Selected thermotolerant bivoltine hybrids of *Bombyx mori* L. exhibit desirable heterosis for quantitative traits under *Beauveria bassiana* infection

Abstract

Four thermotolerant bivoltine silkworm parents *viz*., B1, B4, B6 and B8 and their 12 hybrids *viz*., B1×B4, B1×B6, B1×B8, B4×B1, B4×B6, B4×B8, B6×B1, B6×B4, B6×B8, B8×B1, B8×B4 and B8×B6 were used in the experiments. One set of the parents and their hybrids were inoculated with *Beauveria bassiana* spore suspension (9.04 × 10^4 spores / ml), on the first day of the fifth instar before first feed and another set were exposed to high temperature (36 ± 1^o C temp. & 85 ± 5 % RH) and their responses were studied in terms of heterotic expression of quantitative traits. Significantly highest positive relative heterosis under normal condition was recorded in B1×B4 hybrid for five characters (fifth instar larval weight, cocoon yield by weight, single cocoon weight, shell weight and filament length) and under muscardine inoculation significantly highest positive relative heterosis was recorded in the hybrid B4×B6 for ten characters (ERR, cocoon yield by number, fifth instar larval weight, cocoon yield by weight, single cocoon weight, shell weight, shell ratio, pupal weight, filament length and filament weight). Heterosis scoring over all the traits under muscardine inoculation revealed that the hybrid B4×B6 scored high overall status of heterotic effects across the characters. Thus, B1 and B8 among parents and B1×B8 and B4×B6 among hybrids could be selected for dual stress tolerance against high temperature and muscardine disease.

Key words: high temperature, muscardine, tolerance, dual stress, *Bombyx mori L.*

1. INTRODUCTION

Continuous domestication of silkworm, *Bombyx mori* L. has resulted in loss of its certain wild characters including resistance to microbial infections.As a result, silkworm is susceptible to a number of pathogens such as fungi, bacteria, virus and protozoans. Thisis a main constrain in the tropical regions to take up bivoltine silkworm rearing, where the prevailing high temperature and high humidity conditions further deteriorates both the qualitative and quantitative traits. In India, CSR&TI, Mysore (Suresh Kumar *et al*., 2003) and APSSRDI, Hindupur (Lakshmi *et al*., 2011) have evolved thermotolerant bivoltine silkworm breeds to make them adoptable to such conditions.Their tolerance to high temperature also makethem tolerate temperature fluctuations.These thermotolerant bivoltine breeds have inherent resistance to viral diseases prevalent during high temperature conditions. In our previous study (Keerthana *et al*., 2019 a few thermotolerant bivoltine breeds were found to have resistance to white muscardine diseasecaused by the fungus, *Beauveria bassiana*,that prevailsduring winter season.Analysis of quantitative traitsin such breeds under fungal infection may throw light on genetic mechanism of multiple stress tolerance in mulberry silkworm, *i.e*., to tolerate both thermal stress and fungal infection (Gautam et al., 2022).Though, the susceptibility in silkworm breeds to *B. bassiana* is genetically determined by two major genes, *mus* located on the 11^{th}chromosome and *cal* located on the 7^{th} chromosome (Shimada, 1999),Zafar *et al*. (2013)haveshownthattheresistanceto *B. bassiana* is quantitatively inherited. Toyoma in 1906 initiated studies on heterosis for quantitative traits under normal rearing conditions. However, such studies as an indicator of silkworm's resistance to diseases is still limited. In the present study, we attempted to determine heterosis for different quantitative traits among selected thermotolerant bivoltine breeds of silkworm under *B. bassiana* infection, todetect and exploit their ability to perform equallywell under both thermal and fungal stress. Further, the possibility of utilizing such breeds for both summer and winter rearingsin tropics could be thought of.

2. MATERIAL AND METHODS

2.1 Silkworm parental strain

Four thermotolerant bivoltine breeds identified to have resistant to muscardine from our previous experiments (Keerthana, 2018) v*iz*. B1, B4, B6 and B8 from CSRTI, Mysore, India were used in this study.

