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Per se performance of six generation crosses of muscardine resistant thermotolerant bivoltine silkworm breeds of silkworm, *Bombyx mori* L. provides a lead for genetic analysis of their resistance to muscardine disease

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Abstract
Three muscardine resistant thermotolerant bivoltine breeds *viz.*, B1, B4 and B8 were crossed with a productive but muscardine susceptible bivoltine breed CSR_4 to raise six generations *viz.*, P1, P2, F_1, F_2, BC_1 and BC_2 which were all reared at once. A batch of silkworms of all the generations were inoculated with 9.04 × 10^4 spores / ml of *B. bassiana* @ 0.5 ml per silkworm to know their performance under fungal stress and another batch was reared under normal condition. The results revealed that among the non-segregating generations of the thermotolerant bivoltine silkworms, B1 breed and F_1 of (B1 × CSR_4) performed better for cocoon parameters *viz.*, cocoon, shell and pupal weights and pupation rate and silk parameters *viz.*, filament length and filament weight under normal conditions. However, B4 breed and F_1 of (B4 × CSR_4) performed better for these traits under muscardine inoculation. Whereas, CSR_4 breed and F_1 of (B8 × CSR_4) performed better for denier under both normal and muscardine disease conditions. Among segregating generations, F_2 of (B1 × CSR_4), BC_1 of (B1 × CSR_4) × B1 and BC_2 of (B1 × CSR_4) × CSR_4 crosses performed better for the cocoon parameters studied *viz.*, cocoon, shell and pupal weights and pupation rate and silk parameters *viz.*, filament length and filament weight under normal conditions. While under muscardine infection, F_2 of (B4 × CSR_4), BC_1 of (B4 × CSR_4) × B4 and BC_2 of (B4 × CSR_4) × CSR_4 crosses performed better for these traits. Whereas, F_2 of (B8 × CSR_4), BC_1 of (B1 × CSR_4) × B1 and BC_2 of (B8 × CSR_4) × CSR_4 crosses performed better for denier under both normal and muscardine disease conditions. Thus, it could be concluded that thermotolerant bivoltine silkworm breed B4 could be used for further improvement of the breeds for dual stress tolerance.

Keywords: Bivoltine, *Bombyx mori* L., six generations, muscardine resistance, cocoon and silk parameters

Introduction
The word Sericulture symbolizes the culturing of richness. It is an important agro-based cottage industry where art, agriculture and industry meet. There are several commercial species of silkworms, among which *Bombyx mori* L. is the most widely used and intensively studied and techniques for its rearing are most improved.

To improve the cocoon yield and silk quality, introduction of productive bivoltine breeds from temperate countries to India is envisaged (Dandin *et al.*, 2006) [2]. For the success of the introduced bivoltine breeds, it is necessary to have stability in cocoon crop under high temperature environment of tropics as well tolerate diseases. The main constrain is lack of high temperature and high humidity tolerant bivoltine breeds as the economically important qualitative and quantitative traits are affected by rearing them under such conditions. To overcome such problem CSR&TI, Mysore (Suresh Kumar *et al.*, 2003) [13] and APSSRDI, Hindupur (Lakshmi *et al.*, 2011) [7] evolved thermotolerant bivoltine silkworm breeds adoptable to high temperature conditions prevailing in summer. However, their resistance to diseases is not yet assessed and therefore, studies on muscardine disease resistance in thermotolerant bivoltine silkworm breeds was initiated at the Department of Sericulture, UAS, Bangalore.

Studies so far conducted on muscardine disease resistance in thermotolerant bivoltine breeds revealed that, B1, B4, B6 and B8 breeds from CSRTI, Mysore had relatively higher tolerance to white muscardine disease from among ten breeds evaluated (Keerthana, 2018) [6].

Similarly, among the hybrids of these thermotolerant bivoltine breeds, B1×B4 and B1×B8 exhibited better *per-se* performance, while B4×B6, B6×B4 and B1×B6 exhibited better heterotic performance under muscardine infection (Jayashree, 2019) [4]. These results indicate that there is a possibility of evolving bivoltine silkworm breeds with multiple/dual tolerance *viz.*, to both high temperature and fungal infection. In an attempt to understand the genetics of muscardine disease resistance by employing six generation mean analysis of promising muscardine resistant thermotolerant bivoltine breeds, we first studied their *per-se* performance and the results are presented in this article.

