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Development of Resistance to *Bombyx mori* Densonucleosis Virus into a Susceptible Silkworm Breed

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Seeing inadequate disinfection and unhygenic condition in rearing area, use of disease resistant silkworm variety is the best option. In order to this, an attempt has been made to develop the resistance to *Bombyx mori* densonucleosis virus (BmDNV-2) into a susceptible silkworm breed Zhenon1 by cross breeding with a resistant silkworm breed SU12 and exposing the subsequent generations to BmDNV-2 followed by the selection of individuals from the surviving batches. After seven generation the evolved DNV-2 resistant strain showed the significantly higher resistance to BmDNV-2 than control Zhenon1. The economic characters of both of the breeds were almost on par.

Key words: Silkworm, *Bombyx mori* densonucleosis virus (BmDNV-2), Breeding, Resistance

Introduction

The silkworm diseases are the main factors which seriously affect the cocoon production (Watanabe, 1986). Hence, prevention of silkworm diseases becomes one of the most important aspect in the success of commercial sericulture. In order to obtain high and stable cocoon yield it is necessary to make efforts firstly to decrease pathogen quantity, pathogenicity and secondly to strengthen the larval constitute by increasing its disease resistance ability. It is generally recommended to disinfect the rearing houses and the appliances before the commencement of rearing, but it is not necessarily adequate to prevent the occurance of diseases. Moreover, among many measures of silkworm disease controlling and prevention, the utilisation of disease resistant silkworm variety along with disinfection would be a most effective step in the direction of disease prevention.

Off all silkworm diseases, which cause damage, viral diseases are the most serious (Samson *et al.*, 1990; Subba Rao *et al.*, 1991; Shivaprakasham and Rabindra, 1995). *Bombyx mori* densonucleosis virus cause considerable crop loss (Sato, 1992), hence a need has felt to develop silkworm breed which are resistant to BmDNV.

Very few studies have been made on the development of resistance of insects to pathogens. David and Gardiner (1960) have obtained a stock of *Pieris brassicae* resistant to granulosis virus following laboratory selection. Resistance of silkworm to nuclear polyhedrosis and cytoplasmic polyhedrosis virus (NPV and CPV) and infectious flacherie virus (IFV) is controlled by polygenes (Watanabe, 1986, 1991). Whereas, resistance of *B. mori* to densonucleosis virus (BmDNV) is controlled by a single major gene (Watanabe and Maeda, 1978, 1981; Eguchi *et al*, 1986). Uzigawa and Aruga (1966) have developed a strain of silkworm resistant to infectious flacherie virus (BmIFV) after selecting survivors from infectious flacherie virus fed larvae for five generations. Watanabe (1967) had evolved a silkworm strain, resistant to cytoplasmic polyhedrosis by selecting survivors from virus fed larvae for eight generations. Similarly Ratna Sen *et al.* (1999) have developed a silkworm strain DR-1 comparatively tolerant to nuclear polyhedrosis virus (BmNPV). Recently, Eguchi *et al.* (1998) have succeded in evolving a silkworm hybrid "Taisei" which is resistant to *B. mori* densonucleosis virus type 1 (BmDNV-1). Adopting the same procedure, an attempt was made to develop the resistance for BmDNV- 2 into a susceptible silkworm breed.

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Materials and Methods

Silkworm Breeds

A commercially used silkworm breed Zhenon1, Chinese bivoltine having plane larvae and white oval shape cocoon with moderate economic characters and low resistance to BmDNV-2 was selected to improve its resistance to BmDNV-2. Another Japanese bivoltine breed SU12 having normal marked larvae and dumbbell shaped white cocoon and whose resistance to BmDNV-2 (measured as survival percent on challenge with virus) was found to be five times higher than Zhenon 1 was selected as the donor parent.

Bombyx mori densonucleosis virus (BmDNV-2) inoculum

Fresh BmDNV-2 inoculum was prepared from diseased silkworms and infectivity was determined by bioassay. The silkworm larvae of 3rd instar were orally inoculated with BmDNV-2 suspension. After 7 days of per-oral inoculation, the gut of diseased silkworm was removed, dried at 25°C for 24 hrs and powdered and stored at 5°C. The powder of infected gut was used to prepare the fresh BmDNV-2 inoculum. Three densities (6.0, 0.6 and 0.06%) of BmDNV-2 inoculum were used for the peroral inoculation of the silkworm. 0.3 g powder of BmDNV-2 infected silkworm gut was dissolved in 5.0 ml double distilled autoclaved water and purified following the method of Maeda *et al.* (1977). The original suspension was 6.0%, it was diluted 10 times and 100 times with double distilled autoclaved water to make 0.6 and 0.06% density of the suspension.

