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Inheritance of Resistance to Nuclear Polyhedrosis Virus in Silkworm, *Bombyx mori*

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Inheritance pattern of resistance to *Bombyx mori* nuclear polyhedrosis virus (BmNPV) was studied in an Indian silkworm stock TX by single back-cross test method. The resistant parent [TX], susceptible parent [HM], their F1, F2, and F1 progeny back-crossed to TX [BC(R)] and HM [BC(S)] were inoculated *per os* with a fixed concentration of BmNPV (0.5 x 10^7 PIB/ml) on the first day of second stadium. The cumulative mortality was recorded until day 10^{th} post-inoculation. The results show that the resistance to BmNPV in TX follow mono Mendelian inheritance pattern. The resistance dominated over the susceptibility at F1. At F2 , the resistant and susceptible offspring segregated in 3:1 ratio whereas at BC(S), the resistant and susceptible offspring segregated in 1:1 ratio. The response of BC(R) was more or less like the resistant parent TX which confirms the involvement of a major dominant gene conferring resistance to BmNPV in TX. The possible mechanism of inheritance of resistance in TX is discussed.

Key words : Inheritance, Resistance, Susceptibility, Dominant gene, Nuclearpolyhedrosis, *Bombyx mori*

Introduction

Virus susceptibility of silkworm, although varies depending upon the internal and external environmental factors, the fundamental resistance is determined genetically (Watanabe, 1986). Earlier work in our laboratory has shown the existence of genetic variability among the germplasm stock of silkworm *Bombyx mori* in response to *Bombyx mori* nuclear polyhedrosis virus (BmNPV) (Sen *et al.*, 1997). A low yielding non-diapausing stock known as TX was found to be highly resistant to BmNPV. Such resistant stocks could be utilized as a donor parent in breeding of silkworm for disease resistance. The knowledge of the mode of inheritance of resistance is essential before utilizing such stocks for breeding work. Aratake (1973) and Watanabe (1986) have reported that resistance to BmNPV is controlled in general, by polygenes. However Zhiqhi Meng (1982) has shown that in a Chinese breed Lan wu, a single major effect gene with a few minor effect genes present in Z chromosome controls the resistance to BmNPV. No information on the inheritance of resistance in Indian silkworm stock is available. The present study is an attempt to determine the mode of inheritance of resistance to BmNPV in Indian silkworm stock.

Although there are several methods to determine the inheritance pattern, single back cross test has been used widely to distinguish between resistance controlled by a single locus (mono-genic) and the resistance controlled by more than one locus (poly-genic) owing to its simple and less time-consuming approach (Georghiou, 1969; Tabashnik, 1991). In the present study, the inheritance of resistance to BmNPV has been determined by single back cross test.

Materials and Methods

Silkworm Parental Stock

A low yielding (12-14% cocoon shell ratio), non-diapausing stock namely TX which spins turmeric yellow coloured, spindle shaped cocoon and has about 25 fold more LC_{50} value for BmNPV as compared to susceptible stock has been selected as resistant parent [R]. Another low yielding (13-14% cocoon shell ratio), non-diapausing stock namely Hosa Mysore [HM] which spins greenish yellow coloured, spindle shaped cocoon and very sensitive to BmNPV has been selected as susceptible parent [S].

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Table 1. Progenies raised from the resistant [TX] and susceptible [HM] silkworm stocks

Genetic Cross	Progeny
TX x HM	F_1
HM x TX	RF_1
(TX x HM) x (TX x HM)	F_2
(TX x HM) x HM	BC(S)
(TX x HM) x TX	BC(R)

Table 2 shows the characteristics of these two stocks. The progenies, which were raised utilizing these two parental stocks are shown in Table 1.

BmNPV inoculum

BmNPV was extracted from the haemolymph of infected larvae and purified by repeated washing and centrifugation (Govindan *et al.*, 1998) followed by sucrose gradient centrifugation using Hitachi ultracentrifuge. The concentration of the stock solution was determined by counting polyhedral inclusion bodies (PIB) on Neubauers chamber slide under Leitz diaplan microscope. The stock solution of virus inoculum was diluted to desired concentrations by adding autoclaved distilled water. The concentration is expressed as PIB/ml.

