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Research article

EVALUATION AND SELECTION OF POTENTIAL PARENTS TOLERANT TO *BOMBYX MORI* NUCLEAR POLYHEDRO VIRUS (*BM*NPV) FOR DEVELOPMENT OF DISEASE RESISTANT HYBRIDS

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ABSTRACT: *Bombyx mori* Nuclear polyhedrosis virus (*Bm*NPV) causes nuclear polyhedrosis in silkworm which is one of the dominant viral diseases noticed in almost all sericultural areas in the country. The tolerance in silkworm breeds have physiological and genetic origins that are controlled by polygenes. The present work relates to the evaluation and selection of silkworm breeds tolerant to *Bm*NPV among the germplasm stocks available at Andhra Pradesh state Sericulture Research and Development Institute (APSSRDI), Hindupur. The silkworm breeds were inoculated with the viral suspension of (2 x 10^6/ml) *Bm*NPV PIB's by smearing onto mulberry leaves @ 1ml/100 larvae. Eleven bivoltine oval and nine bivoltine peanut silkworm breeds exhibiting real tolerance to *Bm*NPV infection were shortlisted and further evaluated under three trials. Based on the overall mean performance and economic merit over the three trials, 5 each of bivoltine oval and peanut silkworm breeds were identified as potential resource material for further breeding programme.

Key words: Silkworm germplasm, *Bm*NPV, disease resistance/tolerance, susceptibility.

INTRODUCTION

Nuclear polyhedrosis in silkworm is caused by *Bombyx mori* Nuclear polyhedrosis virus (*Bm*NPV), so- called as 'grasserie', 'Jaundice', 'milky disease' and 'hanging disease' are synonyms for nuclear polyhedrosis. It is one of the dominant viral diseases noticed in almost all sericultural areas in the country. Nuclear polyhedrosis alone accounts for 33-55 % crop loss in Karnataka, India [8]. It is well known that silkworm diseases are the main factors which seriously affect the cocoon production. Rearing of silkworm breeds/hybrids which are resistant/tolerant to diseases is economical where inadequate disinfection methods are practiced. Among the major silkworm diseases, grasserie caused by *Bombyx mori* Nuclear Polyhedrosis Virus (*Bm*NPV) is controlled by polygenes [1]. In order to obtain high and stable cocoon yield it is necessary to reduce the incidence which in turn decrease pathogen load in the environment. Moreover, among many measures of controlling and prevention of silkworm diseases, the utilization of disease resistant varieties is the most economical and effective way in earning better cocoon yield. Larval susceptibility to viral disease greatly differed in various breeds/ hybrids. Many researchers have reported that, inter-breed/strain differences were noticed in susceptibility to *Bm*NPV [15, 16, 1, 17, 5, 7, 9] but very few studies have been carried out on the development of resistance of insects to pathogens [5]. The resistance in silkworm breeds have physiological and genetic origins that are controlled by polygenes [6, 3]. The present work relates to the identification of silkworm breeds tolerant to *Bm*NPV among the germplasm stocks available at Andhra Pradesh State Sericulture Research and Development Institute (APSSRDI), Hindupur and utilized for the selection of potential resource materials for the silkworm breeding programme.

MATERIALS AND METHODS

Propagation of *Bm*NPV: A popular susceptible bivoltine silkworm breed *viz*., NB18 was used to induce nuclear polyhedrosis infection and was per orally inoculated immediately after IV moult with 20,000 *Bm*NPV polyhedra/larvae @ 1ml per 100 larvae by smearing onto mulberry leaf.

Preparation of inoculum: Purified *Bm*NPV polyhedra maintained at APSSRDI, Hindupur were used to prepare the *Bm*NPV inoculum. The *Bm*NPV polyhedra concentration was determined by using Neubar double heamocytometer and suitably diluted to a polyhedral suspension of 1×10^8 polyhedra/ml.