2.2 Preparation of hybrids

The four identified breeds B1, B4, B6 and B8 were hybridized in a diallele fashion to develop sixteen crosses comprising of four parents *viz*., B1, B4, B6, B8 and 12 hybrids B1× B4, B1× B6, B1× B8, B4× B1, B4× B6, B4× B8, B6× B1, B6× B4, B6× B8, B8× B1, B8× B4 and B8× B6.The salient features of the parents are presented in Table 1.

Table 1: Larval and cocoon features of the thermotolerant breeds utilized in the experiments

Sl. No	Parents	Characters
1	B1	Plain larva spinning oval shaped cocoon
2	B4	Plain larva spinning oval shaped cocoon
3	B6	Marked larva spinning peanut cocoon
4	B8	Marked larva spinning peanut cocoon

2.3 Determination of LC_{50}

Silkworm rearing was conducted up to spinning stage by following standard rearingpractices (Dandin and Jayswal, 2014). Newly moulted fifth instar larvae in each cross were divided into batches for inoculating with different doses of fungal spore suspension. Three replications comprising 50 worms each were maintained under each such treatment. The silkworms were topically inoculated with the fungus by spraying stock (3.17×10^5 spores / ml) and 10^{-2}, 10^{-4}, 10^{-6} and10^{-8}diluted spore suspensions at the rate of 0.5 ml per worm.Daily larval mortality was recorded from first day of post inoculation to spinning and LC_{50} was calculated as per Reed and Muench (1938) using SPSS software.

2.4 Estimation of Heterosis under thermal and fungal pathogen stress

One set of all the sixteen crosses with three replications each, were topically inoculated with the fungus on the first day of fifth instar with the dose equivalent to LC_{50} for most tolerant hybrid (9.04×10^4 spores / ml), determined as mentioned above. Similarly, another set of all the sixteen crosses were subjected for thermal treatment at 36 ± 1^o C temperature and 85 ± 5 per cent relative humidity. Thermal exposure was for five consecutive days from second day to seventh day of fifth instar, for the duration of 6 hours daily (10.00 to 16.00hours IST), after which the worms were reared under normal rearing conditions. The observations were recorded replication-wise for different traits in larval, cocoon and post-cocoon stages and relative heterosis estimated for the hybrids following diallele analysis employing Griffing's method- I (Singh and Choudhary, 2012)in the software WINDOSTAT VERSION 9.1. The scoring of hybrids for heterotic expression under both fungal infection and thermal exposure was done by employing the procedure given by Arunachalam and Bandyopadhyay (1979).

2.5 Statistical analysis

The statistical analysis of the experimental data was carried out using computer software OPSTAT. The data obtained from the laboratory experiments were analysed statistically with Completely Randomized Design (CRD). Different treatments were compared using critical difference (CD) value at 0.05 (1%) level of significance.

3. RESULTS AND DISCUSSION

3.1 LC_{50}

When the silkworm crosses were inoculated with different doses of fungal spores, B8 breed among the parents showed highest LC_{50} (7.12×10^4 spores / ml)and B6 recorded the lowest(3.91×10^4).While among the hybrids, B1× B8 showed highest LC_{50} value (9.04×10^4 spores / ml)and lowest was inB6 × B8 (3.98×10^4 spores/ml) (Fig. 1). In the second experiment all the crosses were inoculated with fungal spore suspension of 9.04×10^4 spores / ml (the highest recorded in B1 X B8) to study the heterotic expression of quantitative traits under muscardine disease.

