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Resistance to *Bombyx mori* Densonucleosis Virus Type 1 and Its Inheritance in Silkworm, *Bombyx mori* L.

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***Bombyx mori* densonucleosis virus type 1 (BmDNV1) - a non occluded virus causes flacherie disease in the susceptible stocks of the silkworm, *Bombyx mori*. However, some stocks are non-susceptible. Non-susceptibility to BmDNV1 in *B. mori* is a unique case where the virus infection is completely inhibited by a single gene of the host. A survey conducted by this institute in some parts of Karnataka state has revealed that, 43.05% of the total incidence of flacherie disease caused by non-occluded viruses, are due to the synergistic infection of *B. mori* densonucleosis and infectious flacherie virus. Earlier study indicated that rearing of BmDNV1 resistant silkworm stock is effective in protecting silkworm against BmIFV also. In the present study the response of 78 silkworm stocks which include 42 of non-diapausing and 36 of diapausing groups, to BmDNV1 is investigated. Newly ecdysed third instar larvae were inoculated *per-os* with 10% inoculum of BmDNV1 extracted from the mid-gut of infected silkworm. One non-diapausing and three diapausing silkworm stocks were found to be resistant to BmDNV1. Eleven silkworm stocks were found to possess moderate resistance whereas rest sixty three were found to be susceptible to BmDNV1. Genetic analysis has shown that the resistance to BmDNV1 is autosomally inherited and controlled by a major dominant or a major recessive gene in different silkworm stocks. These resistant stocks can be utilized as the resource material to develop BmDNV1 resistant commercial hybrids. The selection strategies, depending upon the mode of inheritance of resistance in the resource material chosen, are discussed.**

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Introduction

Development of silkworm stocks with resistance to important diseases is the need of the hour in the country like India where maintenance of germfree environment in rearing site is very difficult if not impossible. Avoidance of extreme susceptibility to important diseases is the major objective of any silkworm breeding programme now. Utilization of silkworm stocks, resistant to various pathogens in breeding programme is essential to develop genetically stable resistant silkworm hybrids. It is rather easy to transfer the resistance from silkworm stocks in which the resistance to a disease is controlled by a single gene as compared to polygene. The flacherie disease caused by *Bombyx mori* densonucleosis virus type1 (BmDNV1) is one such disease in which the resistance has been reported to be inherited by a single gene.

BmDNV1 multiplies in the nuclei of columnar cells of the mid-gut epithelium of silkworm, *B. mori* (Watanabe *et al.*, 1976; Maeda and Watanabe, 1978; Watanabe and Kurihara, 1988). The larvae infected with BmDNV1 show the major sign of the flaccid body and die in about 8 – 10 days after the virus inoculation (Watanabe and Kurihara, 1988). The infected columnar cells with hypertrophied nuclei are readily discharged into the gut lumen and the alimentary canal is found to be pale yellow in colour and almost devoid of any contents (Watanabe *et al.*, 1976; Maeda and Watanabe, 1978; Abe *et al.*, 1993).

Non-susceptibility to BmDNV1 infection was found to be controlled by a single recessive gene, *nsd-1* (Watanabe and Maeda, 1981) or a dominant gene, *nid-1* (Eguchi *et al.*, 1986). Thus it makes a unique case where infection is completely inhibited by a single gene of the host animal

(Watanabe, 1994). Therefore it may be possible to breed BmDNV1 resistant silkworm hybrids by introducing the resistant gene into a productive, but otherwise susceptible, parental stocks.

It is reported that BmIFV (*Bombyx mori* infectious flacherie virus) and BmDNV1 are synergistically infectious to BmDNV1 susceptible silkworm stocks (Watanabe and Shimuzu, 1981), therefore rearing of resistant stocks to BmDNV1 may be effective for protection against BmIFV infection too (Watanabe, 1994). But before taking up any breeding programme in this direction, it is essential to assess the breeding resource materials for resistance to BmDNV1. Knowledge of the genetic basis of resistance is especially important for the breeder. With this objective we have screened seventy eight silkworm stocks of our germplasm for resistance to BmDNV1. The mode of inheritance of resistance was also studied by single back cross test and log concentration probit regression analysis.

Materials and Methods

Silkworm stock

Seventy-eight silkworm stocks, presently maintained in silkworm breeding and genetics division of Central Sericultural Research and Training Institute, Mysore were utilized in this study. Out of 78 stocks, 42 were non-diapausing (multivoltine) and 36 were diapausing (bivoltine) at embryonic stage. The larvae collected from a pool of 8 – 10 disease free laying of each silkworm stock were screened for resistance to BmDNV1.