***Bm*NPV polyhedra purification:** *Bm*NPV polyhedra were purified by following the method described by [14]. The heamolymph from *Bm*NPV infected silkworm larvae was collected on ice in the presence of few specks of phenylthiourea, allowed for bacterial degradation for 24 hours and filtered through two layers of cheese cloth. The polyhedra were pelleted by centrifugation at 5000 rpm for 10min at 4ºC. *Bm*NPV polyhedra were washed thoroughly thrice with distilled water and then thrice with 1M NaCl by centrifugation at 5000 rpm for 10min at 4ºC. The polyhedra pellet was suspended in distilled water and then layered on to a cushion 40% of sucrose (w/v) and centrifuged at 15,000 rpm for 15min in a refrigerated centrifuge. The purified polyhedra thus obtained were washed thoroughly with distilled water thrice by centrifugation at 5000 rpm for 10min at 4ºC. The polyhedral pellet was finally suspended in distilled water and stored at 4ºC for further use.

Screening of silkworm germplasm: 70 bivoltine silkworm genetic stocks (40 ovals and 30 peanuts) available at APSSRDI, Hindupur were screened against *Bm*NPV for identifying the *Bm*NPV tolerant and susceptible breeds. The silkworm larvae out of 2nd moult (3rd instar) were used for *Bm*NPV viral stress. On the first day of third instar 300 larvae per each replication were taken and inoculated per orally. The silkworm breeds were inoculated with the virus suspension of (2 x 10^{6}/ml) *Bm*NPV PIB's by smearing onto mulberry leaves @ 1ml/100 larvae by using non-absorbent cotton and fed to the silkworms. The larvae were allowed to feed onto inoculum-smeared leaf for 6 hrs. All the silkworm breeds were reared in three replications. These pure breeds were reared according to standard rearing practices [4]. The *Bm*NPV infection was confirmed by light microscopy of the heamolymph for *Bm*NPV polyhedra. The data on mortality due to nuclear polyhedrosis was recorded up to moth emergence. The degree of tolerance was estimated from the mortality recorded during pupal and moth stages. The relative tolerance was categorized into. a) Apparent tolerance: The inoculated larvae complete larval period and form cocoons, but do not metamorphoses into pupae. b) Real tolerance: The inoculated larvae complete larval period, form cocoons, metamorphoses into pupae and moths emerge from cocoons. c) Susceptibility: The inoculated larvae succumb to death due to nuclear polyhedrosis during larval period itself. The data was statistically analysed for mean, standard deviation (S.D) and co-efficient of variation (CV) to indicate the extent of variation between the replications. Individual Evaluation index (EI) and Subordinate function (SF) values were obtained for each of the selected traits of each breed and the average cumulative index value over the eight characters under study was calculated. The shortlisted silkworm breeds showing real tolerance were evaluated for three trials under *Bm*NPV exposure. Based on statistical analysis by employing EI and SF for the overall mean performance of the three trials, 5 each of bivoltine oval and bivoltine peanut silkworm breeds were selected as potential resource material for further breeding.

RESULTS

Screening of Bivoltine Oval and Peanut Silkworm Breeds: Seventeen bivoltine oval silkworm breeds showed complete mortality during larval stage and twelve silkworm breeds showed apparent tolerance to *Bm*NPV. Eleven bivoltine oval silkworm breeds *viz*., AP02, SDO1, APDR105, APDR117, SPO, SDO5, BBO2, APSR, D43O, APS33, APR exhibiting real tolerance to *Bm*NPV infection were shortlisted and further evaluated under three trials (Table 1).