Material and methods

Silkworm breeds used for the experiment

The muscardine resistant thermotolerant bivoltine breeds B1, B4 and B8 identified from the previous studies and one productive CSR_4 breed obtained from CSRTI, Mysore crosses were affected and reared by following appropriate rearing practices (Dandin and Giridhar, 2014) [1]. The characteristic features of the parents utilized in the study are provided in table 1.

Table 1: Characteristic features of the bivoltine breeds used in the experiment

Sl. No.	Genotypes	Breed traits	Response to muscardine infection
1	B1	Plain larva spinning oval shaped cocoon	Thermotolerant and resistance to muscardine infection
2	B4	Plain larva spinning oval shaped cocoon	
3	B8	Marked larva spinning peanut cocoon	
4	CSR_4	Plain larva spinning peanut cocoon	Productive but susceptible to muscardine infection

Rearing of six generations of silkworm breeds

To develop six generation of the selected parents, three rearings were conducted, first by rearing the parents, next by rearing the parents and hybrids and finally obtained all the generation of crosses in the third rearing. The six generation of silkworm crosses were reared at once and divided into two batches, one was inoculated with muscardine fungus and other batch was maintained as control. Treatments were done to segregating batches in such a way that half the brood was inoculated with muscardine disease and the other half of the brood was raised under normal conditions and the worms in each batch were grouped into three replications randomly. For non-segregating generations the treatment was imposed for thirty worms each in three replications. Inoculation with fungus load of 9.04×10^4 spores / ml @ 0.5 ml per silkworm (Jayashree, 2019) [4] was done immediately after the fourth moult, before resuming the feed in the V instar.

Two rearings of six generations with treatments as indicated above was done, once during February-April 2021 and again during December 2021 -February 2022. Pooled mean data of the two rearing on cocoon weight, pupal weight, shell weight, pupation rate, filament length, filament weight and denier were recorded. The data so collected was analysed using a completely randomised design (Sundarraj *et al.*, 1972) [12]. The per cent data was analysed after arcsine transformation by using the formula sin-1√p/100. The mean values were compared by using Duncan's Multiple Range Test (DMRT) (Duncan, 1955) [3].

Results and Discussion

Cocoon weight (g)

Among the parents, B1 breed recorded significantly highest cocoon weight of 1.90g under normal condition while B4 breed recorded significantly highest cocoon weight of 1.00 g under muscardine inoculation (Table 2). Among F_1's significantly highest cocoon weight of 2.01 g was observed in the hybrid, B1 × CSR_4 under normal condition and B4 × CSR_4 recorded significantly highest cocoon weight of 1.43 g under muscardine inoculation. In segregating generations, among F_2's significantly maximum cocoon weight of 1.90 g was recorded in B1 × CSR_4 cross, under normal conditions, whereas, significantly maximum cocoon weight of 1.14 g was recorded in B4 × CSR_4 cross under *B. bassiana* infection. Under normal conditions, among BC_1's, significantly highest cocoon weight of 1.87 g was recorded in (B1 × CSR_4) × B1 cross and significantly maximum cocoon weight of 1.34 g was recorded in (B4 × CSR_4) × B4 cross under muscardine inoculation. In the batch without any fungal treatment, among BC_2's, significantly maximum cocoon weight of 1.91 g was recorded in (B1 × CSR_4) × CSR_4 cross and in muscardine treated batch, significantly highest cocoon weight of 1.11 g was recorded in (B4 × CSR_4) × CSR_4 cross.

Among all the generations, significantly highest cocoon weight under normal condition was observed in F_1 of B1 × CSR_4 with 2.01 g, followed by F_1 of B4 × CSR_4 (1.98 g) and least cocoon weight was recorded in F_2 of (B8 × CSR_4) (1.08 g). Similarly, significantly highest cocoon weight under muscardine inoculation was recorded in F_1 of B4 × CSR_4 (1.43 g), followed by BC_1 of (B4 × CSR_4) × B4 (1.34 g) and least cocoon weight of 0.51 g was recorded in CSR_4 breed.

