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Screening of Silkworm Breeds for Tolerance to *Bombyx mori* Nuclear Polyhedro Virus (BmNPV)

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BmNPV (*Bombyx mori* nuclear polyhedrosis virus) causes nuclear polyhedrosis in silkworms. The tolerance of silkworms to BmNPV is controlled by polygenes. This paper reports on the relative tolerance of silkworm breeds among the germplasm maintained at Andhra Pradesh State Sericultural Research & Development Institute (APSSRDI), Hindupur, India. The silkworm larvae out of second moult were *per orally* inoculated with BmNPV polyhedra (1×10^7/ml) and reared upto spinning. The response to BmNPV had been categorized into apparent tolerance, real tolerance and susceptibility. Among the 145 silkworm breeds screened, 18 bivoltines and 16 polyvoltines were found to have real tolerance to BmNPV.

Key words: *Bombyx mori*, Germplasm, Nuclear polyhydrosis virus, Tolerance

Introduction

Bombyx mori nuclear polyhedrosis virus (BmNPV), which belongs to Baculoviridae, causes nuclear polyhedrosis in silkworms. Nuclear polyhedrosis is the most common viral disease and is prevalent in almost all the sericultural areas in India. The incidence of nuclear polyhedrosis in India is reported to be 20−40% (Chitra *et al.*, 1987) and was estimated as 33−55% in different seasons in Karnataka, India (Nataraju *et al.*, 1998). The persistence of BmNPV polyhedra, high temperature and humidity are the major factors that contribute to the crop losses due to nuclear polyhedrosis at farmers level in India.

The best approach to prevent an infectious disease such as nuclear polyhedrosis may be to use relatively tolerant silkworm breeds. This is due to the fact that the resistance to BmNPV is controlled by polygenes (Aratake, 1973). The response by different genetic stocks of silkworm to BmNPV is differential and established clearly. This feature, the susceptibility level of the breed, is the most important character influencing the causation, initiation and development of epizootic of infectious diseases such as nuclear polyhedrosis in silkworms (Watanabe, 1987). Many researchers reported the inter-breed/strain difference in susceptibility to BmNPV (Watanabe, 1966, 1986; Aratake, 1973; Liu, 1984; Furuta, 1995; Nataraju, 1995; Sen *et al.*, 1997) available in different R&D institutions. The screening of breeds for their relative tolerance/susceptibility for BmNPV would be helpful in identifying the silkworm breeds that are less susceptible and they might be exploited commercially to evolve tolerant breeds/hybrids through breeding plans for increased silk productivity. The silkworm germplasm being maintained at Andhra Pradesh State Sericulture Research & Development Institute (APSSRDI), Hindupur comprises of several inbred lines derived from several commercial silkworm hybrids of Chinese, Japanese and Madagascar origin, indigenously developed lines and tropical breeds. No information was available with regard to the tolerance to BmNPV among the silkworm germplasm stocks in APSSRDI, Hindupur. Hence, the present study has been carried out to screen the APSSRDI germplasm for their relative tolerance to BmNPV.

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Materials and Methods

Propagation of BmNPV

The BmNPV was propagated in a susceptible breed

(NB_{18}) of silkworms by per oral inoculation of BmNPV polyhedra during 4th instar. The haemolymph of the infected larvae was collected and BmNPV polyhedra were purified as described by Sugimori *et al.* (1990).

Purification of BmNPV polyhedra
The haemolymph is collected on ice from the BmNPV infected larvae, allowed for bacterial degradation for 24 hrs and centrifuged at 5000 rpm for 10 min for pelleting the BmNPV polyhedra. The polyhedra were washed thrice in 1 M NaCl followed by distilled water. The polyhedra were then suspended in distilled water and quantified by haemocytometry. The purified polyhedra were stored at 4°C for further use.

Screening of silkworm germplasm
145 silkworm (78 bivoltine and 67 polyvoltine) breeds maintained in the germplasm bank of Andhra Pradesh State Sericulture Research & Development Institute (APSSRDI), Hindupur, India, was screened for determining their relative tolerance/susceptibility to BmNPV.

