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INHERITANCE IN SELECTED BIVOLTINE SILKWORM BREEDS RESISTANT TO NUCLEAR POLYHEDROSIS VIRUS (BmNPV).

T.S. SOWMYASHREE, P. SUDHAKARA RAO AND B. NATARAJU
CENTRAL SERICULTURAL RESEARCH AND TRAINING INSTITUTE,
SRIRAMPURA, MYSORE-570 008, INDIA.
(e-mail : sowmyashreetss@yahoo.com)

The knowledge of mode of inheritance of resistance in silkworm is essential before utilizing such stocks for breeding work. The popular bivoltine breeds such as CSR18 and 61N and one susceptible bivoltine breed CSR2 were selected for the study and crosses were made between Resistant × susceptible (R × S) and their hybrids. The resistant and susceptible parents are crossed and F_1 is prepared, their reciprocal F_1 (R S and S R), F_1 and F_2 hybrids back crossed with resistant (F_1 R = BCR) and susceptible (F_1 S = BCS) parents were inoculated with a fixed LC_{50} concentration of BmNPV. In the present study, the results have shown that NPV resistance is controlled by four dominant genes , N1, N2, N3 & N4 and each is contributing about 25% resistance (tolerance). CSR18 and 61N is having genetic constitution of N1, N2, N3 & N4 having 50% resistance and CSR2 has n1, n2, N3 and n4 with 25% tolerance . F_1 will have a genetic constitution of N1, N2, N3 & n4 with 75% tolerance. In F_2 and F_1, the expected survival percentage is calculated as a total survival percentage of the population of resistant breeds CSR18 and 61N and F_1 population of susceptible breed CSR2. The total observed survival percentage of F_2 in resistant breeds comes to 74.17 & 66.67 which is very close to the expected survival percentage 75.00. The chi square value calculated based on the observed and expected numbers. Thus, the observed values in F_1 are insignificant and deviating from the expected values, thus strengthen the hypothesis to be a major dominant gene in the resistant breeds. The present study is an attempt to determine the mode of inheritance of resistance to BmNPV in Indian silkworm stocks for using them in future breeding programme.

Key words: Silkworm breeds, bivoltine , BmNPV, resistance, susceptible, Inheritance

INTRODUCTION

An understanding of the inheritance of character is of fundamental significance in the study of evolution and in the application of genetics to animal and plant breeding. Based on the two fields, the quantitative genetics received the chief impetus for its growth. Quantitative genetics is mainly concerned with the inheritance of differences between individuals witch are quantitative rather than qualitative. Qualitative difference between individuals which are inherited depends on the genes whose effects are meager with respect to the variations caused from other sources. Further, quantitative differences are usually influenced by gene differences at many loci and Mendelian ratios does not fit in. While qualitative differences clearly distinguish particular characters which are usually under the control of single gene whose Mendelian ratios acts. 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 by polygenes. Not much information is available on the inheritance pattern of resistance in Indian silkworm breeding stocks except that Ratna Sen *et al.* (2004) studied the inheritance pattern against BmNPV. Although there are several methods to determine the inheritance pattern, single back cross method has been widely used to distinguish between resistance controlled by a single locus (mono-genic) and the resistance controlled by more than one locus (polygenic) owing to its simple and less

time- consuming approach (Georghiou, 1969; Tabashinik, 1991). Recently Sudhakara Rao *et al.* (2006) has established resistance pattern against BmDNV1 and introduced dominant / recessive genes available in the low yielding donors (A and C. Nichi) to productive, but susceptible bivoltine silkworm stocks against BmDNV1. Thus an attempt was made in the present study to establish the genetic resistance in the newly developed bivoltine breeds CSR18 and 61N.

MATERIALS AND METHODS

The resistant and susceptible parents are crossed and F_1 is prepared, their reciprocal F_1 (R ♀ S and S ♀ R), F_1 and F_2 hybrids back crossed with resistant (F_1 ♀ R = BCR) and susceptible (F_1 ♀ S = BCS) parents were inoculated with a fixed LC_{50} concentration of BmNPV (Tabashnik, 1991; Ratna Sen *et al.*, 2004). The cumulative mortality due to BmNPV infection was daily recorded 10^{th} days of post inoculation. BmNPV was extracted from the hemolymph of infected larvae and purified by repeated washing centrifugation followed by sucrose gradient centrifugation (Sugimori *et. al.*, 1990) using Hitachi Ultra centrifuge. The concentration of the stock was determined by counting the polyhedral inclusion bodies (PIB) under Leitz-diaplan microscope. The stock solution of virus inoculum was diluted to desired concentration by adding autoclaved distilled water. The concentration is expressed as PIB/ml.