Shell weight (g)

In the batch without fungal treatment, among the parents B1 recorded significantly highest shell weight of 0.45 g, while under muscardine inoculation, the thermotolerant bivoltine breed B4 recorded significantly highest shell weight of 0.23 g (Table 2). Among F_1's under normal conditions, significantly highest shell weight of 0.47g was observed in the hybrid, B1 × CSR_4, while, B4 × CSR_4 F_1 hybrid recorded significantly highest shell weight of 0.33 g under muscardine inoculation. In segregating generations, among F_2's significantly maximum shell weight of 0.43 g was recorded in B1 × CSR_4 cross under normal conditions and significantly maximum shell weight was recorded in B4 × CSR_4 and B1 × CSR_4 (0.23 g each) crosses under *B. bassiana* infection. Under normal conditions, among BC_1's, significantly highest shell weight of 0.44 g was recorded in (B1 × CSR_4) × B1 cross, whereas, in muscardine inoculated batch significantly maximum shell weight of 0.30 g was recorded in (B4 × CSR_4) × B4 cross. Among BC_2's, significantly maximum shell weight was recorded in (B1 × CSR_4) × CSR_4 cross (0.43 g) under normal conditions, while, significantly maximum shell weight was recorded in (B4 × CSR_4) × CSR_4 cross (0.24 g) under *B. bassiana* infection.

Table 2: Cocoon weight (g) and shell weight (g) as affected by muscardine inoculation among six generations of crosses between muscardine resistant thermotolerant bivoltine silkworm breeds and muscardine susceptible productive bivoltine silkworm breed

Breeds and generations		Cocoon weight (g)		Shell weight (g)	
		Normal	Muscardine inoculation	Normal	Muscardine inoculation
Parents	B1	1.90^{bc}	0.88^{defg}	0.45^{abc}	0.21^{de}
	B4	1.86^{cd}	1.00^{cdef}	0.44^{bc}	0.23^{cd}
	B8	1.54^{f}	0.84^{efg}	0.35^{g}	0.19^{de}
	CSR_4	1.78^{de}	0.51^{h}	0.38^{f}	0.15^{e}
F_1	B1 × CSR_4	2.01^{a}	1.23^{abc}	0.47^{a}	0.28^{abc}
	B4 × CSR_4	1.98^{ab}	1.43^{a}	0.46^{ab}	0.33^{a}
	B8 × CSR_4	1.40^{g}	0.89^{defg}	0.30^{h}	0.20^{de}
F_2	B1 × CSR_4	1.90^{bc}	1.11^{bcde}	0.43^{cd}	0.23^{cd}
	B4 × CSR_4	1.78^{de}	1.14^{bcd}	0.41^{de}	0.23^{cd}
	B8 × CSR_4	1.08^{i}	0.69^{gh}	0.21^{i}	0.16^{e}
BC_1	(B1 × CSR_4) × B1	1.87^{c}	0.94^{cdefg}	0.44^{bc}	0.25^{bcd}
	(B4 × CSR_4) × B4	1.75^{e}	1.34^{ab}	0.40^{ef}	0.30^{ab}
	(B8 × CSR_4) × B8	1.51^{f}	0.95^{cdefg}	0.31^{h}	0.25^{bcd}
BC_2	(B1 × CSR_4) × CSR_4	1.91^{bc}	1.04^{cdef}	0.43^{cd}	0.20^{de}
	(B4 × CSR_4) × CSR_4	1.74^{e}	1.11^{bcde}	0.39^{ef}	0.24^{cd}
	(B8 × CSR_4) × CSR_4	1.18^{h}	0.75^{fgh}	0.24^{i}	0.16^{e}
F test		**	**	**	**
S.Em ±		0.027	0.089	0.009	0.018
CD @5%		0.078	0.257	0.026	0.053
CV (%)		2.765	15.583	4.091	14.058

Figures with same superscript are statistically on par
*-Significant @5%; ** - significant @1%; NS-Non-significant

Among all the generations, significantly highest shell weight under normal condition was observed in F_1 of B1 × CSR_4 with 0.47 g, followed by F_1 of B4 × CSR_4 (0.46 g) and least shell weight was recorded in F_2 of (B8 × CSR_4) (0.21 g). Similarly, significantly highest shell weight under muscardine inoculation was recorded in F_1 of B4 × CSR_4 (0.33 g), followed by BC_1 of (B4 × CSR_4) × B4 (0.30 g) and least shell weight of 0.15 g was recorded in CSR_4 breed.