The silkworm larvae out of 2nd moult (3rd instar) were *per orally* inoculated with BmNPV polyhedra (1×10^7/ml) by smearing onto mulberry leaf (0.25 ml/25 cm^2 leaf/25 larvae). The larvae were allowed to feed onto inoculum-smeared leaf for 6 hrs. The larvae were reared under standard rearing conditions. The mortality due to nuclear polyhedrosis was recorded during larval and pupal stages. A mock-inoculated batch of each breed was maintained as control. A known susceptible breed, NB_{18}, was also maintained as check variety. Fifty larvae were maintained in 3 replications in each batch. The BmNPV infection was confirmed by light microscopy of the haemolymph for BmNPV polyhedra. The tolerant population in each batch was collected. The moths emerged from the survivors were processed for egg laying and the eggs were used for reproducing BmNPV tolerant stocks.

The degree of tolerance was estimated from the mortality recorded during larval and pupal stages. The relative tolerance was categorized into (1). Apparent tolerance (the inoculated larvae complete larval period and form cocoons but do not metamorphose into pupae), (2). Real tolerance (the inoculated larvae complete larval period, form cocoons and metamorphose into pupae and moths emerge from the cocoons) and (3). Susceptibility (the inoculated larvae succumb to death due to nuclear polyhedrosis during larval period itself). The mortality data collected for each breed was analyzed statistically and the variation between the replications was expressed in terms of standard deviation (S.D.) over the mean values.

Results

The results indicate wide range of variability among different silkworm breeds screened for their tolerance/susceptibility to BmNPV.

Bivoltine silkworm breeds
Apparent tolerance: Four silkworm breeds (2 Chinese and 2 Japanese) showed apparent tolerance to BmNPV among the 78 breeds screened (Table 1). The Chinese type breeds, CC_1 and LPKO, showed apparent tolerance to BmNPV and the degree of tolerance recorded was 41.67% and 58.33%, respectively. The apparent tolerance exhibited by DNNB and DNBD (Japanese type) was 45.67% and 4.33%, respectively.
Real tolerance: Among the breeds screened, a total of eighteen (5 Chinese and 13 Japanese) breeds showed real tolerance to BmNPV (Table 1). Real tolerance was observed in $D_{43}O$, APS_9, AP_9 and SPO among the Chinese type silkworm breeds. The tolerant population by larval stage was highest (56.67%) in SPO followed by 51.00% in $D_{43}O$, whereas the tolerant population by pupal stage was highest (50.67%) in $D_{43}O$ followed by 46.63% in SPO. In APS_9, lowest tolerant population by larval (28.67%) and pupal (15.33%) stages was observed among the Chinese type breeds.

Thirteen Japanese type breeds exhibited real tolerance to BmNPV and they include NB_4D_2-B, APS_8, NB_4D_2-A, SMPD, NMPW, SMPW, SPMD, NMPL, SMPL NAMD, HNSD, $APSD_4$-P and GPC. The tolerant population observed was highest by larval (75.67%) as well as pupal (47.00%) stages in NB_4D_2-B. The degree of tolerance ranged from 20.67% in $APSD_4$-P to 44.33% in SMPW by larval stage and from 14.67% in $APSD_4$-P to 34.67% in SMPW among the Japanese type breeds.

The cocoons of breeds exhibiting real tolerance were assessed for cocoon weight, cocoon shell weight and shell ratio. Among the Chinese type breeds, maximum cocoon weight (1.502 g) and cocoon shell weight (0.292 g) was recorded in SPO and shell ratio in AP4 (21.03%). A Maximum of 1.654 g cocoon weight was recorded in SMPL and HNSD, shell weight of 0.324 g in HNSD and shell ratio of 20.75% in GPC among the Japanese type of breeds (Table 1).
Susceptibility to BmNPV: 56 bivoltine silkworm breeds were found to be completely susceptible to BmNPV during the larval stage itself among the 78 breeds screened. They include 25 Chinese (AP_1, AP_2, AP_3, AP_8, AP_{10}, TR_1O, SMO, NB_{10}, HTPO, KA, CSR_2, APS_5, TLO_4, HTMO, SDO_1, SDO_2, SDO_3, SDO_4, SDO_5, APO_1, APO_2, APO_3, APO_4, APO_5 and APO_6) and 31 Japanese (KK_1, KK_2, KM_1, FNN_9, FNF_9, NB_{18}, FC_9, CTV, LPKC, LPKD,