The larvae of identified resistant donor Bivoltine silkworm breeds (Sowmyashree and Nataraju, 2007) *viz.*, CSR18 and 61N and susceptible bivoltine breed CSR2 were selected from the pooled population of 10-12 disease free layings were taken for inoculation in case of parental stocks and their F_1 hybrids as all the individuals are supposed to be genetically identical. In case of F_2 and back cross progeny, all the larvae hatched from a full brood laid one female moth was taken for the experiment since the resistant and the susceptible individual are supposed to be segregated in one brood of F_2 and back cross progeny. The hatched larvae from the parents and their progeny were inoculated with BmNPV along with the first feed after second moult and inoculated with 1 ml inoculum of LC_{50} concentration for CSR18 as 1 ml $10^{4.684}$ PIB/ml, 61N as 1 ml $10^{5.717}$ PIB/ml and susceptible breed CSR2 as 1 ml $10^{4.679}$ PIB/ml. The inoculum was smeared on to the mulberry leaf surface and fed to the larvae. These larvae were reared following the standard method. 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 and the cumulative mortality due to infection was recorded on 10^{th} day post inoculation. The experiment was carried out thrice to confirm the results. The average data of replications were taken for analysis.

Chi square test (Snedecor and Cocharan, 1980) was employed to determine the deviation between the observed and the expected mortality. The expected mortality in F_2 BC (S) and BC(R) progeny were calculated based on the observed mortality of the parents and their F_1 hybrid assuming non genetic inheritance pattern (Tabashnik, 1991). The data of three determinations were subjected to homozygocity. Chi square test in order to check the deviations if any, between the determinations and the inheritance pattern was established.

Table I : Progeny raised from the resistant and susceptible silkworm stocks. CSR18 and 61N is resistant breed (R), susceptible breed CSR2(S).

Genetic cross	Progeny
R S	F1
S R	RF1
(R S) (R S)	F2
(R S) S	BC(S)
(R S) R	BC(R)

RESULTS

Response of parents and progenies to BmNPV (Bivoltine breeds) : Response of Bivoltine parents and their progenies to BmNPV or inheritance pattern against BmNPV in bivoltine breeds is presented in Table II and III. The results presented in Table 2 shows the mortality rate of resistant breed CSR18 and susceptible breed CSR2 and their progeny of F_1, F_2 and back cross progenies in reciprocal crosses to a BmNPV. The mortality rate of the resistant breed CSR18 was recorded as 47.50% where as the same was observed as 83.66% in case of susceptible breed CSR2. The F_1 and RF_1 have recorded a mortality of 30.16% and 25.66% respectively. The mortality rate for F_2 and RF_2 was found to be 25.83% and 22.83% respectively. The progeny (BCT) derived from the female of F_1, crossed with resistant donor parents of CSR18 male was recorded a mortality % of 14.33%. When the female of CSR18 was crossed with F_1 male (TBC), the mortality rate was found to be 15.00%. The progeny of female of RF_1 crossed with CSR18 male (RBCT), has shown a mortality rate of 13.66%. The progeny (TRBC), derived from CSR18 female and RF_1 male has recorded a mortality rate of 14.33%. The progeny (BCS), derived from the cross of F_1 female and CSR2 male has shown a mortality rate of 38.33% where as the same in reciprocal cross *i. e.*, female CSR2 mated with male of F_1 (SBC) was 38.66%. The progeny (RBCS) of female mated with CSR2 male has recorded a mortality of 40.00% and the same in reciprocal cross (SRBC) *i e.* female of CSR2 and male of RF_1 (SRBC) was 39.16%. The results revealed that more than 3.84 value chi square analysis considered that significant and less than the value of 3.84 is considered to be insignificant which recorded in F_1 progeny.

The mortality rate of resistant breed 61N and susceptible breed CSR2 and their F_1, F_2 progeny and back cross progenies in reciprocal crosses to BmNPV were indicated in Table III. The mortality rate of the resistant breed 61N was recorded as 45.55% where as the same was observed as 83.66% in case of susceptible breed CSR2. The F_1 and RF_1 have recorded a mortality of 26.95% and 28.57%, respectively. The mortality rate for F_2 and RF_2 was found to be 28.80% and 23.08%, respectively. The progeny (BCT) derived from the female of F_1, crossed with resistant donor parents of 61N male was recorded a mortality rate of 13.51%. When the female of 61N was crossed with F_1 male (TBC), the mortality rate was found to be 14.36%. The progeny of female of RF_1 crossed with 61N male (RBCT), has shown a mortality rate of 15.59%. The progeny (TRBC), derived from 61N female and RF_1 male has recorded a mortality rate of 13.79%. The progeny (BCS), derived from the cross of F_1 female and CSR2 male has shown a mortality rate of 37.81% where as the same in reciprocal cross *i.e.* female CSR2 mated with male of F_1 (SBC) was 38.52%. The progeny (RBCS) of female mated with CSR2 male has also recorded a

Table II : Chi square analysis for the assessment of inheritance of resistance to BmNPV following the Mono Mendelian Inhritance model.

