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Development of a Robust Polyvoltine Breed "NP_1" of the Mulberry Silkworm, *Bombyx mori* L.

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A breeding programme was initiated during 2001 utilizing two polyvoltine silkworm breeds *viz.* BL_{69}, an evolved breed tolerant to high temperature and MAR, comparatively resistant to *Bombyx mori* nuclear polyhedrosis virus (BmNPV) with the objective to develop robust polyvoltine breeds and hybrids. The breed NP_1 was developed by exposing the fifth instar larvae to high temperature ($36 \pm 1°C$), high Relative Humidity ($85 \pm 5\%$ R.H.) and inoculating third instar larvae with BmNPV inoculum. At F_{12}, the breed was tested for hybrid forming ability utilizing six bivoltine silkworm breeds *viz.* CSR_2, CSR_4, CSR_{17}, CSR_{18}, CSR_{19} and NB_4D_2. The hybrid "$NP_1 \times CSR_{17}$" exhibited its superiority by recording 97.2% survival, 1.892 g cocoon weight, 0.406 g cocoon shell weight, 21.5% cocoon shell ratio, 16.6% raw silk percentage and 890 m filament length whereas the control ($PM \times CSR_2$) has recorded 90.2% survival, 1.599 g cocoon weight, 0.304 g cocoon shell weight, 18.9% cocoon shell ratio, 13.1% raw silk percentage and 768 m filament length. Commercial exploitation of the new polyvoltine × bivoltine hybrid in sericulture industry has been discussed.

Key words: *Bombyx mori* L., BmNPV, Breeding, High temperature, Polyvoltine × bivoltine hybrid

Introduction

In India, a viral disease commonly known as "grasserie" caused by *Bombyx mori* nuclear polyhedrosis virus (BmNPV), is the most serious among silkworm diseases (Samson *et al.,* 1990; Subba Rao *et al.,* 1991; Shivaprakasam and Rabindra, 1995). The less cocoon yield under Indian tropical conditions is attributed due to high temperature in addition to grasserie disease. Studies have been carried out to develop silkworm breeds and hybrids comparatively resistant to BmNPV (Uzigawa and Aruga, 1966; Aratake, 1973; Ratna Sen *et al.,* 1999) and tolerant to high temperature (Shao *et al.,* 1987, 1989, 1990; He *et al.,* 1989, 1991; Datta *et al.,* 2001; Suresh Kumar *et al.,* 2002). In the present study, an attempt has been made to develop polyvoltine silkworm breeds and hybrids which not only can withstand high temperature stress condition but also comparatively resistant to BmNPV.

Materials and Methods

Thirty four polyvoltine breeds maintained in the germplasm at Central Sericultural Research and Training Institute, Mysore were challenged with a dose of 1.0×10^7 POB/ml/100 larvae and their susceptibility status was determined. Seven breeds *viz.,* GNP, MAR, BL_{37}, Mysore Princess, Moria, 96 C and BL_{36} showed mortality percentage < 50%. Similarly, forty four polyvoltine breeds were exposed to high temperature ($36 \pm 1°C$) and $85 \pm 5\%$ RH from third day of fifth instar and fed with mulberry leaves. Thermal exposure was given daily for a period of 6 hr till spinning. 100 larvae were kept in each bed with three replications. Eight breeds *viz.,* BL_{30}, BL_{36}, BL_{45}, BL_{62}, BL_{65}, BL_{67}, BL_{68} and BL_{69} were found comparatively tolerant to high temperature based on their pupation rate. In the present study, two plyvoltine silkworm breeds *viz.,* BL_{69}, tolerant to high temperature and MAR, comparatively resistant to BmNPV were utilized as breeding resource materials. Larvae were also reared at room tem-

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perature (25 ± 1°C) and 65 ± 5% RH. Post cocoon parameters were determined from the cocoons obtained from rearing at 25 ± 1°C.