Table 1. Characteristics of bivoltine silkworm breeds tolerant to BmNPV

Breed	Larval character	Cocoon colour	Degree of tolerance (%)		Cocoon wt. (g)	Shell wt. (g)	Shell ratio (%)
			By larval stage	By pupal stage			
Chinese type							
CC_1	Plain	White	41.67±3.51	0.00			
LPKO	Plain	White	58.33±1.53	0.00			
$D_{43}O$	Plain	White	51.00±3.61	50.67±3.79	1.172	0.227	19.37
APS_9	Plain	White	28.67±3.06	15.33±1.15	1.406	0.260	18.49
AP_9	Plain	White	38.00±1.73	23.67±3.51	1.399	0.267	19.09
SPO	Plain	White	56.67±2.52	46.63±2.08	1.502	0.292	19.44
AP_4	Plain	White	35.67±2.52	23.67±3.51	1.165	0.245	21.03
Japanese type							
DNNB	Plain	White	45.67±4.51	0.00			
DNBD	Plain	White	4.33±1.15	0.00			
NB_4D_2-B	Plain	White	75.67±4.04	47.00±3.61	1.302	0.245	18.82
APS_8	Marked	White	34.33±4.73	16.00±3.46	1.342	0.248	18.48
NB_4D_2-A	Plain	White	28.33±4.73	19.33±3.06	1.302	0.242	18.59
SMPD	Plain	Yellow	37.67±5.51	32.00±2.00	1.430	0.284	19.86
NMPW	Plain	White	44.00±3.61	31.33±4.16	1.386	0.254	18.33
SMPW	Plain	White	44.33±3.22	34.67±3.06	1.390	0.274	19.71
SPMD	Marked	Yellow	30.33±2.52	24.33±3.51	1.457	0.292	20.04
NMPL	Plain	Flesh	41.33±3.06	30.00±2.00	1.590	0.315	19.81
SMPL	Plain	Flesh	36.00±2.65	26.33±3.79	1.654	0.307	18.56
NAMD	Marked	Yellow	20.33±2.52	14.67±3.06	1.632	0.298	18.26
HNSD	Plain	White	46.00±3.46	37.00±2.65	1.654	0.324	19.59
$APSD_4$-P	Plain	White	20.67±2.52	14.67±3.06	1.531	0.312	20.38
GPC	Plain	White	22.33±2.08	15.67±2.08	1.494	0.310	20.75
NB_{18}	Plain	White	0.00				

*Values represent an average of 3 replications±S.D.

HMTC, CSR_4, APS_4, TR_5C, $D_{43}C$, TLD_4, SDD_1, SDD_2, SDD_3, SDD_4, SDD_5, APD_1, APD_2, APD_3, APD_4, APD_5, APD_6, TL_1D, TL_5D, TL_5DM and TL_1DM) type breeds.

Polyvoltine silkworm breeds

Apparent tolerance: Among the 67 polyvoltine silkworm breeds screened, a total of thirteen (9 Chinese and 4 Japanese) silkworm breeds showed apparent tolerance to BmNPV (Table 2). The apparent tolerance to BmNPV was observed in CSPROP, CS_2O-W, CS_2WY, CS_2O-C, WO, MDPCY, POWSY, POPM and POWBL among the Chinese type breeds. The degree of tolerance ranged from 6.33% in CS_2O-C to 71.33% in POPM. Among Japanese type breeds, $MDNBCD_1$, MDNB-PW, MD_2-P and WDP showed apparent tolerance to BmNPV. The degree of tolerance ranged from 6.67% in $MDNBCD_1$ to 26.33% in MDNB-PW.