Progeny		Observed				Expected				Chi square value			
		Survival (No.)	Survival (%)	Mortality (No.)	Mortality (%)	Survival (No.)	Survival (%)	Mortality (No.)	Mortality (%)	Survival	Mortality	Total	Significance (at 5%)
CSR18		315	52.50	285	47.50	-	-	-	-	-	-	-	-
CSR2		98	16.33	502	83.66	-	-	-	-	-	-	-	-
F1	CSR18 x CSR2 (RxS)	419	69.98	181	30.16	450	75	150	25	2.2050	6.2017	8.407	**
RF1	CSR2 x CSR18 (SxR)	446	74.33	154	25.66	450.	75	150	25	0.0450	0.0817	0.127	NS
F2	CSR18 x CSR2 (RxS) F2	445	74.17	155	25.83	450	75	150	25	0.0672	0.1350	0.202	NS
RF2	CSR2 x CSR18 (SxR) RF2	463	77.16	137	22.83	450	75	150	25	0.3472	1.2150	1.562	NS
BCT	(CSR18 x CSR2) x CSR18	514	85.67	86	14.33	525	87.50	75	12.50	0.2519	1.4700	1.722	NS
TBC	CSR18 x (CSR1 x CSR2)	510	85.00	90	15.00	525	87.50	75	12.0	0.4576	2.8033	3.261	NS
RBCT	(CSR2xCSR18) x CSR18	518	86.33	82	13.66	525	87.50	75	12.50	0.1071	0.5633	0.670	NS
TRBC	CSR18 x (CSR2xCSR18)	514	85.67	86	14.33	525	87.50	75	12.50	0.2519	1.4700	1.722	NS
BCS	(CSR18 x CSR2) x CSR2	370	61.67	230	38.33	375	62.50	225	37.50	0.0807	0.0900	0.171	NS
SBC	CSR2 x (CSR18xCSR2)	368	61.33	232	38.66	375	62.50	225	37.50	0.1500	0.1878	0.338	NS
RBCS	(CSR2xCSR18) x CSR2	360	60.00	240	40.00	375	62.50	225	37.50	0.6407	0.9344	1.575	NS
SRBC	CSR2 x (CSR2xCSR18)	365	61.83	235	39.16	375	62.50	225	37.50	0.2940	0.4011	0.695	NS

Table III : Chi square analysis for the assessment of inheritance of resistance to BmNPV following the Mono -Mendelian inheritance model.

Progeny		Observed				Expected				Chi square value			
		Survival (No.)	Survival (%)	Mortality (No.)	Mortality (%)	Survival (No.)	Survival (%)	Mortality (No.)	Mortality (%)	Survival	Mortality	Total	Significance (at 5%)
	61N	326	54.33	274	45.55	-	-	-	-	-	-	-	-
	CSR2	98	16.33	502	83.66	-	-	-	-	-	-	-	-
F1	61N x CSR2 (RxS)	434	72.33	166	26.95	450	75	150	25	0.6050	1.6017	2.207	NS
RF1	CSR2 x 61N (SxR)	420	70.00	180	28.57	450	75	150	25	2.0672	5.8017	7.869	**
F2	61N x CSR2 (RxS)	418	69.66	182	28.80	450	75	150	25	2.3472	6.6150	8.962	**
RF2	CSR2 x 61N (SxR)	465	77.50	135	23.08	450	75	150	25	0.4672	1.6017	2.069	NS
BCT	(61N x CSR2) x 61N	518	86.33	82	13.51	525	87.50	75	12.50	0.1071	0.5633	0.670	NS
TBC	61N x (61NxCSR2)	512	85.33	88	14.36	525	87.50	75	12.50	0.3471	2.0833	2.430	NS
RBCT	(CSR2x61N) x 61N	503	83.83	97	15.59	525	87.50	75	12.50	0.9643	6.1633	7.128	**
TRBC	61N x (CSR2x61N)	516	86.00	84	13.79	525	87.50	75	12.50	0.1719	0.9633	1.135	NS
BCS	(61N x CSR2) x CSR2	372	62.00	228	37.81	375	62.50	225	37.50	0.0327	0.0278	0.060	NS
SBC	CSR2 x (61NxCSR2)	365	60.83	235	38.52	375	62.50	225	37.50	0.2940	0.4011	0.695	NS
RBCS	(CSR2x61N) x CSR2	365	60.83	235	38.52	375	62.50	225	37.50	0.2940	0.4011	0.695	NS
SRBC	CSR2 x (CSR2x61N)	363	60.50	237	38.73	375	62.50	225	37.50	0.4167	0.5878	1.004	NS

mortality of 38.52% and the same in reciprocal cross (SRBC) *i.e.* female of CSR2 and male of RF_1 (SRBC) was 38.73%. The Chi square value being less than 3.84 showing insignificant deviation between the observed and the expected values in FR_1, F_2 and RBCT progeny.