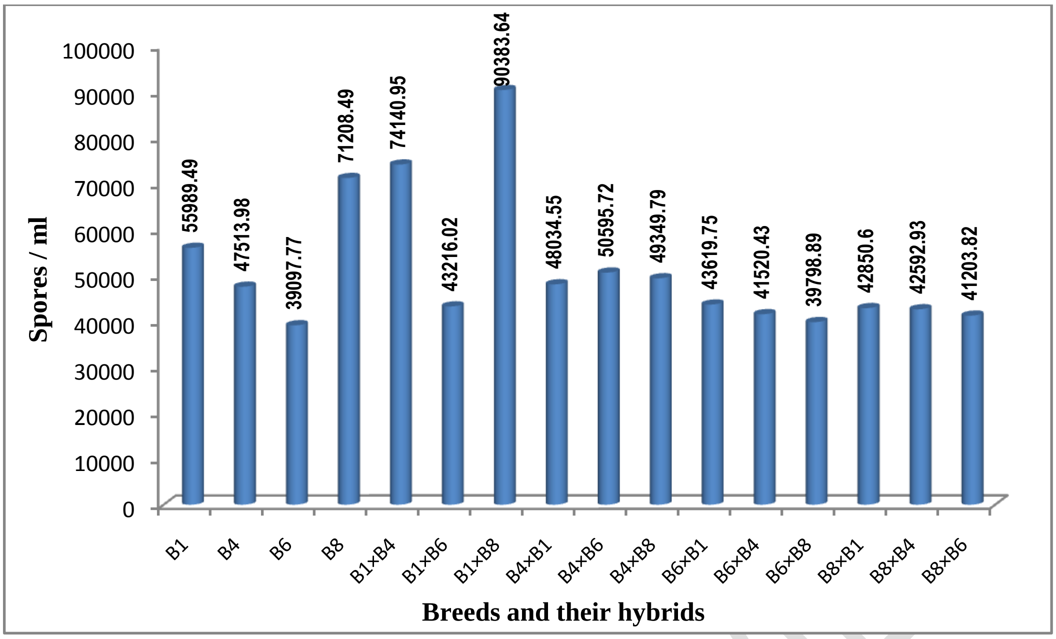

Fig. 1: LC_{50} values for *B. bassiana* inoculation among thermotolerant bivoltine silkworm breeds and their hybrids

3.2 Relative heterosis for survival parameters

The survival parameters denote the ability of the silkworm to thrive under a given environmental conditions and their values recorded under different treatments indicate the ability of the breeds to continue their life and spin cocoons or lay substantially good number of eggs which are fertile. Relative heterosis was computed for four such parameters*viz*., ERR, larval duration, cocoon yield by number and pupation ratein 12 bivoltine thermotolerant hybridsunder normal rearing conditions and under muscardine fungal inoculation and depicted in table 2. Under normal rearing conditions none of the parents or hybrids showed significant differences for ERR, cocoon yield by number and pupation rate and hence, heterosis was not computed for these traits. However, significantly highest negative heterosis, as desired, was reported for larval duration in B4×B6 (-1.47) hybrid. Under muscardine inoculation the hybrid B4×B6 expressed significantly highest positive heterosis for ERR (118.18 %) and cocoon yield by number (118.18 %). None of the hybrids showed significant heterosis for larval durationunder muscardine fungal infection in desired direction. The heterosis for pupation rate,which denotes the survival potential of the silkworm, was significantly highest and positive in the hybrid B1×B6 (131.58 %).

Significant scafor ERR in the hybrid CSR_2 × CSR_4 under *Bm*NPV stress condition has been reported by Manjunath Gowda *et al*. (2011) and Asha and Bhaskar (2014). As these sca effects are due to non-additive gene action, B4×B6 hybrid which showed significant positive relative heterosis is worth for improving ERR under muscardine stress. Similarly, for pupation rate significantly highest sca (2.343) was recorded in the hybrid Pure Mysore × CSR_2, followed by C-nichi × Daizo (1.28) under *Bm*NPV stress condition (Manjunath Gowda *et al*., 2011). In the present study under muscardine inoculation the hybrid B1×B6 recorded significantly highest positive heterosis of 131.58 per cent so, this could be better hybrid for improving pupation rate under muscardine infection.

Table 2. Relative heterosis (%) of survival parameters in thermotolerant bivoltine silkworm hybrids under normal rearing condition and muscardine inoculation

Hybrids	ERR		Larval duration		Cocoon yield by number		Pupation rate	
	Control	Muscardine inoculation	Control	Muscardine inoculation	Control	Muscardine inoculation	Control	Muscardine inoculation
B1 × B4	NA	66.67**	-1.42**	-6.45	NA	66.67**	NA	18.18**
B1 × B6	NA	-28.21	-1.32**	119.35**	NA	-28.21	NA	131.58**
B1 × B8	NA	33.33**	-1.18**	-8.20*	NA	33.33**	NA	25.58**
B4 × B1	NA	-13.89	-0.69	3.23	NA	-13.89	NA	20.91**
B4 × B6	NA	118.18**	-1.47**	93.55**	NA	118.18**	NA	115.20**
B4 × B8	NA	-50.54**	-0.6	1.64	NA	-50.54**	NA	5.31
B6 × B1	NA	-23.08	-0.17	119.35**	NA	-23.08	NA	-5.26
B6 × B4	NA	-15.15	0.02	125.81**	NA	-15.15	NA	28.00**
B6 × B8	NA	-100.00**	-0.12	-100.00**	NA	-100.00**	NA	-100.00**
B8 × B1	NA	-87.88**	-0.6	14.75**	NA	-87.88**	NA	-16.28**
B8 × B4	NA	-54.84**	0.64	4.92	NA	-54.84**	NA	-30.61**
B8 × B6	NA	-70.00**	0	133.33**	NA	-70.00**	NA	16.67