Inoculation of BmDNV-2

One hundred µl of BmDNV-2 suspension was pipetted out on a fresh mulberry leaf cut into a square of 2" side and smeared evenly with a flat tipped glass rod. Every 50 lavae of 1st instar were fed with such leaf. After 24 hrs, the larvae were fed with uncontaminated fresh leaf twice a day. These larvae were reared under 28 ± 1°C temperature and 85 ± 5% relative humidity. After 72 hrs of inoculation each larva was examined visually for the symptoms of disease daily for 15 days. The cumulative mortality and the number of healthy larvae were recorded.

Breeding

Female of Zhenon1 breed was mated with male of SU12. Rearing of subsequent generations was conducted following standard rearing method as advocated by Krishanaswami (1978). The F1 population reared *en masse* was tested for its resistance to BmDNV-2 by inoculating 50 newly hatched larvae sample from 10 families each by BmDNV-2 at a concentration of 0.06%. The cumulative mortality was recorded up to 15 days post inoculation. The larvae from the batches that showed more than 50% survival (the batches which recorded survival much above the average of the population) were selected to continue the life cycle and F2 population was raised. Again, 50 newly hatched larvae each from 45 families of F_2 generation (total 2,250 larvae) were challenged with the same concentration of BmDNV-2. The larvae from the batches which have shown more than 80% survival (much above the average of the population) were selected to continue the life cycle. Single cocoon assessment was done to pick up the individuals with cocoon characters, which fell above the average level. They were back crossed with Zhenon1 males and the third generation population (BF2) was raised which was inoculated with BmDNV-2 at a concentration of 0.6%. The larvae from the batches showed more than 50% survival (much above the average of the population) were selected and reared *en masse*. At 4th generation, again 50 larvae from each of the 50 families (2,500 larvae) were challenged with BmDNV-2 (0.6%). Larvae from the batches showed more than 70% survival (much above the average of the population) were selected for the next generation. Single cocoon assessment and selection of above average individuals were resorted. During progeny selection, due attention was given to select the cocoons of uniform shape and size. Only those cocoon which resemble that of the breed Zhenon1 were selected in every generation. Population at 3rd generation was inoculated with a larger concentration (6.0%) of BmDNV-2 and the individuals which fell well above the average survival percent of the population were selected. The same process of challenging with BmDNV-2 followed by the selecting high survival batches was continued until 5th generation.

To compare the resistance of evolved BmDNV-2 resistant strain and Zhenon1, every batch of 50 larvae from both the breeds was fed with a leaf cut into a square of 4"side, smeared with 200 µl of each of 3 concentration of BmDNV-2 (0.06, 0.6 and 6.0%). After the inoculation, they were reared as described above. Cumulative mortality was recorded from day 4 to day 15 post inoculation. This operation was repeated three times and the average cumulative mortality was subjected for anova test using Completely Randomised Design (Snedecor and Cochran, 1967) to determine the significant difference between the resistance of Zhenon1 and evolved BmDNV-2 resistant strain.

Results

Characteristics of the breeding resistance materials *i.e.*, Zhenon 1 and SU12 are shown in Table 1. Though the

Table 1. Characteristics of breeding resource silkworm varieties Zhenon1 and SU12 (average of 9 generations)

Breed	Egg/laying	Larval duration	Pupation rate (%) based on 3rd moult	Yoeld/10000 larvae after 3rd moult	Single cocoon weight (g)	Single cocoon shell weight (g)	Cocoon shell ratio (%)	Survival % in response to BmDNV-2 (0.06% conc.)
Znenon 1	592 ± 56.21	22 : 12	90.56 ± 4.18	18.596 ± 1.09	1.923 ± 0.13	0.39 ± 0.03	20.00 ± 1.32	16.55 ± 3.92
SU12	512 ± 49.12	23 : 20	92.38 ± 3.91	15.952 ± 1.02	1.732 ± 0.11	0.33 ± 0.02	20.56 ± 1.29	81.60 ± 9.82