Selection and breeding methods

NP_1: The line NP_1 was developed utilizing two polyvoltine silkworm breeds *viz.,* BL_{69} and MAR. Ten breeding plans were initiated utilizing identified BmNPV resistant and thermotolerant breeds. F_1 to F_5, rearing was conducted 'enmasse'. Thermal exposure was given continuously upto 6 generations whereas BmNPV inoculum was given at alternate generations. Backcrossing was adopted and from F_5 onwards, cellular rearing was resorted to. Batches showing higher performance for pupation rate, cocoon colour, cocoon shape, cocoon yield and reeling characters were selected. Dose of BmNPV inoculum was increased from 1.0×10^7 (F_1) to 3.0×10^7 POB/ml/100 larvae (F_{11}). More emphasis was given for high viability at high temperature and less mortality following inoculated with BmNPV inoculum. Generation-wise performance of NP_1 reared at 25 ± 1°C and 65 ± 5% RH is given in Table 1. The post cocoon parameters like raw silk %, filament length, denier, reelability and neatness were also considered in each generation.

Characteristics of NP_1: Newly laid eggs are light yellow in colour, chorion white. Newly hatched larvae are dark brown in colour. Fully grown larvae are plain with bluish tinge.Cocoons are greenish yellow and oval with medium grains. Larval duration is 22 to 23 days.

Characteristics of new polyvoltine × bivoltine hybrid "$NP_1 \times CSR_{17}$": Newly laid eggs are light yellow in colour, chorion white. Newly hatched larvae are dark brown. Fully grown larvae are plain with bluish tinge. Cocoons are light greenish yellow, oval shape and larval period is around 23 days. The hybrid possesses high viability, cocoon yield, cocoon weight, cocoon shell weight, cocoon shell ratio, longer filament length, higher raw silk %, reelability and neatness. A breeding plan for the development of new polyvoltine breed NP_1 is presented in (Fig. 1).

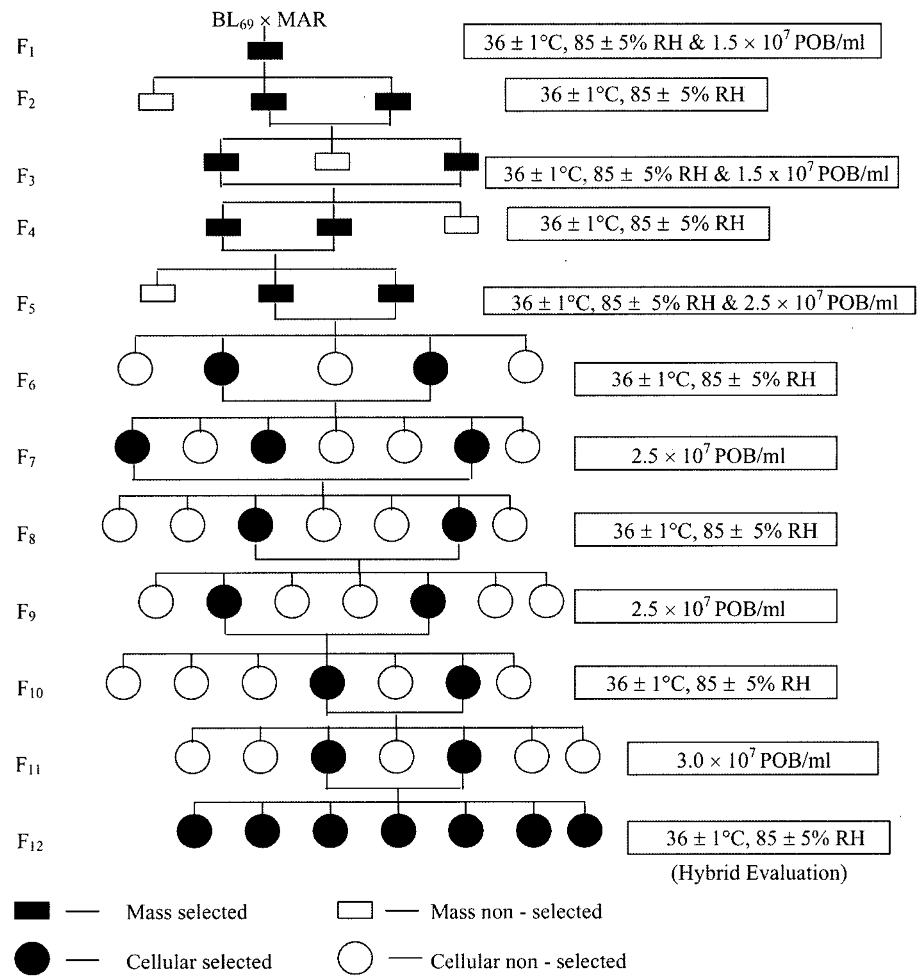

Fig. 1. Breeding plan for NP_1 - A new polyvoltine silkworm breed.