Table 1: Screening of Bivoltine Oval Silkworm Breeds Tolerant to *Bm*NPV

Breed	Larval Character	Cocoon Colour	Degree of Tolerance (%)		Cocoon Weight (g)	Shell Weight (g)	Shell Ratio (%)	EI	SF
			Pupal Stage	Moth Stage					
BBO3	PW	White	28.33 ± 2.37	0.00 ± 0.00	1.300 ± 0.072	0.231 ± 0.072	17.77 ± 0.12	43.66	2.64
BBO4	PW	White	20.67 ± 245	0.00 ± 0.00	1.315 ± 0.025	0.253 ± 0.025	19.24 ± 0.53	45.46	3.22
APS9	PW	White	47.67 ± 3.55	29.00 ± 5.68	1.343 ± 0.053	0.249 ± 0.053	18.54 ± 0.64	51.70	5.07
SDO1	BW	White	46.33 ± 2.08	30.00 ± 8.52	1.355 ± 0.028	0.251 ± 0.028	18.52 ± 0.35	50.60	4.73
BBO5	BW	White	21.33 ± 1.02	0.00 ± 0.00	1.329 ± 0.025	0.238 ± 0.025	17.91 ± 0.74	44.46	2.89
APDR105	PW	White	57.00 ± 2.3	39.33 ± 7.21	1.408 ± 0.047	0.289 ± 0.047	20.55 ± 0.76	63.90	8.47
TR20	PW	White	28.00 ± 6.25	0.00 ± 0.00	1.342 ± 0.064	0.234 ± 0.064	17.41 ± 0.47	45.69	3.16
APDR117	PW	White	57.33 ± 4.12	44.33 ± 6.25	1.412 ± 0.045	0.283 ± 0.045	20.04 ± 0.85	63.82	8.45
APS67	PW	White	30.33 ± 4.16	0.00 ± 0.00	1.321 ± 0.058	0.248 ± 0.058	18.77 ± 0.44	48.38	3.90
SPO	PW	White	54.33 ± 1.89	40.67 ± 2.68	1.354 ± 0.032	0.277 ± 0.032	20.46 ± 0.52	61.09	7.67
BBO6	BW	White	24.67 ± 2.38	0.00 ± 0.00	1.352 ± 0.047	0.232 ± 0.047	17.16 ± 0.31	44.84	2.96
AC9	BW	White	31.67 ± 2.65	0.00 ± 0.00	1.334 ± 0.081	0.241 ± 0.081	18.04 ± 0.46	45.84	3.25
AP71	BW	White	29.33 ± 1.23	0.00 ± 0.00	1.325 ± 0.042	0.238 ± 0.042	17.96 ± 0.85	44.83	2.94
GPO	BW	White	25.67 ± 7.65	0.00 ± 0.00	1.333 ± 0.017	0.235 ± 0.017	17.63 ± 0.62	42.98	2.50
AP9	BW	White	51.00 ± 3.54	27.33 ± 6.21	1.342 ± 0.065	0.262 ± 0.065	19.52 ± 0.25	54.82	5.89
AP1	BW	White	33.33 ± 1.35	0.00 ± 0.00	1.338 ± 0.043	0.234 ± 0.043	17.52 ± 0.44	46.70	3.41
BBO2	PW	White	41.00 ± 3.27	13.33 ± 1.21	1.381 ± 0.036	0.263 ± 0.036	19.04 ± 0.62	53.87	5.55
APSR	PW	White	53.67 ± 4.16	33.33 ± 5.32	1.417 ± 0.038	0.272 ± 0.038	19.20 ± 0.74	58.09	6.89
D43O	PW	White	57.33 ± 2.54	38.33 ± 6.71	1.401 ± 0.061	0.284 ± 0.061	20.27 ± 0.54	60.54	7.50
871 PO	PW	White	30.67 ± 4.48	0.00 ± 0.00	1.314 ± 0.031	0.243 ± 0.031	18.49 ± 0.39	46.30	3.35
APS9	PW	White	27.67 ± 2.03	0.00 ± 0.00	1.309 ± 0.071	0.241 ± 0.071	18.41 ± 0.11	46.06	3.22
APS33	BW	White	46.33 ± 2.36	28.33 ± 6.37	1.387 ± 0.042	0.269 ± 0.042	19.39 ± 0.27	56.04	6.27
APR	PW	White	55.33 ± 3.24	34.33 ± 7.10	1.399 ± 0.019	0.282 ± 0.019	20.16 ± 0.32	58.45	6.91

* Values represent an average of three replications ± SD.
* PW: Plain White: BW: Bluish White

Among the peanuts sixteen silkworm breeds shown complete mortality during larval stage and five silkworm breeds showed apparent tolerance to *Bm*NPV infection. Nine bivoltine peanut silkworm breeds *viz.*, APS20, NB4D2-B, HNSD, APS8, 4KIN, AP10, AP72, SMPW, CT1PP exhibiting real tolerance to *Bm*NPV infection were shortlisted and further evaluated under three trials (Table 2).

