3).

Percentage change in treated larvae over control of F_2 and backcross populations

Lowest reductions of -11.43%, -6.22%, -3.75% and -1.42% were observed in larval weight, cocoon yield / 10,000 larvae by number and cocoon yield / 10,000 larvae by weight and cocoon weight of Nistari x (Nistari x CSR2), respectively. Highest reduction in larval weight and cocoon weight was observed in CSR2 x (Cambodge x CSR2) (-17.17%) and CSR2 x (Nistari x CSR2) (-9.72%), respectively. Highest reduction in cocoon yield / 10,000 larvae by number (-30.57%) and weight (-23.52%) was found in Nistari x CSR2-F_2. Highest and lowest reduction in cocoon shell weight was found in CSR2 x (Nistari x CSR2) (-17.54%) and Nistari x CSR2-F_2 (-6.98%). In cocoon shell percent, highest (-10.52%) and lowest (-5.16%) reductions were found in Cambodge x CSR2 -F_2 and Nistari x CSR2-F_2, respectively (Table 3).

DISCUSSION

Sericulture in India is predominantly practised in hot tropical regions; rearing of silkworms under such conditions will adversely affect rearing traits. Therefore, the success and spread of silkworm rearing is most probably dependent on F_1 hybrids developed by crossing females of native multivoltine with males of exotic bivoltine breeds as they are likely to be more tolerant to high temperature than either of the parent. Rearing of these hybrids is disadvantageous has they spun inferior quality cocoons [2]. Moreover, preparation of layings of cross breeds in each rearing involves an additional cost of rearing both the parents for hybrid preparation, labour and expertise. Hence, development of new silkworm breeds tolerant to high temperature like multivoltine and productive like bivoltine is of vital importance. In order to do so it is important to assess the rearing traits in populations of F_2 and backcross at high temperature conditions to initiate and select the breeding process.

On thermal treatment, all the quantitative traits of silkworms showed a decline in all the tested populations. This result is in accordant with [8]. Insects react to temperature challenges by altering their various behavioural activities, physiological responses and biochemical mechanisms, which requires an additional energy to accomplish these activities [12]. One such important mechanism to buffer the stress comprises the expression of heat shock proteins (HSPs) as an immediate response enhancing survival under heat stress [13]. Therefore, under uncomfortable temperatures the insect will be deprived of energy sources to perform normal biological functioning of the cells. Furthermore, heat stress affects biologically important molecules like DNA, RNA, lipids and halts the mechanism of normal protein synthesis and even causes unfolding of the typical folded functional cellular proteins making them non-functional [14], [15]. These all tend to increase the vulnerability to other biological processes resulting in the decline in the performance of the silkworm.

Decline in the rearing traits like larval weight, cocoon weight, cocoon shell weight, cocoon yield / 10,000 larvae by number and weight of the breeds / hybrids at 36°C was observed. This is probably due to the low feeding activity of the silkworm during high temperature as a fact that increase in temperature reduces the moisture content in the leaf through evaporation thereby drying the leaf sooner making difficult to feed by silkworms [16]. The rearing traits like larval weight (32.69 g), cocoon weight (1.546 g), cocoon shell weight (0.282 g) and cocoon shell percent (18.24%) were highest in CSR2 x (Nistari x CSR2) indicating better silk yield. Therefore, CSR2 x (Nistari x CSR2) is a better performer in terms of silk yield at 36°C. But, one of the target of the silkworm breeders is to direct the farmer with a breed that performs with less variations at different environments. In this study the percentage change in treated larvae over control of different breeds, hybrids and segregating populations was determined. The result indicates that the backcross population Nistari x (Nistari x CSR2) possessed less variations or least percentage reduction over their control in larval weight (-11.43%), cocoon yield / 10,000 larvae by number (-6.22%) and weight (-3.75%) and cocoon weight (-1.45%), and second lowest percentage reduction in all other studied traits *viz*., cocoon shell weight (-7.07%) and cocoon shell percent (-5.76%). Therefore backcross population Nistari x (Nistari x CSR2) can be suggested as more stable than others.

Cocoon shell percent is considered to be most important rearing trait to gauge silk productivity. Generally, among segregating populations, backcross populations performed better than F_2s with regard to cocoon shell percent. Highest cocoon shell percent was recorded with backcross populations developed by using CSR2 as female component indicating the efficiency of the breed in conversion of leaf to silk compared to the females of Nistari or Cambodge. Similarly, the backcross populations developed by using Nistari or Cambodge as female component showed higher survival (cocoon yield / 10,000 larvae by number) compared with the backcross populations developed by using CSR2 as females. One of the reasons is also that the characters like cocoon shell percent and thermotolerance (cocoon yield / 10,000 larvae by number) are highly heritable [17], [18], [19]. Moreover, thermotolerance trait in silkworm is believed to be maternally inherited [9] and hence the backcross populations developed with thermotolerant multivoltines (Nistari and Cambodge) as females are more tolerant than backcross populations developed with the bivoltine (CSR2) as females.

CONCLUSION

In conclusion, backcross populations performed better than the F_2s but Nistari x CSR2-F_2 was stable in terms of cocoon shell weight (-6.98%) and cocoon shell percent (-5.16) with less reduction in treated larvae over control. Therefore, current study neither corroborates nor disproves that inter-mating results in poor performance. Overall, Nistari and CSR2 combinations were proven to be good performers at 36°C. Nistari is an indigeneous breed adapted to tropical conditions and CSR2 is a productive breed of Japanese origin and most of the traits studied are quantitative and genetically inherited. Therefore, the generation backcrossed with CSR2 performed well in productive traits and the generation backcrossed with Nistari performed well in survivability. This study enhances the knowledge on selecting the segregating populations in high temperature breeding of silkworm.

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