Real tolerance: A total of sixteen (14 Chinese and 2 Japanese) silkworm breeds showed real tolerance to BmNPV among the 67 polyvoltine silkworm breeds screened (Table 2). The Chinese type breeds exhibiting real tolerance to BmNPV include MD_2PO, APM_1, EY, PRON, CSNM, EYSY, YPO, MHM_1, MDBYHDY, RON, POCPO, MDPWPOW, SYLWOM and WOM. The degree of tolerance in larval stage was highest at 34.67% in MHM_1 and WOM, whereas it was highest at 27.67% in MHM_1 by pupal stage. The tolerant population ranged from 10.00% in CSNM to 34.00% in MD_2PO by larval stage and from 8.00% in PRON to 26.67% in MD_2PO by pupal stage.

The Japanese type breeds, MCRMC and MD_1Y, were found to have real tolerance to BmNPV. Their tolerance level by larval stage was around 28% in both the breeds and ranged from 19.33% to 22.00% by pupal stage. The cocoons of polyvoltine breeds exhibiting real tolerance were also assessed for cocoon weight, cocoon shell weight and shell ratio. %). A maximum of 1.350 g cocoon weight was recorded in RON, shell weight of 0.284 g in POCPO and shell ratio of 19.92% in MDPWPOW among

Table 2. Characteristics of polyvoltine silkworm breeds tolerant to BmNPV

Breed	Larval character	Cocoon colour	Degree of tolerance (%)		Cocoon wt. (g)	Shell wt. (g)	Shell ratio (%)
			By larval stage	By pupal stage			
			Chinese type				
CSPROP	Plain	GY	17.33±2.52	0.00			
CS_2O-W	Plain	White	10.00±2.00	0.00			
CS_2O-WY	Plain	GY	13.33±3.06	0.00			
CS_2O-C	Plain	White	6.33±1.53	0.00			
W.O	Plain	White	6.67±1.53	0.00			
MDPCY	Plain	Yellow	8.67±1.15	0.00			
POWSY	Plain	GY	68.33±3.21	0.00			
POPM	Plain	GY	71.33±4.16	0.00			
POWBL	Plain	GY	58.67±3.06	0.00			
MD2PO	Plain	White	34.00±5.29	26.67±2.31	1.198	0.183	15.28
APM1	Plain	White	21.33±4.16	16.67±1.15	1.212	0.191	15.76
EY	Plain	GY	19.33±4.16	13.33±3.06	1.254	0.197	15.71
PRON	Plain	Yellow	22.67±3.06	8.00±2.00	1.189	0.183	15.39
CSNM	Plain	GY	10.00±2.00	8.33±1.53	1.197	0.185	15.46
EYSY	Plain	GY	17.33±2.52	12.33±2.52	1.295	0.204	15.75
YPO	Plain	Yellow	28.67±5.03	24.67±4.16	1.273	0.222	17.44
MHM1	Plain	GY	34.67±3.06	27.67±3.21	1.324	0.241	18.20
MDBYHDY	Plain	GY	24.33±2.52	16.67±1.53	1.285	0.185	14.40
RON	Marked	Yellow	24.67±2.89	15.53±2.52	1.350	0.232	17.19
POCPO	Plain	White	22.33±2.08	14.67±3.06	1.690	0.284	16.80
MDPWPOW	Plain	White	20.33±2.08	12.33±2.39	1.229	0.245	19.92
SYLWOM	Plain	GY	33.33±3.06	19.67±1.53	1.191	0.221	18.57
WOM	Plain	GY	34.67±3.06	23.33±2.52	1.214	0.237	19.52
			Japanese type				
$MDNBCD_1$	Plain	GY	6.67±1.53	0.00			
MDNB-PW	Plain	GY	26.33±1.15	0.00			
MD_2-P	Plain	GY	24.67±0.58	0.00			
WD-P	Plain	White	8.33±1.53	0.00			
MCRMC	Marked	White	28.67±3.06	22.00±3.46	1.268	0.216	17.03
MD_1-Y	Marked	Yellow	28.00±4.00	19.33±4.16	1.265	0.207	16.36
Pure Mysore	Plain	GY	0.00				

*Values represent an average of 3 replications±S.D.

the Chinese type of breeds. Both the Japanese type breeds (MCRMC and MD_1Y) recorded a cocoon weight of 1.26 g and shell weight of 0.21 g with a shell ratio of 17% (Table 2).