Table 1. Performance of NP_1 during the process of breeding

Generation	Survival %	Cocoon yield/ 10,000 larvae (kg)	Cocoon wt. (g)	Cocoon shell wt. (g)	Cocoon shell ratio (%)	Raw silk %	Filament length (m)	Denier (d)	Reela-bility (%)	Neatness (p)
F_1	96.11	15.222	1.589	0.283	17.80	11.26	727	2.10	88	91
F_2	87.65	11.277	1.331	0.249	18.70	12.63	656	2.23	89	89
F_3	88.87	11.434	1.360	0.253	18.80	12.52	615	2.59	84	92
F_4	89.17	12.400	1.342	0.248	18.60	12.92	621	2.51	84	92
F_5	94.25	13.000	1.504	0.277	18.41	13.57	672	2.73	89	92
F_6	91.50	13.900	1.530	0.273	17.84	13.33	703	2.61	84	86
F_7	98.34	12.667	1.261	0.221	17.52	11.33	574	2.23	78	85
F_8	88.90	10.967	1.278	0.221	17.29	12.10	581	2.20	88	85
F_9	84.00	11.600	1.447	0.250	17.27	13.29	592	2.91	80	86
F_{10}	86.34	11.117	1.378	0.242	17.56	11.84	560	2.61	84	87
F_{11}	88.34	12.467	1.407	0.246	17.48	11.60	587	2.50	84	88
F_{12}	90.84	11.900	1.322	0.235	17.77	13.20	640	2.64	76	90
Mean	90.77	12.329	1.395	0.249	17.92	12.46	627	2.48	85	89
SD	3.49	1.203	0.098	0.019	0.534	0.787	51	0.23	3.57	2.66

Results

Performance of parental breed at 25 ± 1°C and 65 ± 5% relative humidity

Generation -wise mean values of ten economic characters of the breeding line NP_1 is given in (Table 1). The survival percentage ranged from 86.34 (F_{10}) to 98.34% (F_7). Maximum cocoon yield/10,000 larvae ranged from 10.967 (F_8) to 15.222 kg (F_1). The cocoon weight was recorded minimum at F_7 (1.261 g) whereas it was maximum (1.589 g) at F_1. The cocoon shell weight ranged from 0.221 g (F_7 and F_8) to 0.283 g (F_1). The cocoon shell ratio ranged from 17.27 (F_9) to 18.80% (F_3).

The data recorded for post cocoon parameters show that the raw silk % ranged from 11.26 (F_1) to 13.57% (F_5). The longest filament length (727 m) was recorded at F_1 and it was minimum (650 m) at F_{10}. There was variation in the filament size which ranged from 2.10 d (F1) to 2.91 d (F_9). The maximum reelability percentage (89%) was recorded at F_2 and F_5 whereas it was minimum (78%) at F_7. The maximum neatness (92 p) was observed at F_3 to F_5 and it was minimum (85 p) at F_7 and F_8.

Performance of parental breed at 36 ± 1°C and 85 ± 5% RH

Performance of NP_1 at high temperature during different generations is given in (Table 2). Maximum survival percentage (85.60%) was recorded at F_{10} whereas it was minimum (76.00%) at F_2. Cocoon weight ranged from 1.184 (F_1) to 1.327 g (F_5). Cocoon shell weight was observed maximum (0.247 g) at F_5 whereas it was minimum (0.185 g) at F_1. Cocoon shell ratio ranged from 15.62 (F_1) to 18.61% (F_5).

Table 2. Performance of NP_1 at high temperature (36 ± 1°C) during the process of breeding

Generation	Pupation rate	Cocoon weight (g)	Cocoon shell weight (g)	Cocoon shell ratio (%)
F_1	81.33	1.184	0.185	15.62
F_2	76.00	1.267	0.232	18.31
F_3	84.67	1.191	0.209	17.54
F_4	80.33	1.291	0.233	18.04
F_5	81.67	1.327	0.247	18.61
F_6	79.20	1.254	0.210	16.74
F_8	83.50	1.318	0.236	17.90
F_{10}	85.60	1.235	0.210	17.00
F_{12}	82.19	1.271	0.221	17.38
Mean	81.61	1.259	0.220	17.46
SD	2.75	0.047	0.017	0.863

Mortality of parental line at different generations on challenge with BmNPV

Mortality percentage in NP_1 following inoculation with BmNPV during different generations is given in (Table 3).