BmDNV1 inoculum

BmDNV1 was extracted from the mid-gut of infected larvae and checked serologically against the antiserum of BmDNV1. Inoculum at a dilution of 10^{-1} (10%) was utilized for the screening. The stock solution was serially diluted with sterilized distilled water to make different concentration and used for Probit analysis.

Inoculation with BmDNV1

Hundred larvae from each silkworm stock were fed with mulberry leaf smeared with 500 µl of the inoculum on the first day of third instar for the screening of germplasm. While pipetting out the aliquot, the inoculum was continuously stirred using a cyclomixer. After 24 hrs, the larvae were transferred on fresh uncontaminated mulberry leaf and fed twice a day. The larvae were reared at 25 ± 1°C temperature and 75 ± 5% relative humidity for 10 days during which the symptoms of the disease was observed very carefully and the apparently diseased larvae were checked serologically utilizing antiserum of BmDNV1. An equal number of larvae from each batch were reared simultaneously as control and the data of mortality, if any, caused due to the reason other than the pathogen in question was corrected by using Abbots formula (Finney, 1971).

Serological diagnosis of BmDNV1

The serological diagnosis was based on a micro gel diffusion method, performed on a microscope slide covered with a layer of 1% agarose solution. The template formed at a tetragonal arrangement with a central well, in which the antiserum of BmDNV1 was placed. The peripheral wells contained a suspension of macerated mid-gut from each test larva. The slide was kept at 37°C for two days in a humid box, the bottom of which was layered with a wet filter paper to prevent desiccation. Infection with BmDNV1 was confirmed by the formation of precipitin line between the BmDNV1 antiserum and mid-gut suspension.

Inheritance pattern of resistance to BmDNV1

The inheritance pattern of the resistance to BmDNV1 was determined by single back-cross test (Tabashnik, 1991) and log-concentration probit regression analysis by utilizing 3 resistant silkworm stocks namely C. Nichi, 644 and A and a susceptible stock NB_4D_2. To minimize the heterogeneity, if any, the selected parental stocks were subjected to a programme of inbreeding for 5 consecutive generations and selection before the commencement of the main experiment. The resistant [R] and susceptible [S] parents, their reciprocal F_1, F_2 and F_1 hybrids back-crossed with resistant [$F_1 \times R$ = BCR] and susceptible [$F_1 \times S$ = BCS] parents were inoculated simultaneously with 6 concentrations of BmDNV1. The cumulative mortality due to infection was recorded on the 10^{th} day post inoculation. The infection was confirmed by serological diagnostic test as mentioned above. The data generated from the inoculation with only one concentration (10^{-1}) was utilized to determine the inheritance pattern of resistance by single back-cross test method, in which the expected mortalities for the F_2 and back-cross progeny were calculated assuming single locus inheritance. Observed and expected mortalities in F_2 and back-cross progeny were compared with chi square test. The data obtained from all the six concentrations of BmDNV1 inoculum was subjected to log-concentration-probit regression analysis to confirm the single locus inheritance of the resistance. The relationship between the concentrations of BmDNV1 (X values) and the mortalities converted to probit (Y values) of the parents, F_1, F_2 and back-cross progeny were seen using a software, Curve fit.

Results

Susceptibility status

Table 1 enlists the silk worm stocks which were screened for resistance to BmDNV1. Out of 78 stocks tested, only 4 stocks namely C. Nichi, A, 644 and NJ were found to be resistant where the mortality was in the range of 10 to 22%. Eleven stocks namely Sarupat, GNP, GNM, BL37, 96C, KA, 1HT, B61, 44(B)M, NJ3 and Hu204 were found to have moderate resistance where the mortality was in the range of 40 to 65% and remaining 63 stocks were susceptible in which the mortality was above 80% when challenged with BmDNV1 at the dilution of 10^{-1}.

Mode of inheritance for resistance to BmDNV1

Table 2 shows the mortality % of resistant (C. Nichi, A and 644) and susceptible (NB_4D_2) silkworm stocks and their progeny in response to a fixed concentration (10^{-1}) of BmDNV1 (results obtained in a separate determinations than that of screening). In resistant stocks the mortality ranged from 8 to 12% whereas in susceptible stock it was 100%. The F_1 hybrids of resistant and susceptible parent (R × S) have registered 8% mortality in case of C. Nichi and 644 whereas in case of A, the same was 99%. In reciprocal cross (S × R) of F1 hybrid, the mortality was 12 and 6% in C. Nichi and 644 respectively but in A it was 95%. In F_2 progeny, virus infection was to the tune of 25.56% in C. Nichi and 26.31% in 644, while 72.29% was infected in stock A. In BCS progeny of C. Nichi, 644 and A, the mortality was of the order of 48.37%, 48.91% and 96.95% respectively. The mortalities of BCR progeny were only 8.92% and 11.18% in C. Nichi and 644 respectively but in case of A, it was 50.85%. The chi square test conducted to compare the observed and expected mortalities in F_2 and back-cross progeny has shown an insignificant deviations (Table 2).