NA = Could not be analysed since, the performance was uniform among all crosses in control.
Significance Levels: * = < 0.05, ** = < 0.01

3.3 Relative heterosis for economic parameters

Relative heterosis for economic characters *viz*., larval weight, cocoon yield by weight, single cocoon weight, shell weight, shell ratio, pupal weight, filament length and filament weight are presented in table 3 and 4. Under normal condition,the relative heterosis for larval weight, which contributes to cocoon and shell weightand an indicator of the general health of the larvae, was found to be significantly positive and highest in the hybrid B1×B4 (4.01%). The same hybrid also expressed significantly highest positive heterosis for cocoon yield by weight (2.75 %),single cocoon weight (2.75 %), single shell weight (5.64 %) and filament length (3.07 %).For pupal weight highest significant positive heterosis was expressed in the hybrid B4×B1(3.76 %). None of the hybrids showed significant positive heterosis for shell ratio and filament weight. Under muscardine inoculation the hybrid B4×B6 exhibited significantly highest positive relative heterosis for all the traits evaluated *viz*., fifth instar larval weight (7.96 %),cocoon yield by weight (121.93 %), single cocoon weight (103.26 %), shell weight (104.08 %), shell ratio (100.88 %), pupal weight (103.10 %), filament length (110.88 %) and filament weight (109.30 %).

The intake of food during total larval life is reflected by the weight of mature larvae. The observations made on the larval weight depicted significant variation among the hybrids.The hybridB4×B6 followed by B1×B8 exhibited significant positive heterosis under muscardine inoculation, which is in concurrence with the observations of Manjunath Gowda *et al*. (2011) who reported significant sca of 3.554 for mature larval weight in C-nichi × CSR_2 hybrid, followed by C-nichi × CSR_4 (3.296) under *Bm*NPV stress condition, wherein C-nichi was tolerant to *Bm*NPV infection. So, the above two hybrids could be a better combination for improving mature larval weight under muscardine stress.

Table 3. Relative heterosis (%) of economic traits in thermotolerant bivoltine silkworm hybrids under normal rearing condition and muscardine inoculation

Hybrids	Fifth instar larval weight		Cocoon yield by weight (g / 1000 worms)		Single cocoon weight		Shell weight	
	control	Muscardine inoculation	Control	Muscardine inoculation	Control	Muscardine inoculation	control	Muscardine inoculation
B1 × B4	4.01**	5.36**	2.75*	81.25**	2.75*	8.86**	5.64**	11.54**
B1 × B6	-0.03	0.37	0.1	-38.84*	0.1	70.06**	3.3	67.27**
B1 × B8	-4.07**	7.70**	1.18	44.16**	1.18	8.58**	-3.55	9.73**
B4 × B1	-5.75**	1.76	1.96	-14.62	1.96	-0.81	-5.64**	0
B4 × B6	1.84	7.96**	-0.1	121.93**	-0.1	103.26**	-1.73	104.08**
B4 × B8	-0.53	0.62	-1.6	-52.80**	-1.6	-3.47	-2.13	-2.8
B6 × B1	-0.64	-1.82	-1.13	-31.98*	-1.13	77.07**	-5.49*	49.09**
B6 × B4	-0.21	-3.16**	-2.19	-25.99	-2.19	74.59**	-4.05	55.10**
B6 × B8	-5.78**	-6.85**	-1.78	100.00**	-1.78	-100.00**	-7.43**	-100.00**
B8 × B1	-9.94**	-6.76**	-4.73**	-89.80**	-4.73**	-14.82**	-18.78**	-27.43**
B8 × B4	-2.71	-8.69**	-3.00**	-59.60**	-3.00**	-9.78**	-5.32*	-10.28**
B8 × B6	-0.9	-8.67**	0.52	-75.46**	0.52	63.30**	-6.29**	51.72**