Table 3. Mortality percentage in NP_1 following inoculation with BmNPV in different generations

Generation	Concentration of BmNPV (POB/ml/100 larvae)	Mortality percentage
F_1	1.5×10^7	6.00
F_3	2.5×10^7	13.33
F_5	2.5×10^7	4.00
F_7	2.5×10^7	25.75
F9	2.5×10^7	36.77
F_{11}	3.0×10^7	34.33

Table 4. Mortality percentage in NP1 × CSR_{17} and control, PM × CSR_2 after inoculation with BmNPV at a rate of 1×10^7 POB/ml/100 larvae

Hybrid	NP1 × CSR_{17}	PM × CSR_2 (Control)
Mortality percentage	26.66	23.66

The new breed shows minimum mortality (6.00%) at F_1 when challenged with a dose of 1.5×10^7 POB/ml/100 larvae. Maximum mortality (36.77%) was recorded at F_9 following inoculation of BmNPV at a concentration of 2.5×10^7 POB/ml/100 larvae. Mortality percentage in new polyvoltine × bivoltine hybrid following inoculation with BmNPV is given in (Table 4). The new hybrid shows a mortality of 26.66% as against 23.66% in the control PM × NB_4D_2.

Performance of polyvoltine × bivoltine hybrid

The laboratory performance of new hybrid is presented in (Table 5). The new hybrid, NP_1 × CSR_{17} recorded an average survival of 97.2% over the control hybrid PM × CSR_2 (90.2%). The percent improvement in NP_1 × CSR_{17} was 7.7. The cocoon yield/10,000 larvae of 16.50 kg was recorded in NP_1 v CSR_{17} over the control hybrid (14.27 kg). The cocoon weight of 1.892 g was recorded in NP_1 × CSR_{17} as against the control hybrid PM × CSR2 (1.599 g). The cocoon shell weight was recorded 0.406 g in NP_1 × CSR_{17} whereas it was 0.304 g in the control hybrid. The percent improvement in NP_1 × CSR_{17} was 20.0 over control hybrid. Cocoon shell ratio in NP_1 × CSR_{17} was 21.45% as against 18.99% in control hybrid. The improvement noticed was 13.5 over the control.

Raw silk percentage in NP_1 × CSR_{17} was 16.57% and recorded an improvement of 26.7% over the control. The filament length in NP_1 × CSR_{17} was 890 m as against 768 m in the control hybrid. The percent improvement over control was 15.8 in NP_1 × CSR_{17}. The filament size recorded in NP_1 × CSR_{17} was 3.21 d whereas it was 2.56 d in control. NP_1 × CSR_{17} recorded reelability of 85% and it was 81% in the control hybrid. The new hybrid, NP_1 × CSR_{17} recorded neatness of 90 p and it was 88 p in the control hybrid.

Evaluation of the new hybrid-NP_1 × CSR_{17}

The new hybrid NP_1 × CSR_{17} was tested along with the control hybrid PM × CSR_2 utilizing 200 dfls in each hybrid. The performance is given in (Table 6). The results indicate that NP_1 × CSR_{17} performed better over the control hybrid PM × CSR_2 in terms of pupation rate, cocoon weight, cocoon yield, cocoon shell weight, cocoon shell ratio, raw silk percentage, filament length, reelability and neatness. The percent improvement observed in NP_1 × CSR_{17} for yield/10,000 larvae was 20.5, survival 13.40, cocoon shell weight 17.29, cocoon shell ratio 8.79, raw silk % 13.90, filament length 12.83 and neatness 10.00%.

Discussion

In the present study, an attempt has been made to develop robust polyvoltine silkworm breeds and hybrids comparatively resistant to BmNPV and tolerant to high temper-

Table 5. Performance of NP_1 × CSR_{17} in the laboratory (Mean of 3 trials)