Pupal weight (g)

The thermotolerant bivoltine breed B1 recorded significantly highest pupal weight of 1.45 g among the parents under normal conditions, whereas, in muscardine inoculated batch, B4 breed recorded significantly highest pupal weight of 0.77 g (Table 3). Among the F_1's under normal conditions, significantly highest pupal weight was observed in the hybrid, B1 × CSR_4 (1.54 g) and B4 × CSR_4 recorded significantly highest pupal weight of 1.10 g under muscardine inoculation. In segregating generations, among F_2's significantly maximum pupal weight of 1.46 g was recorded in B1 × CSR_4 cross, while in muscardine treated batch, significantly maximum pupal weight was recorded in B4 × CSR_4 cross (0.90 g). In the batch without any fungal treatment, among BC_1's, significantly highest pupal weight was recorded in (B1 × CSR_4) × B1 cross (1.43 g) and significantly maximum pupal weight was recorded in (B4 × CSR_4) × B4 cross (1.05g) under *B. bassiana* infection. Under normal conditions, among BC_2's, significantly maximum pupal weight was recorded in (B1 × CSR_4) × CSR_4 cross (1.48 g) and significantly highest pupal weight was recorded in (B4 × CSR_4) × CSR_4 cross (0.87g) under muscardine inoculation.

Among all the generations, under normal condition significantly highest pupal weight was observed in F_1 of B1 × CSR_4 with 1.54 g, followed by F_1 of B4 × CSR_4 (1.52 g) and least pupal weight was recorded in F_2 of (B8 × CSR_4) (0.86 g). Similarly, significantly highest pupal weight under muscardine inoculation was recorded in F_1 of B4 × CSR_4 (1.10 g), followed by BC_1 of (B4 × CSR_4) × B4 (1.05 g) and least pupal weight of 0.36 g was recorded in CSR_4 breed.

The thermotolerant silkworm breed B4 and its crosses were found to be better with respect to pupal weight under *B. bassiana* inoculation.

Table 3: Pupal weight (g) and pupation rate (%) as affected by muscardine inoculation among six generations of crosses between muscardine resistant thermotolerant bivoltine silkworm breeds and muscardine susceptible productive bivoltine silkworm breed

Breeds and generations		Pupal weight (g)		Pupation rate (%)	
		Normal	Muscardine inoculation	Normal$	Muscardine inoculation$
Parents	B1	1.45^{bcde}	0.67^{bcdefg}	99.97^{abcd} (89.279)	37.3^{bcde} (37.618)
	B4	1.40^{defg}	0.77^{bcdefg}	99.96^{bcde} (88.885)	41.41^{bcd} (40.034)
	B8	1.19^{h}	0.65^{cdefg}	99.94^{de} (88.57)	34.31^{cdef} (35.797)
	CSR_4	1.38^{efg}	0.36^{h}	99.95^{cde} (88.773)	15.44^{f} (22.922)
F_1	B1 × CSR_4	1.54^{a}	0.95^{ab}	100.00^{a} (90.00)	53.44^{ab} (46.961)
	B4 × CSR_4	1.52^{ab}	1.10^{a}	100.00^{a} (90.00)	53.09^{ab} (46.755)
	B8 × CSR_4	1.10^{i}	0.69^{bcdefg}	99.99^{ab} (89.594)	40.51^{bcde} (39.484)
F_2	B1 × CSR_4	1.46^{bcd}	0.87^{abcd}	99.98^{abc} (89.324)	23.10^{def} (28.509)
	B4 × CSR_4	1.36^{fg}	0.90^{abc}	99.96^{bcde} (88.84)	33.73^{def} (35.161)
	B8 × CSR_4	0.86^{j}	0.53^{gh}	99.94^{de} (88.695)	22.14^{ef} (27.609)
BC_1	(B1 × CSR_4) × B1	1.43^{cdef}	0.69^{bcdefg}	100.00^{a} (90.00)	59.34^{a} (50.536)
	(B4 × CSR_4) × B4	1.35^{g}	1.05^{a}	99.99^{ab} (89.391)	52.60^{abc} (46.605)
	(B8 × CSR_4) × B8	1.20^{h}	0.70^{bcdefg}	99.96^{bcde} (88.824)	35.26^{bcde} (36.328)