Susceptibility to BmNPV: A total of 38 polyvoltine silkworm breeds including 32 Chinese (BL_{24}, MCRMO, POW, MDPRO, PEY, Nistari, Pure Mysore, CPD-O, CS_2CCP, CSPRMO, CS_2WCP, CS_2CP, PCY, MD_2PO, PM-K, PM-SL, ROSY, POWO, SYMDBY, CS_2BL, POCPROW, SYLWOM, MDPWCW, SYLPMSYL, PMWSY, POPOW, POPMW, PMWBL, ROWBL, POSY, WOY and CSWL) and 6 Japanese type (MD_1C, MD_1W, MD_2M, MDNB-CP, MCRPC and CL_2) breeds were found to be completely susceptible to BmNPV by larval stage itself.

Discussion

The crop losses due to the incidence of nuclear polyhedrosis caused by BmNPV are quite frequent under tropical conditions. This is attributed to high humidity and high temperature, and their wide fluctuations coupled with persistence of BmNPV polyhedra in the rearing environment, recurring severe infections form major factors for the incidence and spread of the nuclear polyhedrosis infections.

Among the most reliable aspects for prevention of an infection is the non-susceptibility or low susceptibility of the host. Non-susceptibility (resistance) offers complete protection against specific infectious agent and the less susceptible hosts will be better equipped to fight against infections. The possibility of epizootic level of infection becomes limited with the use of non-susceptible hosts. The identification of silkworm breeds tolerant to BmNPV by per oral inoculation tests were conducted for the silkworm germplasm stocks at NISES (Japan) by Furuta (1995), Liu (1984) in China, Nataraju (1995) and Sen *et al.* (1997) in India.

Among 78 bivoltine and 67 polyvoltine silkworm breeds screened in the present study for their relative tolerance/susceptibility, various levels of tolerance-real and apparent tolerance. A total of 18 bivoltine (5 Chinese and 13 Japanese) and 16 polyvoltine (14 Chinese and 2 Japanese) lines showed real tolerance to BmNPV. The breeds showing apparent tolerance to BmNPV include 4 bivoltines (2 Chinese and 2 Japanese) and 13 polyvoltines (9 Chinese and 4 Japanese). A total of 94 silkworm breeds (56 bivoltines including 25 Chinese and 31 Japanese type; 38 polyvoltines including 32 Chinese and 6 Japanese type) were completely susceptible to BmNPV.

The silkworm breeds with complete susceptibility to BmNPV may be more productive but will be very difficult to rear in the field conditions. The silkworm breeds, which showed apparent tolerance to BmNPV are able to spin cocoons to some extent, failed to pupate completely and succumbed to death. Among the Chinese type bivoltine breeds identified to have real tolerance to BmNPV, the difference in mortality due to BmNPV by larval and pupal stage was almost nil in case of $D_{43}O$ and in others it ranged from 10.04% (SPO) to 14.33% (AP_9). The difference in mortality by larval and pupal stage was highest in NB_4D_2-B (28.67%); 10–20% in APS_8, NMPW and NMPL; less than 10% in other Japanese type bivoltine breeds. Among the polyvoltine Chinese type breeds, the different in mortality due to BmNPV was least in CSNM (1.67%); above 10% in PRON, SYLWOM and WOM; below 10% in other breeds. In the Japanese type polyvoltine breeds, MCRMC and MD_1Y, the difference in mortality due to BmNPV was found to be 6.67% and 8.67%, respectively. The lesser difference in mortality due to BmNPV by larval and pupal stages indicates the higher degree of tolerance to BmNPV and these breeds may be more useful practically. Some of the identified tolerant breeds are having better cocoon characteristics as compared to the susceptible check varieties (NB_{18} and PM) and the tolerance level does not have any correlation with cocoon characteristics.

These BmNPV tolerant stocks are being maintained and used to develop silkworm breeds/hybrids with less susceptibility to BmNPV through breeding programmes implemented at APSSRDI.

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