Log concentration probit regression analysis

Fig. 1, 2 and 3 show the log concentration- probit curve

Table 1. Response of silkworm germplasm stocks to BmDNV1

Sl. no.	Stock	Mortality %	Sl. no.	Stock	Mortality %	Sl. no.	Stock	Mortality %
1	PM	100	27	BL44	99	53	CSR4	100
2	Nistari	100	28	BL45	98	54	CSR5	100
3	Sarupat	65	29	BL51	45	55	CSR12	100
4	Moria	89	30	BL53	99	56	CSR13	100
5	Cambodge	86	31	BL54	99	57	NB1	80
6	C. Nichi	16	32	BL61	100	58	1HT	65
7	A 4e	73	33	BL62	100	59	2HT	89
8	MR1	89	34	BL65	98	60	A60	100
9	GNP	60	35	BL67	99	61	A62	99
10	GNM	56	36	BL68	100	62	A63	99
11	H.M.	86	37	BL69	100	63	A70	95
12	TN White	90	38	96A	100	64	A71	100
13	KW 2	96	39	96C	50	65	B61	53
14	PA 12	98	40	96E	99	66	B63	87
15	BL 22	98	41	96H	100	67	B71	100
16	BL 23	99	42	96N	86	68	MBN2	100
17	BL24	100	43	RD1	90	69	44(B)M	49
18	BL27	100	44	HU204	52	70	NJ	22
19	BL28	100	45	AUZ5	80	71	NJ3	53
20	BL30	100	46	Daizo	100	72	Dong 306	71
21	BL33	100	47	NB4D2	100	73	644	10
22	BL34	100	48	KA	40	74	I-1	100
23	BL35	100	49	IBF	80	75	CDC2	100
24	BL36	100	50	DR1	100	76	B1	100
25	BL37	53	51	A	12	77	7042	100
26	BL43	100	52	CSR2	100	78	KDC	100

Table 2. Inheritance of BmDNV1 resistance in silkworm

Breed/progeny	No. of larvae inoculated	Mortality		Survival		Degree of freedom (n-1)	Chi square
		Observed no.	Expected no.	Observed no.	Expected no.		
NB_4D_2 [S]	100	100	-	0	-	-	-
C. Nichi [R]	100	11	-	89	-	-	-
F1	100	8	-	92	-	-	-
RF1	100	12	-	88	-	-	-
F2	536	137	134	399	402	1	0.089*
BCR	493	44	54	449	439	1	2.079*
BCS	523	253	262	270	261	1	0.620*
644 [R]	100	10	-	-	-	-	-
F1	100	8	-	-	-	-	-
RF1	100	6	-	-	-	-	-
F2	532	140	133	392	399	1	0.490*
BCR	626	70	62	556	564	1	1.145*
BCS	595	291	298	304	297	1	0.328*
A [R]	100	8	-	-	-	-	-
F1	100	99	-	-	-	-	-
RF1	100	100	95	-	-	-	-
F2	581	420	436	161	145	1	2.35*
BCR	590	300	295	290	295	1	0.168*
BCS	557	540	557	17	0	1	0.518*

[S = Susceptible; R = Resistant; F1 = R × S; RF1 = S × R; BCR = F1 × R; BCS = F1 × S]
*Non-significant ($p < 0.05$).