BC$_2$	(B1 × CSR$_4$) × CSR$_4$	1.48^{abc}	0.84^{abcdef}	99.98^{abc} (89.561)	34.50^{cde} (35.8)
	(B4 × CSR$_4$) × CSR$_4$	1.33^{g}	0.87^{abcde}	99.96^{bcde} (88.878)	40.26^{bcde} (39.237)
	(B8 × CSR$_4$) × CSR$_4$	0.93^{j}	0.59^{dfgh}	99.93^{e} (88.506)	34.41^{cde} (35.885)
F test		**	**	**	**
S.Em±		0.023	0.084	0.223	3.39
CD @5%		0.067	0.241	0.645	9.81
CV (%)		3.070	18.980	0.433	15.522

$-Figures in parentheses are angular transformed values; Figures with same superscript are statistically on par; *-Significant @5%; ** -significant @1%

Pupation rate (%)

The thermotolerant bivoltine silkworm breed B4 recorded significantly maximum pupation rate of 99.97 per cent and 41.41 per cent among the parents, under normal conditions and muscardine inoculation, respectively (Table 3). Under normal conditions among the F$_1$'s, significantly 100 per cent pupation rate was observed in F$_1$ hybrids, B1 × CSR$_4$ and B4 × CSR$_4$, whereas, under muscardine inoculation, B1 × CSR$_4$ hybrid recorded significantly maximum pupation rate of 53.44 per cent. In segregating generations, among F$_2$'s significantly maximum pupation rate of 99.98 per cent was recorded in B1 × CSR$_4$ cross under normal condition and significantly maximum pupation rate of 33.73 per cent was recorded in B4 × CSR$_4$ cross under muscardine inoculation. Among BC$_1$'s, significantly hundred per cent and 59.34 per cent of pupation rate was recorded in (B1 × CSR$_4$) × B1 under normal condition and muscardine inoculation, respectively. Among BC$_2$'s, significantly maximum pupation rate was recorded in (B1 × CSR$_4$) × CSR$_4$ (99.98%) under normal conditions, while, in muscardine treated batch, significantly maximum pupation rate was recorded in (B4 × CSR$_4$) × CSR$_4$ (40.26%).

Among all the generations, significantly hundred per cent pupation rate under normal condition was observed in B1 × CSR$_4$, B4 × CSR$_4$ hybrids and BC$_1$ of (B4 × CSR$_4$) × B4 and minimum pupation rate was recorded in BC$_2$ of (B8 × CSR$_4$) × CSR$_4$ (99.93%). Similarly, under muscardine inoculation significantly maximum pupation rate was recorded in BC$_1$ of (B1 × CSR$_4$) × B1 (59.34%), followed by F$_1$ of B1 × CSR$_4$ (53.44%) and least pupation rate of 15.44 per cent was recorded in CSR$_4$ breed.

Since pupation rate indicates the survival ability in the pupal stage, reduction in pupation rate due to muscardine inoculation over control and deviation in pupation rate among muscardine inoculated batches over the mean pupation rate due to infection was calculated and presented in a bi-plot (Fig. 1). The breeds and their crosses which occupy the extreme left and middle corner of the plot, have least per cent reduction in pupation rate over control and also show maximum positive deviation from mean pupation rate under muscardine inoculation. Thus, they are muscardine disease resistant breeds or crosses. Accordingly, among the crosses BC$_1$ of (B1 × CSR$_4$) × B1, followed by F$_1$ of B4 × CSR$_4$ and F$_1$ of B1 × CSR$_4$ showed better resistance to muscardine inoculation. Similarly, among parents B4 showed better resistance to muscardine inoculation. The breed CSR$_4$ which occupy extreme right of the bi-plot will susceptible to muscardine inoculation.