Overall mean performance for all the three trials: The overall mean performance of 11 bivoltine oval and 9 bivoltine peanut silkworm breeds for all the three trials evaluated together for each trait. Considering the economic merit over the three trials, the bivoltine oval and peanut silkworm breeds were further evaluated for confirming their overall superiority and relative merit by employing the Evaluation Index and subordinate function methods. Among oval breeds based on the multiple trait evaluation index method, 5 breeds namely APDR117 (57.51), SPO (56.14), APDR105 (55.02), APR (54.14) and D43O (54.11) recorded top five ranks with cumulative average index values above 50. In the sub-ordinate function method, the cumulative values ranged from the maximum of 8.63 (APDR117) to 5.15 (APS33). The analysis of the data present that the breeds APDR117 (8.63), SPO (7.97), APDR105 (7.87) D43O (7.54), and APR (7.27) were recorded higher values and assigned top five ranks. (Table 3).

Table 2: Screening of Bivoltine Peanut Silkworm Breeds Tolerant to *Bm*NPV

Breed	Larval Character	Cocoon Colour	Degree of Tolerance (%)						Cocoon Weight (g)			Shell Weight (g)			Shell Ratio (%)			EI	SF
			Pupal Stage			Moth Stage													
TR4C	PW	White	28.00	±	0.42	0.00	±	0.00	1.318	±	0.017	0.233	±	0.003	17.68	±	0.22	46.79	3.11
APS20	BW	White	56.33	±	0.96	40.67	±	0.69	1.403	±	0.020	0.281	±	0.012	20.03	±	0.29	61.09	6.75
NB4D2-B	BW	White	48.67	±	0.92	35.67	±	0.57	1.414	±	0.021	0.282	±	0.004	19.94	±	0.23	55.99	5.71
NMPW	BW	White	27.00	±	0.41	0.00	±	0.00	1.361	±	0.023	0.244	±	0.004	17.93	±	0.08	45.80	3.39
HNSD	BW	White	50.67	±	0.86	33.00	±	0.53	1.357	±	0.019	0.266	±	0.003	19.60	±	0.25	50.99	4.37
APS8	BW	White	55.33	±	1.04	37.67	±	0.67	1.403	±	0.022	0.257	±	0.004	18.32	±	0.30	56.40	5.87
KM1	BW	White	30.33	±	0.46	0.00	±	0.00	1.301	±	0.026	0.263	±	0.003	20.22	±	0.32	49.88	3.16
4KIN	BW	White	35.67	±	0.61	21.00	±	0.38	1.322	±	0.027	0.257	±	0.004	19.44	±	0.24	54.17	4.48
AP10	BW	White	48.67	±	0.92	30.00	±	0.51	1.411	±	0.021	0.284	±	0.004	20.13	±	0.15	58.68	6.04
AP72	BW	White	54.67	±	0.83	39.67	±	0.72	1.402	±	0.021	0.264	±	0.003	18.83	±	0.26	55.85	6.14
BBP2	BW	White	24.00	±	0.41	0.00	±	0.00	1.349	±	0.02	0.246	±	0.005	18.24	±	0.16	42.13	2.45
BBP5	BW	White	26.33	±	0.5	0.00	±	0.00	1.300	±	0.019	0.229	±	0.003	17.62	±	0.27	45.33	2.86
SMPW	PW	White	34.00	±	0.51	22.33	±	0.38	1.403	±	0.018	0.285	±	0.004	20.31	±	0.22	52.09	4.54
CTIPP	BW	White	6.33	±	0.58	23.67	±	0.45	1.384	±	0.020	0.271	±	0.004	19.58	±	0.09	49.48	3.91

* Values represent an average of three replications ± SD.