Inoculation with BmNPV

The larvae from the pooled population of 10-12 disease free laying were taken for inoculation in case of parental stocks and their F1 hybrids, as all the individuals are supposed to be genetically identical. In case of F2 and back cross progeny, all the larvae hatched from a full brood laid by one female moth was taken for the experiment since the resistant and the susceptible individuals are supposed to be segregated in one brood of F2 and back cross progeny.

The larvae from the parents and their progeny were inoculated with BmNPV along with the first feed in second stadium. Fresh mulberry leaf cut in to a size of 6.2 cm diameter was smeared with 200 μl of BmNPV suspension of 0.5 x 10^7 PIB/ml concentration with the help of a flat tipped glass rod. A cyclomixer was used to mix the inoculum while pipetting out the aliquot. A group of 50 larvae were fed with one leaf disc and reared in a plastic box of 100 x 80 cm size with perforated lid. The temperature and humidity of the rearing room was maintained at 27 ± 1°C and 85 ± 5% relative humidity. After 24 hrs of inoculation, the larvae were fed with fresh mulberry leaf twice a day for 10 days. The symptoms of the disease were apparent from 72 hrs onwards after inoculation. Terminally infected larvae were counted. The cumulative mortality due to infection was recorded on 10^{th} day post inoculation. The experiment was done thrice to confirm the results.

Statistical Analysis

Chi square test (Snedecor and Cocharan, 1980) was employed to determine the deviations between the observed and the expected mortalities. The expected mortalities in F2, BC(S) and BC(R) progeny were calculated based on the observed mortalities of the parents and their F1 hybrid assuming mono-genic inheritance pattern (Tabashnik, 1991). The data of three determinations were

Table 2. Characteristics of parental stocks (mean of six rearings ±SD)

Stock	Eggs/ laying	Larval period Days:Hrs	Weightt of 10 mature larvae (g)	Yield/10,000 larvae		Cocoon Weight (g)	Shell Weight (g)	Shell ratio %	LC_{50} for BmNPV (PIB/ml)
				No.	Wt (Kg)				
TX	380±25	22:00±0:12	28.46±1.95	8161±746	9.100±1.62	1.120±0.08	0.154±0.02	13.83±0.53	$3.0x10^7$
HM	460±40	21:00±0:20	35.46±2.22	9063±529	12.89±2.61	1.320±0.04	0.186±0.01	14.10±0.81	$1.2x10^6$

Table 3. Chi square analysis for the assessment of inheritance of resistance to BmNPV (mean of three determinations ±SD)

Test material	No. of larvae inoculated	No. of larvae died	No. of larvae survived	Mortality %	Survival %	Chi square	Homogeneity Chi square
TX (R)	600±0.00	129.00±49.56	470.67±31.87	21.55	78.44	-	-
HM (S)	600±0.00	520.00±119.75	80.00±66.50	86.66	13.33	-	-
F1 (RxS)	600±0.00	123.00±97.04	476.66±91.50	20.55	79.44	-	-
RF1(SxR)	600±0.00	130.00±104.00	470.33±98.55	21.61	78.38	-	-
F2	1,306±177	485.66±116.64	820.33±222.64	37.18	62.81	0.0195 (NS)	4.94 (NS)
BC(S)	1,355±219	708.00±142.06	647.33±237.55	52.25	47.75	1.0058 (NS)	1.74 (NS)
BC(R)	1,336±313	263.66±93.03	1,072.00±331.38	19.74	80.26	1.330 (NS)	0.32 (NS)

Parent	TX Rnp/Rnp 21.555%		X ↓		HM $+^{Rnp}/+^{Rnp}$ 86.666%	**Expected mortality**	**Observed mortality**
F1			Rnp/$+^{Rnp}$ 20.555%				
F2	25% like TX Rnp/Rnp 5.388%	+ +	50% like F1 Rnp/$+^{Rnp}$ 10.277%	+ +	25% like HM $+^{Rnp}/+^{Rnp}$ 21.666%	=37.331%	37.187%
BC(S)		50% likeF1 Rnp/$+^{Rnp}$ 10.277%	+ +	50% likeHM $+^{Rnp}/+^{Rnp}$ 43.333%		=53.610%	52.251%
BC(R)		50% likeF1 Rnp/$+^{Rnp}$ 10.277%	+ +	50% likeTX Rnp/Rnp 10.777%		=21.054%	19.740%

Fig. 1. Segregation of resistant and susceptible offspring in F2 and backcross progeny on the assumption of monogenic inheritance of resistance to BmNPV in *Bombyx mori.*

subjected to homogeneity Chi square test in order to check the deviations, if any, between the determinations.