Significance Levels: * = < 0.05, ** = < 0.01

Table 4. Relative heterosis (%) of economic traits in thermotolerant bivoltine silkworm hybrids under normal rearing condition and muscardine inoculation

Hybrids	Shell ratio		Pupal weight		Filament length		Filament weight	
	Control	Muscardine inoculation	Control	Muscardine inoculation	Control	Muscardine inoculation	Control	Muscardine inoculation
B1 × B4	2.89	2.62	2.06	8.32**	3.07**	9.72**	1.25	4.44
B1 × B6	3.62	96.65**	-0.63	70.66**	-0.05	72.36**	0	65.96**
B1 × B8	-4.68	1.15	2.33	8.33**	1.37**	11.09**	0.65	14.58**
B4 × B1	-7.35**	1.07	3.76*	-0.97	-1.72**	-0.46	-7.5**	0
B4 × B6	-1.5	100.88**	0.25	103.10**	-1.26*	110.88**	-5.63	109.30**
B4 × B8	-0.51	0.88	-1.48	-3.61	-2.53**	-4.12**	-5.81*	0
B6 × B1	-4.07	68.33**	-0.13	83.01**	-0.63	66.56**	-2.86	61.70**
B6 × B4	-1.74	77.62**	-1.78	78.29**	-3.37**	78.94**	-5.63	81.40**
B6 × B8	-5.58*	-100.00**	-0.51	-100.00**	-3.92**	-100.00**	-15.56**	-100.00**
B8 × B1	-14.76**	-14.83**	-1.35	-12.12**	-4.12**	-23.66**	-4.58	-27.08**
B8 × B4	-2.43	-0.03	-2.46	-9.68**	-2.12**	12.68**	-3.23	-17.39**
B8 × B6	-6.60*	85.75**	2.06	65.80**	-4.56**	63.68**	-12.59**	58.18**

Significance Levels: * = < 0.05, ** = < 0.01

3.4 Overall status of heterotic effects under normal rearing condition and muscardine inoculation

The relative heterosis effects, significant at 5 per cent level was used as a norm for that trait and significant relative heterosis effects equal to or greater than the norm were given the score of +1 and relative heterosis of hybrids whose score was less than the norm was given a score of -1. Non-significant heterosis effects received zero score. Final score was obtained by totalling the scores over all the 12 traits. The mean of the final score was taken as final norm and the hybrids whose value exceeded the final norm were given high (H) overall status and the hybrids whose final score was less than the final norm were given low (L) status.With this criteria, under normal rearing conditions, seven of the twelve hybrids had high overall heterotic status and remaining had low overall heterotic status (Table 5 & 6).

Among the seven hybrids,B1×B4 and B4×B1 secured highest score of foureach and the lowest score was observed in B8×B1 (-6). Under muscardine inoculationonly five combinationshad high overall heterotic status and remaining had low overall heterotic status (Table 7). Highest score of 12 was secured by B4×B6 hybrid followed by B1×B6 and B6×B4 (7 each).Lowest score was observed in B6×B4, B8×B1 and B8×B4(-10 each).

Since, report on scoring of heterosis is not available in case of silkworm results are compared in relation to sca scoring in plants. Ramesh (1996) reported high overall sca status for 15 traits among 24 crosses of sesame and low overall sca status in remaining 27 crosses. Arunachalam and Bandyopadhyay (1980) in *B.compestris* and Lokprakash *et.al* (1993) in rice reported high overall sca status across the characters. In the present study, under muscardine treatment the hybrid B4×B6 scored high overall status of heterotic effects across the characters.