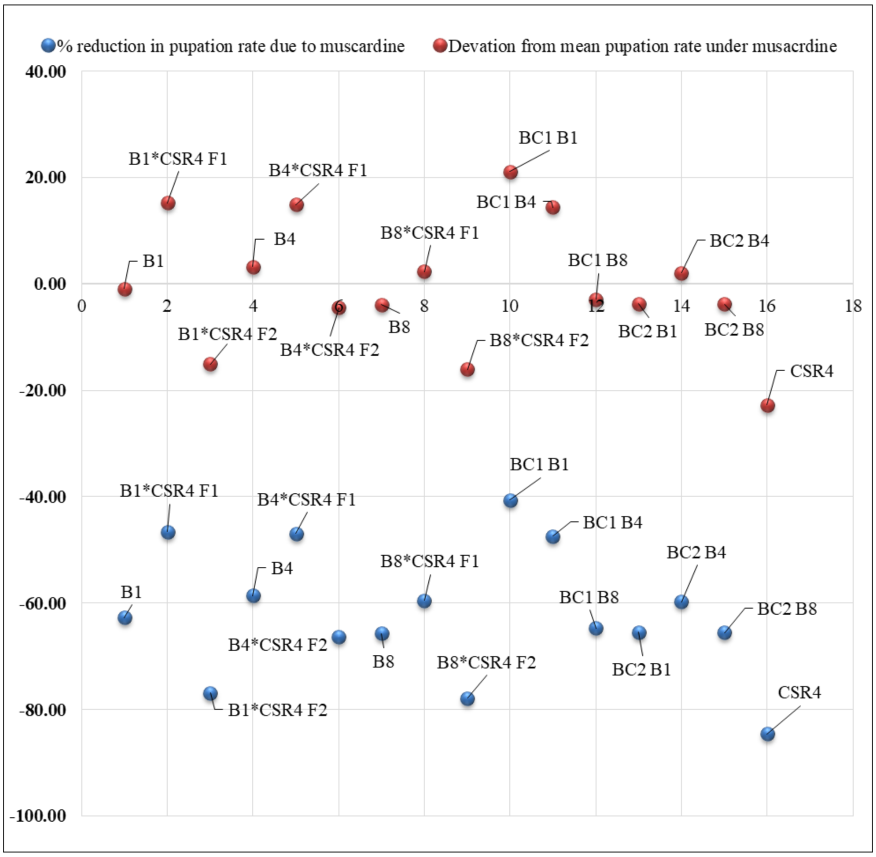

Fig 1: Biplot for *per se* performance of parents and crosses between thermotolerant bivoltine silkworm breeds (B1, B4 and B8) and CSR$_4$ for pupation rate under normal condition and muscardine inoculation

Filament length (m)

In the batch without fungal treatment, among the parents, B1 recorded significantly longest filament length of 1207.93 m, whereas, under *B. bassiana* infection, the thermotolerant bivoltine breed B4 recorded significantly longest filament length of 615.40 m (Table 4). Under normal conditions, significantly highest filament length was observed in the F_1 hybrid, B1 × CSR_4 (1299.11 m) while B4 × CSR_4 F_1 hybrid recorded significantly highest filament length of 862.83 m under muscardine inoculation. In segregating generations, among F_2's, significantly maximum filament length of 1183.12 m was recorded in B1 × CSR_4 cross under normal conditions and significantly maximum filament length was recorded in B4 × CSR_4 (619.16 m) cross under *B. bassiana* infection. Under normal conditions, among BC_1's, significantly highest filament length was recorded in (B1 × CSR_4) × B1 cross (1219.39 m), while significantly maximum filament length was recorded in (B4 × CSR_4) × B4 cross (770.13 m) under muscardine inoculation. Among BC_2's, significantly maximum filament length was recorded in (B1 × CSR_4) × CSR_4 cross (1172.00 m) under normal conditions, whereas, under muscardine inoculation, significantly highest filament length of 575.81 m was recorded in (B4 × CSR_4) × CSR_4 cross.

Among all the generations, under normal condition significantly longest filament length was observed in F_1 of B1 × CSR_4 with 1299.11 m, followed by F_1 of B4 × CSR_4 (1247.74 m) and lowest filament length was recorded in F_2 of (B8 × CSR_4) cross (737.74 m). Similarly, under muscardine inoculation significantly maximum filament length was recorded in F_1 of B4 × CSR_4 (862.83 m), followed by BC_1 of (B4 × CSR_4) × B4 cross (770.13 m), while lower filament length of 274.95 m was recorded in CSR_4 breed.

Filament weight (g)

Among the parents B1 recorded significantly highest filament weight (0.28 g) under normal conditions, whereas, in muscardine treated batch, B4 recorded significantly highest filament weight of 0.19 g (Table 4). Under normal conditions among F_1's, significantly highest filament weight was observed in the F_1 hybrid, B1 × CSR_4 (0.33 g) while both B4 × CSR_4 and B1 × CSR_4 hybrids recorded significantly highest filament weight of 0.23 g each under muscardine inoculation. In segregating generations, among F_2's, significantly maximum filament weight of 0.28 g was recorded in B1 × CSR_4 cross under normal conditions, whereas, under muscardine inoculation, significantly maximum filament weight of 0.18 g was recorded in B4 × CSR_4 cross.