Results

Since the homogeneity Chi square test revealed no significant deviation, the average of the three determinations was taken for the calculation. The response of parents, their reciprocal F1 hybrid and back cross progeny to a fixed concentration of BmNPV (0.5 x 10^7 PIB/ml) is shown in Table 3. The resistant parent TX has recorded a mortality of 21.555% while in case of susceptible parent HM, the value was 86.666%. The F1 and its reciprocal (RF1) registered the mortality of 20.50% and 21.611% respectively. The F2 progeny has recorded a mortality of 37.187% whereas the same was 52.251% in case of BC(S) and 19.740% in case of BC(R).

The Chi square values calculated on the assumption of a major dominant gene controlling the resistance to BmNPV with the segregation of resistant and susceptible offspring in 3:1 ratio for F2 and 1:1 ratio for BC(S) progeny showed that the deviation between the observed and the expected mortality in F2, BC(S) and BC(R) progeny was insignificant. Further, the homogeneity Chi square values were also found to be insignificant (Table 3).

Discussion

In the present study we investigated the inheritance of resistance to BmNPV in silkworm *Bombyx mori*. Fig. 1 is a diagramatic representation of the genotype of the parents, their F1 and the segregating population of F2 and backcross progeny drawn on the basis of the hypothesis of mono-genic inheritance (Tabashnik, 1991). We consider that the resistant parent TX is homozygous for the locus Rnp (resistant to nuclear polyhedrosis) and the susceptible parent Hm is homozygous for the alternate allele $+^{Rnp}$. The observed mortality of TX (Rnp/Rnp) was 21.555% and HM ($+^{Rnp}/+^{Rnp}$) was 86.666%. The observed mortality in F1 (Rnp/$+^{Rnp}$) was 20.50% which is very close to the mortality of TX suggesting the dominance of the locus Rnp. As the mortality of F1 hybrid in reciprocal cross between TX and HM did not differ significantly, maternal effect is not evident. In F2, which is a self cross of F1, the expected mortality is calculatd as the sum of the mortalities of 25% of the population resembling the resistant parent TX, 50% of the population resembling F1 and another 25% of the population resembling the susceptible parent HM. The total expected mortality comes to 37.331% (487.543 larvae).This data is very close to the observed mortality of 37.187% (485.666 larvae) in F2 hybrid. Chi square value being 0.195 which shows that the deviation between the observed and the expected mortalities in F2 is insignificant.

Similarly in BC(S) progeny, the expected mortality is the sum of the mortalities of 50% of the population which resemble F1 hybrid and another 50% of the population which resemble the susceptible parent HM. Thus the total expected mortality comes to 53.610% (726.415 larvae) which is very close to the observed mortality of 52.251%

(708 larvae). The Chi square value being 1.006, showing the insignificant deviation between the observed and the expected mortalities in BC(S) progeny.

The expected mortality in BC(R) progeny is the sum of the mortalities of 50% of the population resembling F1 hybrid and another 50% of the population resembling the resistant parent TX. Thus the total expected mortality comes to 21.054% (281.116 larvae) which is very close to the observed mortality of 19.740% (263.666 larvae). The Chi square value being 1.330, again showing the insignificant deviations between the observed and the expected mortality in BC(R) progeny. Since, some difference was observed in the mortalities of the parents and their progeny in three determinations, homogeneity Chi square analysis was employed to check if the deviations are statistically significant. The homogeneity Chi square values for F2, BC(S) and BC(R) progeny were found to be 4.941, 4.816 and 3.762 (Table 3) respectively. These values indicate that the deviations observed in three experiments conducted in different times are statistically insignificant.

Thus, the analysis of the mortality data of the parents, F1, F2 and backcross progeny in response to BmNPV clearly suggests the possibility of a major effect dominant gene conferring resistance to BmNPV in TX. The results have confirmed for the first time, the existence of monogenic control of BmNPV resistance in Indian silkworm stock as against the poly-genic inheritance, presumed earlier. The findings of this study will greatly help in designing appropriate breeding protocols for development of BmNPV resistant hybrids by intro-gressing the resistant gene from TX into the background of productive silkworm stocks which are susceptible to BmNPV.

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