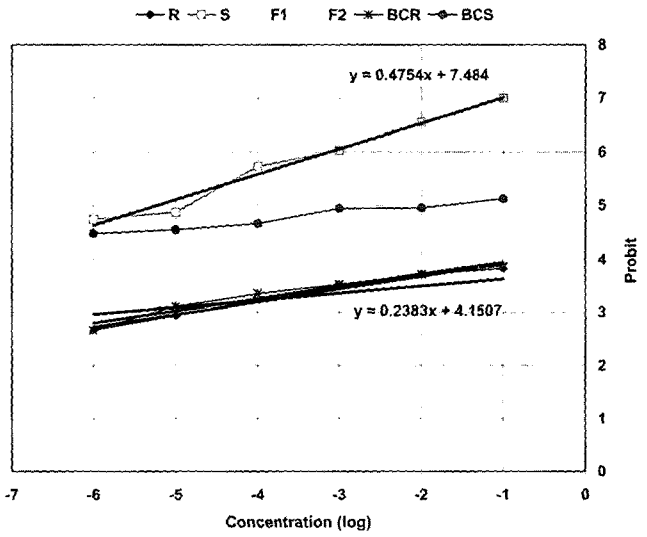

Fig. 1. Concentration (*Bombyx mori* densonucleosis virus) - probit regression for the resistant strain C. Nichi [R], susceptible strain NB_4D_2 [S], the F1 and F2 hybrids between the resistant and susceptible strain and the back-crossed F1 hybrids to the resistant [BCR] and susceptible [BCS] parent strains.

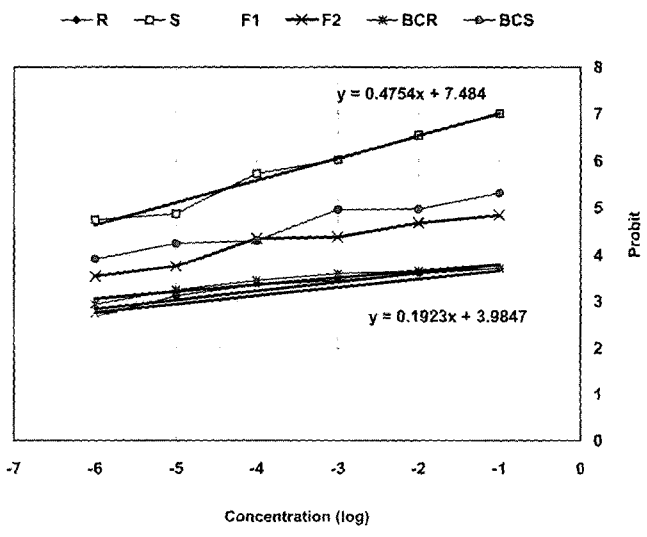

Fig. 2. Concentration (*Bombyx mori* densonucleosis virus) - probit regression for the resistant strain 644 [R], susceptible strain NB_4D_2 [S], the F1 and F2 hybrids between the resistant and susceptible strain and the back-crossed F1 hybrids to the resistant [BCR] and susceptible [BCS] parent strains.

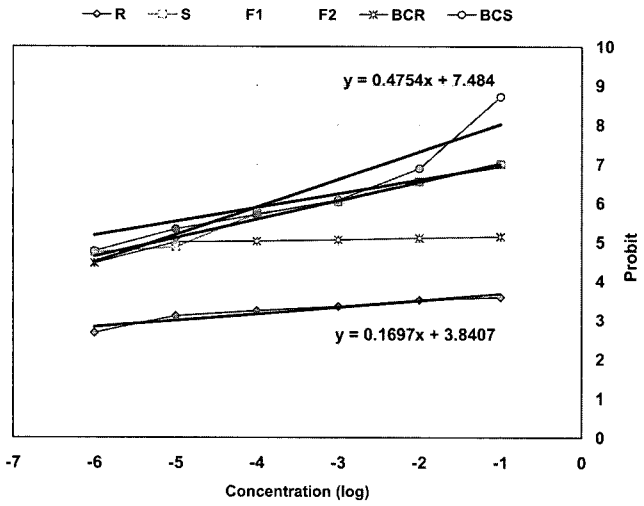

Fig. 3. Concentration (*Bombyx mori* densonucleosis virus) - probit regression for the resistant strain A [R], susceptible strain NB_4D_2 [S], t he F1 and F2 hybrids between the resistant and susceptible strain and the back-crossed F1 hybrids to the resistant [BCR] and susceptible [BCS] parent strains.

drawn for the resistant (C. Nichi, 644, A) and susceptible (NB_4D_2) parental stocks and their progeny. A linear relationship was observed between the log concentrations (X values) and mortalities converted to probit (Y values) in the parents, F_1, BCR in case of C. Nichi and 644 and BCS in A whereas a non-linear relationship was observed in F_2, BCS in C. Nichi and 644 and BCR in A. In C. Nichi and 644, the slope of the linear curve was more or less same for parents, F_1 and BCR while in F_2, the non-linear curve formed a plateau at 25% mortality level (probit 4.32). In BCS the non-linear curve formed a plateau at 50% mortality level (probit 5.00), whereas in case of A, the slope of the linear curve of F_1 and BCS were more or less similar to that of the susceptible parent NB_4D_2. In F_2 the non-linear curve formed a plateau at around 75% mortality level (probit 5.67) and in BCR the plateau was observed at 50% mortality level.