Table 5.Heterosis scoring for different larval, cocoon and filament parameters of thermotolerant bivoltine silkworm hybrids under normal rearing condition

Hybrids	ERR	Larval weight	Larval duration	Cocoon yield by number	Cocoon yield by weight	Single cocoon weight	Pupal weight	Pupation rate	Shell weight	Shell ratio	Filament length	Filament weight	Total	Over all status
B1×B4	-	1	-1	-	1	1	0	-	1	0	1	0	5	H
B4×B1	-	-1	0	-	0	0	1	-	1	1	1	1	4	H
B1×B8	-	1	1	-	0	0	0	-	0	0	1	0	3	H
B1×B6	-	0	1	-	0	0	0	-	0	0	0	0	1	H
B6×B1	-	0	0	-	0	0	0	-	1	0	0	0	1	H
B4×B6	-	0	-1	-	0	0	0	-	0	0	1	0	0	H
B4×B8	-	0	0	-	0	0	0	-	0	0	-1	1	0	H
B6×B4	-	0	0	-	0	0	0	-	0	0	-1	0	-1	L
B8×B4	-	0	0	-	-1	-1	0	-	1	0	-1	0	-2	L
B8×B6	-	0	0	-	0	0	0	-	-1	1	-1	-1	-2	L
B6×B8	-	-1	0	-	0	0	0	-	-1	1	-1	-1	-3	L
B8×B1	-	-1	0	-	-1	-1	0	-	-1	-1	-1	0	-6	L

Table 6.Heterosis scoring for different larval, cocoon and filament parameters of thermotolerant bivoltine silkworm hybrids under muscardine inoculation

Hybrids	ERR	Larval weight	Larval duration	Cocoon yield by number	Cocoon yield by weight	Single cocoon weight	Pupal weight	Pupation rate	Shell weight	Shell ratio	Filament length	Filament weight	Total	Over all status
B4×B6	1	1	1	1	1	1	1	1	1	1	1	1	12	H
B1×B6	0	0	1	0	-1	1	1	1	1	1	1	1	7	H
B6×B4	0	-1	1	0	0	1	1	1	1	1	1	1	7	H
B6×B1	0	0	1	0	-1	1	1	0	1	1	1	1	6	H
B8×B6	-1	-1	1	-1	-1	1	1	0	1	1	1	1	3	H
B1×B4	1	1	0	1	1	-1	-1	-1	-1	0	-1	0	-1	L
B1×B8	1	1	-1	1	1	-1	-1	1	-1	0	-1	-1	-1	L
B4×B1	0	0	0	0	0	0	0	-1	0	0	0	0	-1	L
B4×B8	-1	0	0	-1	-1	0	0	0	0	0	-1	0	-4	L
B6×B8	-1	-1	-1	-1	1	-1	-1	-1	-1	-1	-1	-1	-10	L
B8×B1	-1	-1	1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-10	L
B8×B4	-1	-1	0	-1	-1	-1	-1	-1	-1	0	-1	-1	-10	L

H: High heterotic status ; L : Low heterotic status;
1 : Heterotic value above the mean score of that trait; 0 : Heterotic value non-significant;
-1 : Heterotic value below the mean score of that trait

Table 7. Comparative Ranking of hybrids under normal rearing condition and muscardine inoculation

Hybrids	B1×B4	B4×B1	B1×B8	B1×B6	B6×B1	B4×B6	B4×B8	B6×B4	B8×B4	B8×B6	B6×B8	B8×B1
Normal condition	H	H	H	H	H	H	H	L	L	L	L	L
Muscardine inoculation	L	L	L	H	H	H	L	H	L	H	L	L

4. Conclusion

The mulberry silkworm (*Bombyx mori* L.) is one among the few completely domesticated insects that has attracted breeders from time immemorial due to its economic importance. It has been reported that most of the economically important characters in silkworms are quantitative and polygenic in nature (Kobari and Fujimoto, 1966). Almost all the economic traits of silkwormexhibit heterosis (Gautam *et al*., 1998). Systematic procedures developed for the estimation and exploitation of hybrids through hybridization in silkworm for economic traits on the basis of mid parent value (Relative heterosis) has brought a revolutionary change in overall qualitative and quantitative silk output (Malik *et al*., 2002).Thus, based on these facts it can be concluded that relative heterosis and overall heterotic status for survival and economic traits, three hybrid combinations *viz*., B4× B6, B1× B6 and B6× B4 performed better under muscardine inoculation. Thus, it may be inferred that these thermotolerant hybrids may possess dual tolerance for high temperature treatment and muscardine infection as well.

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