* PW: Plain White; BW: Bluish White

Table 3: Overall Mean Performancefor Three Trials of Bivoltine Oval Silkworm Breeds Tolerant to *Bm*NPV

Breed	Degree of Tolerance (%)						Cocoon Weight (g)			Shell Weight (g)			Shell Ratio (%)			EI	SF
	Pupal Stage			Moth Stage													
APO2	47.33	±	1.30	28.33	±	0.78	1.356	±	0.035	0.264	±	0.007	19.47	±	0.69	49.49	5.46
SDO1	46.78	±	1.06	32.67	±	0.87	1.337	±	0.029	0.241	±	0.006	18.02	±	0.50	51.21	5.25
APDR 105	54.11	±	3.65	33.89	±	2.20	1.430	±	0.089	0.293	±	0.019	20.51	±	1.41	55.02	7.87
APDR 117	57.56	±	4.19	47.11	±	3.43	1.418	±	0.096	0.292	±	0.020	20.59	±	1.49	57.51	8.63
SPO	56.22	±	2.70	41.56	±	1.99	1.379	±	0.061	0.279	±	0.013	20.23	±	0.99	56.14	7.97
SDO5	46.11	±	1.13	21.67	±	0.53	1.360	±	0.030	0.267	±	0.006	19.61	±	0.96	50.23	5.83
BBO2	42.67	±	1.91	18.44	±	1.37	1.392	±	0.067	0.270	±	0.037	19.42	±	1.14	48.76	5.39
APSR	49.67	±	1.13	29.78	±	0.98	1.377	±	0.036	0.256	±	0.006	18.59	±	0.48	49.96	5.20
D43O	54.11	±	0.95	39.22	±	1.75	1.424	±	0.025	0.292	±	0.005	20.49	±	0.36	54.11	7.54
APS33	49.00	±	3.31	30.22	±	1.55	1.369	±	0.098	0.257	±	0.020	18.77	±	1.41	49.58	5.15
APR	57.22	±	3.29	36.22	±	1.94	1.409	±	0.086	0.282	±	0.017	20.01	±	1.17	54.14	7.27

* Values represent an average of three trials ± SD.

Among the peanut breeds, in the Evaluation Index method, 5 breeds namely, APS8 (60.20), APS20 (60.13), AP72 (54.15), NB4D2-B (54.01) and HNSD (52.28) which recorded top five ranks with cumulative average index value above 50 suggest that these breeds possessing economic merit. In the sub-ordinate function method, the cumulative values ranged from the maximum of 8.55 (APS20) to 4.54 (SMPW). The analysis of the data present that the breeds APS20 (8.55), APS8 (7.95), AP72 (7.17), NB4D2-B (6.32), and HNSD (6.06) were recorded higher values and assigned top five ranks (Table 4). Based on the overall mean performance and economic merit over the three trials, 5 each of bivoltine oval (APDR117, SPO, APDR105, D43O and APR) and peanut breeds (APS8, APS20, AP72, NB4D2-B and HNSD) have been identified as potential resource material for further breeding programme

Table 4: Overall Mean Performancefor Three Trials of Bivoltine Peanut Silkworm Breeds Tolerant to *Bm*NPV

Breed	Degree of Tolerance (%)						Cocoon Weight (g)			Shell Weight (g)			Shell Ratio (%)			EI	SF
	Pupal Stage			Moth Stage													
APS20	61.00	±	1.98	49.33	±	1.18	1.421	±	0.046	0.288	±	0.024	20.28	±	0.66	60.13	8.55
NB4D2-B	50.67	±	1.49	36.00	±	1.18	1.441	±	0.048	0.279	±	0.007	19.39	±	0.48	54.01	6.32
HNSD	55.78	±	1.59	38.56	±	1.35	1.363	±	0.050	0.267	±	0.008	19.58	±	0.67	52.28	6.06
APS8	57.33	±	2.48	43.44	±	1.54	1.438	±	0.047	0.290	±	0.011	20.16	±	0.80	60.20	7.95
4KIN	30.78	±	0.68	14.89	±	0.38	1.344	±	0.032	0.267	±	0.008	19.85	±	0.57	49.74	4.76
AP10	43.56	±	1.73	23.89	±	0.60	1.408	±	0.054	0.264	±	0.028	18.75	±	0.76	51.24	5.50
AP72	57.33	±	2.36	42.78	±	1.70	1.396	±	0.070	0.258	±	0.014	18.51	±	0.59	54.15	7.17
SMPW	35.44	±	1.04	21.44	±	0.73	1.390	±	0.017	0.270	±	0.008	19.43	±	0.57	48.85	4.54
CT1PP	36.00	±	1.29	22.33	±	0.70	1.379	±	0.041	0.269	±	0.008	19.48	±	0.67	49.18	4.76