Table 4: Filament length (m), filament weight (g) and denier as affected by muscardine inoculation among six generations of crosses between muscardine resistant thermotolerant bivoltine silkworm breeds and muscardine susceptible productive bivoltine silkworm breed

Breeds and their crosses		Filament length (m)		Filament weight (g)		Denier	
		Normal	Muscardine inoculation	Normal	Muscardine inoculation	Normal	Muscardine inoculation
Parents	B1	1207.93^{abc}	521.91^{de}	0.28^{bc}	0.16^{bc}	2.03^{cd}	2.04^{cde}
	B4	1174.13^{bcd}	615.40^{cd}	0.27^{bcd}	0.19^{abc}	2.06^{bcd}	2.10^{cde}
	B8	1001.17^{fg}	401.68^{efg}	0.23^{ef}	0.13^{c}	2.04^{bcd}	2.22^{cde}
	CSR_4	1065.59^{ef}	274.95^{g}	0.26^{cde}	0.13^{c}	2.13^{abcd}	3.18^{a}
F_1	B1 × CSR_4	1299.11^{a}	700.38^{bc}	0.33^{a}	0.23^{a}	2.27^{abcd}	2.23^{cde}
	B4 × CSR_4	1247.74^{ab}	862.83^{a}	0.32^{a}	0.23^{a}	2.28^{abcd}	1.80^{de}
	B8 × CSR_4	928.18^{g}	409.74^{efg}	0.24^{def}	0.17^{abc}	2.28^{abcd}	2.84^{ab}
F_2	B1 × CSR_4	1183.12^{bcd}	570.72^{cd}	0.28^{bc}	0.14^{c}	2.12^{abcd}	1.72^{e}
	B4 × CSR_4	1130.78^{cde}	619.16^{cd}	0.27^{bcd}	0.18^{abc}	2.18^{abcd}	2.00^{cde}
	B8 × CSR_4	737.74^{i}	335.97^{fg}	0.21^{f}	0.14^{c}	2.47^{a}	2.85^{ab}
BC_1	(B1 × CSR_4) × B1	1219.39^{abc}	579.54^{cd}	0.33^{a}	0.21^{ab}	2.41^{ab}	2.47^{bc}
	(B4 × CSR_4) × B4	1153.5^{bcde}	770.13^{ab}	0.26^{bcde}	0.23^{a}	2.02^{d}	2.06^{cde}
	(B8 × CSR_4) × B8	840.27^{h}	572.06^{cd}	0.23^{ef}	0.19^{abc}	2.40^{abc}	2.26^{cde}
BC_2	(B1 × CSR_4) × CSR_4	1172.00^{bcd}	441.39^{ef}	0.30^{ab}	0.15^{bc}	2.32^{abcd}	2.33^{bcd}
	(B4 × CSR_4) × CSR_4	1106.91^{de}	575.81^{cd}	0.27^{bcd}	0.18^{abc}	2.22^{abcd}	2.10^{cde}
	(B8 × CSR_4) × CSR_4	772.10^{hi}	295.52^{g}	0.20^{f}	0.13^{c}	2.36^{abcd}	2.80^{ab}
F test		**	**	**	**	*	**
S.Em±		29.570	40.467	0.011	0.019	0.109	0.172
CD @5%		85.181	116.570	0.033	0.055	0.316	0.497
CV (%)		4.753	13.121	7.308	19.187	8.526	12.913

Figures with same superscript are statistically on par

*-Significant @5%; ** - significant @1%

Among BC_1's under normal conditions, significantly highest filament weight of 0.33 g was recorded in (B1 × CSR_4) × B1 and significantly maximum filament weight was recorded in (B4 × CSR_4) × B4 cross (0.23 g) under muscardine inoculation. Among BC_2's, significantly maximum filament weight was recorded in (B1 × CSR_4) × CSR_4 cross (0.30 g) under normal conditions and in muscardine treated batch, significantly highest filament weight of 0.18 g was recorded in (B4 × CSR_4) × CSR_4 cross.

Among all the generations, significantly highest filament weight under normal condition was observed in F_1 of B1 × CSR_4 and BC_1 of (B1 × CSR_4) × B1 with 0.33 g each, followed by F_1 of B1 × CSR_4 (0.31 g) and least filament weight of 0.20 g was recorded in BC_2 of (B8 × CSR_4) × CSR_4. Similarly, significantly highest filament weight of 0.23 g was recorded in F_1 of B1 × CSR_4, B4 × CSR_4 and BC_1 of (B4 × CSR_4) × B4, followed by BC_1 of (B1 × CSR_4) × B1 (0.21 g) and least filament weight of 0.13 g was recorded in CSR_4 and B8 breeds, BC_2 of (B8 × CSR_4) × CSR_4 under muscardine infection.