DISCUSSION

In the present study, the results have shown that NPV resistance is controlled by four dominant genes , N1, N2, N3 & N4 and each is contributing about 25% resistance (tolerance). CSR18 & 61N is having genetic constitution of N1, N2, n3 & n4 having 50% resistance and CSR2 has n1, n2, N3 & n4 with 25% tolerance . F_1 will have a genetic constitution of N1, N2, N3 and n4 with 75% tolerance. The genotype of the segregating population of F_2 and back cross progenies on the basis of the hypothesis of mono Mendelian inheritance pattern (Tabashnik, 1991). The CSR18 & 61N are bivoltine breeds homozygous for N1, N2, n3, n4 (resistance to nuclear polyhedrosis virus) CSR2 n1, n2, N3 & n4 (susceptible to nuclear polyhedrosis virus). The CSR18, 52.50 & 61N 54.33 the observed survival percentage in F_1 (N1, N2, N3 & n4) CSR18 69.83 & 61N 72.33. This indicates that F_1 will have a genetic constitution of N1, N2, N3 & n4 with 75% tolerance. On the survival percentage of progeny derived from reciprocal crosses of RF_1, RF_2 TBC, TRBC, SBC & SRBC between the tolerance breeds and susceptible breeds (Table II and III) the result suggest that resistant strain may have a dominant major gene controlling the resistance . No marked difference in the resistance to polyhedrosis infection was observed in the reciprocal crosses there was also no indication of maternal effect on the inheritance of resistance and also did not differ significantly in the straight crosses and reciprocal crosses Watanabe (1966) . In F_2 and F_1, the expected survival percentage is calculated as a total survival percentage of the population of resistance breeds CSR18 and 61N and population of F_1 population susceptible breeds CSR2. The total observed survival percentage of F_2 comes to 74.17 & 66.67 which is very close to the expected survival percentage 75.00 (Table II and III). The chi square value calculated based on the observed and expected numbers. Thus, the observed values are insignificant and deviating from the expected values, thus strengthens the hypothesis to be a major dominant gene in all the resistance breeds. Similarly, in the population of F_1 progeny back crossed with the resistant breed (BCT) the sum of the survivability of 50% population resembling F_1 hybrid and another 50% resembling the resistant breeds like CSR18 and 61N. The observed survival percentage of BCT comes to 85.67 & 86.33 (Table II and III) as the expected survival percentage is 85.67 which is very close to the observed values again showing the insignificant deviation between the expected and observed values in BCT progeny .Thus this observations further strengthen the hypothesis of mono Mendelian inheritance of resistance to BmNPV in CSR18 and 61N. In case of F_1 progeny back crossed with susceptible breed CSR2 (BCS), the observed survival percentage is the total of the survivability of 50% of the population resembling F_1 and another 50% resembling susceptible breed (CSR2). The studies carried out on the inheritance pattern of resistance to BmNPV in silkworm by Aratake (1973) and Watanabe (1986) have shown that the resistance to BmNPV is controlled by many minor effect genes (polygenic). The Chi-square value being less than 3.84 showing insignificant deviation between the observed and the expected values in BCS progeny. Since some differences was observed in the survivability of the parents and their progeny. Similar results were observed by Watanabe

(1966) and many workers have studied the genetic system of inheritance of resistance to various diseases of silkworms, but there are difference of opinion in the pattern of inheritance in one of the most resistant silkworm breeds to *per oral* infection with BmCPV in Daizo, while the Okuso breed is highly susceptible as investigated the mode of inheritance of resistance in the Daizo breed (Watanabe, 1966 & 1965). Zhiqhi Meng (1982) indicated that the resistance against the *per oral* infection of nuclear polyhedrosis virus was found to be controlled by pair of major dominant genes and some minor effect genes. Seki (1984) studied the genetic resistance of the silkworm to the flacherie virus infection in Japanese and Chinese races as well as their hybrids. Resistance to the viral infection is a recessive genetic character which is probably controlled by a major gene. The findings of the present study will greatly help in designing appropriate breeding protocols for development of BmNPV resistant hybrids by introgression of the resistant genes into the productive silkworm stocks which are susceptible to BmNPV to enhance cocoon production in tropical areas.

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