Hybrid	Pupation rate	Cocoon yield/ 10,000 larvae (kg)	Cocoon wt. (g)	Cocoon shell wt. (g)	Cocoon shell ratio (%)	Raw silk (%)	Filament length (m)	Denier (d)	Reela-bility (%)	Neatness (p)
NP_1 × CSR_{17}	97.2	16.50	1.892	0.406	21.45	16.57	890	3.21	85.0	90.0
PM × CSR_2 (Control)	90.2	14.27	1.599	0.304	18.99	13.07	768	2.56	81.0	88.0
CD at 5%	2.6	0.40	0.066	0.02	0.86	0.92	37.0	0.12	1.40	1.5
CV%	3.31	1.12	1.66	2.69	1.54	2.67	1.98	1.82	0.99	0.75
Percent improvement over control hybrid										
	7.7	15.6	5.8	20.0	13.5	26.7	15.8	25.3	3.9	2.9

Table 6. Performance of $NP_1 \times CSR_{17}$ under in house testing at rearing technology and innovation laboratory at CSR&TI, Mysore

Hybrid	Pupation rate	Cocoon yield/ 10,000 larvae (kg)	Cocoon wt. (g)	Cocoon shell wt. (g)	Cocoon shell ratio (%)	Raw silk (%)	Filament length (m)	Denier (d)	Reela-bility (%)	Neatness (p)
$NP_1 \times CSR_{17}$	86.56	16.302	1.884	0.373	19.80	13.67	897	2.98	85.0	90
$PM \times CSR_2$ (Control)	76.33	13.530	1.744	0.318	18.20	12.01	795	2.83	83.0	87
			Percent improvement over control hybrid							
	13.4	20.5	8.02	17.29	8.79	13.90	12.83	5.30	2.40	10.00

ature through selection of survived individuals on challenge with BmNPV and exposure of fifth instar larvae at high temperature for several generations. At F_1, the concentration of BmNPV inoculum was 1.5×10^7 POB/ml/100 larvae whereas it was increased to 3.0×10^7 POB/ml/100 larvae at F_{11} in order to exert selection pressure during the process of breeding. BmNPV inoculum was given at alternate generations and fifth instar larvae were exposed to high temperature continuously upto 6 generations and thereafter at alternate generations. Silkworm breeds have been developed by selecting the survived progeny following exposure of larvae to virus (Uzigawa and Aruga, 1966; Aratake, 1973; Ratna Sen *et al.*, 1999). Baig *et al.* (1991) have studied susceptibility status of different silkworm breeds to NPV and observed that Pure Mysore is comparatively resistant to grasserie disease.

Efforts have been made to develop robust silkworm breeds through hybridization, back-crossing selection and continuous rearing of larvae at high temperature and humidity for several generations (Shao *et al.*, 1987, 1989; He *et al.*, 1989, 1991). Petkov and Nguyen (1987) studied that quantitative characters were genetically conditioned when the silkworms were reared at high temperature (26.2 to 29.4°C) and relative humidity (79 to 88%) and dominant and epistatic effects had relatively higher share in the inheritance of cocoon weight and cocoon shell weight. Robust silkworm breeds and hybrids suitable for rearing in summer autumn have been developed. Hong *et al.* (1988) have developed a high yielding silkworm "Eunbackjam" with 8% more silk and 3% higher survival as compared to the control and suitable for summer autumn rearing. A silkworm variety "Samkwangjam" suitable for summer-autumn rearing with 5% more cocoon yield and 6% more raw silk as compared to the control and resistant to flacherie virus was developed (Sohn *et al.*, 1990). Midorikawa and Yokozuka (1988) bred a silkworm hybrid "Honen × Kempaku" and Zhang *et al.* (1994) bred "Qiufeng × baiyer" through hybridization and pedigree separation suitable for summer autumn seasons.

Attempts have been made to develop robust bivoltine silkworm breeds through exposure of fifth instar larvae to high temperature (36 ± 1°C) and high humidity (85 ± 5%) (Datta *et al.*, 2001; Suresh Kumar *et al.*, 2002). Shirota (1992) has selected healthy silkworm breed through rearing of fifth instar larvae at high temperature (30°C) and humidity (90%). Thermal sensitivity and heat shock response of different silkworm breeds have been analysed by Joy and Gopinathan (1995) and they observed that C.Nichi and Pure Mysore showed better survival as compared to bivoltine breed NB_4D_2 when exposed to 41°C.

The performance of the new hybrid $NP_1 \times CSR_{17}$ demonstrated its superiority in almost all the quantitative and qualitative characters in comparison to the control hybrid. The hybrid is comparatively resistant to BmNPV and tolerant to high temperature and can be exploited throughout the year. Presently, the hybrid $NP_1 \times CSR_{17}$ is under testing at different centres located in different regions in South India.

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