* Values represent an average of three trials ± SD.

DISCUSSION

Bombyx mori Nuclear polyhedrosis virus (*Bm*NPV) causes nuclear polyhedrosis in silkworm which is one of the dominant viral diseases noticed in almost all sericultural areas in the country. The objectives of disease resistance breeding of silkworms is to develop a silkworm race resistant to adverse environmental conditions and epidemic situations. Several silkworm genetic resources in China, Japan, Iran, Thailand and India have been screened for their resistance to silkworm viruses *viz*., *Bm*CPV, *Bm*NPV, *Bm*DNV and *Bm*IFV. Studies on susceptibility of different Indian silkworm races to *Bm*NPV have shown that they vary a great deal in the susceptibility [2]. The results confirm the observations that the silkworm breeds differ in susceptibility to *Bm*NPV [1].

The relative tolerance/ susceptibility to *Bm*NPV among 40 bivoltine oval and 30 bivoltine peanut silkworm breeds was studied based on the real and apparent tolerance levels. 17 bivoltine oval and 16 bivoltine peanut silkworm breeds showed complete mortality during larval stage, 12 bivoltine oval and 5 bivoltine peanut silkworm breeds showed apparent tolerance to *Bm*NPV. The silkworm breeds exhibiting real tolerance includes 11 bivoltine oval and 9 bivoltine peanut silkworm breeds. The status of germplasm stocks to specific pathogens for their relative resistance / tolerance / susceptibility provides an insight into the performance of the parental stocks as well as their hybrids. Various researchers have demonstrated the difference of susceptibility to *Bm*NPV among silkworm [15, 16, 1, 17, 5, 7, 9, 13]. During screening the pupation rate in bivoltine oval silkworm breeds ranged from a maximum of 57.33 ± 4.12 % (APDR117) and 57.33 ± 2.54 % (D43O) to a minimum of 20.67 ± 2.37 % (BBO4) while in peanut silkworm breeds it ranged from 56.33 ± 0.96 % (APS20) to a minimum of 24.00 ± 0.01 % (BBP2). The overall mean performance of the real tolerant breeds under three trials evaluation exhibits highest pupation rate in APDR117 (57.56 ± 4.19%) and lowest pupation rate in BBO2 (39.11 ± 2.25%) respectively in bivoltine oval silkworm breeds. Highest pupation rate was observed in APS20 (61.00 ± 1.98 %) and lowest in 4KIN (30.78 ± 0.68 %) in bivoltine peanut silkworm breeds. Among the top five ranked oval breeds the difference in mortality between pupation rate and moth emergence percentage was observed highest in APR (21.00%), followed by 20.22% (APDR105), 14.89% (D43O) and 14.67% (SPO) and 10.44% (APDR117) respectively. Among the top ranked peanut breeds, highest in AP10 (19.67%), followed by 17.22% (HNSD), 14.67% (NB4D2-B), 13.78% (AP72) and 11.67% (APS20) was observed respectively. In the present study the differences in mortality due to *Bm*NPV among the silkworm breeds corroborates with various studies on inter-strain/breed differences in susceptibility, or relative tolerance/resistance to *Bm*NPV, *Bm*CPV, *Bm*IFV and *Bm*DNV [17, 5, 9, 10, 12, 13, 11]. The breeds showing lesser difference in mortality due to *Bm*NPV reveals that these breeds possess comparatively higher degree of tolerance and can be utilized as potential resource material for further breeding programme.

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