Denier

Among the parents, CSR_4 recorded significantly highest denier of 2.13 and 3.18 under normal conditions and muscardine inoculation, respectively. Under normal conditions among F_1's, significantly highest denier of 2.28 was observed in the hybrids, B1 × CSR_4 and B4 × CSR_4, whereas, B8 × CSR_4 hybrid recorded significantly highest denier of 2.84 under muscardine inoculation. In segregating generations, among F_2's, significantly highest denier of 2.47

and 2.85 was recorded in B8 × CSR_4 cross under normal and muscardine disease conditions, respectively. Among BC_1's, significantly highest denier was recorded in (B1 × CSR_4) × B1 and (B8 × CSR_4) × B8 crosses (2.41 each) under normal conditions and significantly maximum denier was recorded in (B1 × CSR_4) × B1 cross (2.47) under muscardine inoculation. Among BC_2's, significantly maximum denier of 2.36 and 2.80 was recorded in (B8 × CSR_4) × CSR_4 cross under normal and muscardine disease conditions, respectively.

Among all the generations, significantly highest denier under normal condition was observed in F_2 of B8 × CSR_4 with 2.47, followed by BC_1 of (B1 × CSR_4) × B1 and (B8 × CSR_4) × B8 crosses (2.4 each) and least denier was recorded in BC_1 of (B4 × CSR_4) × B4 (2.02). Similarly, under muscardine inoculation significantly highest denier of 3.18 was recorded in CSR_4 breed, followed by F_2 of (B8 × CSR_4) cross (2.85) and least denier of 1.72 was recorded in F_2 of B1 × CSR_4.

The results of Raghavaiah and Jayaramaiah (1990) [8] in similar studies revealed that NB_7 breed spun cocoons with maximum cocoon weight (1.027 g) compared to NB_{18} (0.940 g) when infected with muscardine fungus. Venkataramana Reddy (1978) [14] reported that, NB_7 larvae spun the cocoons with maximum shell weight compared to NB_4D_2, KA and NB_{18} bivoltine breeds, when infected with different doses of *B. bassiana* spores during fifth instar. Rajitha and Savithri (2015) [9] reported a reduction in pupal weight in silkworm hybrid PM×CSR_2 treated with sub lethal concentration of *B. bassiana* conidial suspension. Keerthana *et al.*, (2020) [5] in their studies with ten thermotolerant bivoltine silkworm breeds reported that B4 thermotolerant breed gave significantly higher cocoon weight, shell weight, pupal weight, filament length and filament weight both under normal and muscardine inoculation. Similarly, Jayshree (2019) has reported better performance of B4 breed and B1 × B4 and B1 × B8 hybrids under muscardine conditions. Results of the current study are also in conformity with the above findings and also those of Sreejith (2019) [11] and Sahana *et al.* (2021) [10] with respect to performance of selected B-series thermotolerant bivoltine breeds under *B. bassiana* infections. In the present study, B4 and B1 breeds showed higher shell weight average filament length and highest filament weigh among the thermotolerant bivoltine silkworm breeds and B4 crosses produced better shell weight under *B. bassiana* infection. Furhter the performance of F_1's was also in conformity with the earlier findings of Jayashree (2019) [4] under *B. bassiana* infections.

Conclusion

Three thermotolerant bivoltine silkworm breeds *viz.*, B1, B4 and B8 crossed with productive but susceptible bivoltine silkworm breed CSR_4, for muscardine disease resistance revealed that B1 and their crosses showed better *per-se* performance under normal conditions while, B4 and their crosses showed better *per-se* performance under muscardine inoculation. The thermotolerant bivoltine silkworm breed B4 can be a potential parent with resistance to *B. bassiana* infection. Therefore, B4 thermotolerant bivoltine silkworm breed can be further utilized as a source of breeding material to impart muscardine disease resistance to other productive thermotolerant silkworm breeds. Further, genetics of muscardine disease resistance in thermotolerant bivoltine silkworm breeds needs to be understood to plan an appropriate breeding plan to utilize the potential dual resistant breeds.

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