Discussion

Report of Watanabe (1994) has revealed that most of the silkworm stocks of Japan were non-susceptible when inoculated per orally with BmDNV1 while some stocks were highly susceptible to BmDNV1 infection. Contrary to this report our results show only 4 out of 78 silkworm stocks tested, were resistant to BmDNV1 at a dilution of 10^{-1}. A few silkworm stocks, which exhibited moderate resistance, may be in heterozygous state for the allele conferring resistance to BmDNV1.

The response of reciprocal F_1 hybrids to BmDNV1 in C. Nichi and 644 shows that in these two stocks the resistance is dominant over the susceptibility but in A the resistance is dominated by the susceptibility (Table 2). Since no appreciable difference in the mortality of the reciprocal hybrids was observed, maternal effect on resistance was not evident. This result indicates that the genetic basis of resistance to BmDNV1 is autosomal rather than sex-linked. This finding is in agreement with the earlier reports of Watanabe and Maeda (1981) and Eguchi *et al.* (1986).

Single backcross test showed that the resistant and susceptible offspring in F_2 population of C. Nichi and 644 segregated in roughly 3 : 1 ratio and in BCS in 1 : 1 ratio (Table 2). In BCR progeny of C. Nichi and 644, the response was found to be resembling to that of the resistant parents. This clearly indicates that a major dominant gene confers the resistance to BmDNV1 in C. Nichi and 644 (Tabashnik, 1991).

Contrary to these two silkworm stocks, in A, the resistant and susceptible offspring in F_2 population segregated in roughly 1 : 3 ratio. Almost all the offspring of BCS progeny succumbed to BmDNV1 infection, just like the susceptible parent. In BCR, the segregation of resistant and susceptible offspring was in 1 : 1 ratio. This indicates that a major recessive gene confers the resistance to BmDNV1 in the silkworm stock A (Tabashnik, 1991).

The linear relationship between the log concentrations (X values) and the mortalities converted to probit (Y values) in the resistant and susceptible parents, their F_1 and BCR of C. Nichi (Fig. 1) and 644 (Fig. 2) and BCS of A (Fig. 3) clearly indicates that the population in these progeny are homogenous but the same is not the case in F_2 of the three stocks, BCS of C. Nichi (Fig. 1) and 644 (Fig. 2) and BCR of A (Fig. 3). Here the plateaus at certain mortality levels indicate that the population in these progeny are heterogenous (Watanabe, 1986). The plateau at 25% mortality level (probit 4.32) in F_2 population of C. Nichi (Fig. 1) and 644 (Fig. 2) and at 75% mortality level (probit 5.67) of A indicates that the resistant and susceptible offspring in F_2 segregated in 1 : 3 ratio in C. Nichi and 644 and in 3 : 1 ratio in stock A. Formation of a plateau at 50% mortality level (probit 5) in the BCS population of C. Nichi (Fig. 1) and 644 (Fig. 2) and in BCR population of A (Fig. 3) indicates that the resistant and susceptible offspring segregated in 1 : 1 ratio. Since the resistant and susceptible offspring at F_2 and backcross progeny segregated in a ratio which fit in the mono-Mendelian inheritance pattern, it can be concluded that a major dominant gene confers the resistance to BmDNV1 in C. Nichi and 644 whereas a major recessive gene is responsible for resistance to BmDNV1 in the stock A.

The present findings of genetic analysis are in agreement with the previous reports where some silkworm stocks were found to possess a dominant gene, *nid-1* (Eguchi *et al.*, 1986) and others were found to possess a recessive gene *nsd-1* which confers the resistance to BmDNV1.

Eguchi *et al.* (1998) have successfully introduced the dominant resistant gene *nid-1* from a low yielding silkworm stock namely No. 908 into a productive but susceptible stock, N 150. The economic traits were improved in the resultant stock by recurrent backcross with N 150. Similarly the resistant stocks C. Nichi, 644 and A can be utilized to develop the parental stocks of resistant hybrids. It is needless to mention here that if the breeding resource material is a stock in which the resistance is controlled by a recessive gene, the resistance should be introduced in both the parents in order to develop the resistant hybrid. And if the resource material is a stock in which the resistance is controlled by a dominant gene, introduction of resistance in only one parent can bring the character into the hybrid. Of course during the breeding process, breeder has to carefully choose the selection strategy based on the genetic make-up of the breeding resource material in order to pick up the resistant population from the segregating progeny.

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