Table 2. Mean survival percent of population at succeeding generations and the selected batches on challenge with BmDNV-2

Generation	Concentration of BmDNV-2 (%)	Larval age at inoculation	Survival of population (%)	Survival of selected batches (%)
Zhenon1	0.06	1st instar	16.55 ± 3.27	–
SU12	0.06	1st instar	81.60 ± 8.92	–
F1 (Zhenon1 × SU12)	0.06	1st instar	50.37 ± 6.35	> 60
F2	0.06	1st instar	75.76 ± 12.72	> 80
BF2 (F2 × Zhenon1)	0.6	1st instar	62.26 ± 10.51	> 65
G4	0.6	1st instar	80.38 ± 10.67	> 85
G5	6.0	1st instar	75.28 ± 11.12	> 80
G6	6.0	2nd instar	98.92 ± 10.02	> 95
G7	6.0	2nd instar	97.26 ± 9.93	> 95

cocoon characters of SU12 are comparatively inferior, but it has shown five times higher survival (81.60 ± 9.82%) than the Zhenon1 (16.55 ± 3.92%) when challenged with the same concentration of BmDNV-2 (0.06%). The results of selection of BmDNV-2 resistant strain in succeeding generations are presented in Table 2. The mean survival on challenge with BmDNV-2 (0.06%) at F1 generation was recorded (50.37 ± 6.35%) which is intermediate value of BmDNV-2 resistant breed SU12 (81.60 ± 8.92%) and the BmDNV-2 sensitive breed Zhenon1 (16.55 ± 3.27%).

In F2 generation, the survival percent on challenge with BmDNV-2 (0.06%) was found to vary 65 – 90%, the average being 75.76 ± 12.72%. The batches showed survival ratio of 8 : 10 (80%) or above were selected to back cross with Zhenon1. Average survival percent of BF2 (back crossed progeny) was recorded to be 62.26±10.51% when challenged with BmDNV-2 at 0.6% concentration. The average survival percent in the next generation (G4) was increased to 80.38±10.87% when challenged with the same concentration of BmDNV-2 (0.6%). The concentration of BmDNV-2 was further increased to 6.0% from G5 onwards. The average survival percent on challenge with BmDNV-2 at concentration of 6.0% was decreased to 75.28 ± 11.12% at G5. The average survival percent was further increased to 98.92 ± 10.02 and 97.26 ± 9.93% at G6 and G7, respectively. The survival percent at G6 and G7 was almost constant. After G7 generation, the evolved DNV-2 resistant strain was compared with the control

Table 3. Mortality percent in Zhenon1 and evolved BmDNV-2 resistant strain

BmDNV-2 Conc. (%)	Mortality (%)		CD at 5% Between breeds
	Zhenon1	BmDNV-2 resistant strain	
0.06	81.33 (54.746)	1.00 (0.573)	14.99**
0.60	89.00 (63.467)	3.33 (1.910)	14.99**
6.00	98.00 (80.754)	6.67 (3.823)	14.99**

Values in brackets are Aresine transformation.
**Significant at 5% level.

Table 4. Performance of BmDNV-2 resistant strain and Zhenon1 (average of 3 generations)

Parameters	BmDNV-2 resistant strain	Zhenon1
Eggs/laying	512 ± 43.26	566 ± 56.23
Larval duration (D : H)	22 : 18 ± 0.14	22 : 12 ± 0.14
Pupation (%) based on 3rd moult	94.36 ± 4.91	94.82 ± 4.35
Single cocoon weight (g)	1.943 ± 0.16	1.962 ± 0.17
Single cocoon shell weight (g)	0.381 ± 0.01	0.394 ± 0.01
Cocoon Shell Ratio (%)	20.06 ± 1.12	19.78 ± 1.17
Filament length (m)	980 ± 42	986 ± 35
Denier	2.31 ± 0.11	2.50 ± 0.12
Reelability (%)	91.0 ± 2.0	92.6 ± 2.6
Neetness (Points)	94.12 ± 3.0	93.31 ± 2.6

breed Zhenon1 for the significant difference in mortality by using anova test and results are presented in Table 3. Significantly low mortality ($P<0.05$) in evolved BmDNV-2 resistant strain than control (Zhenon1) indicates the high resistance to BmDNV-2 in evolved silkworm strain. Rearing performance of the evolved strain was on par with Zhenon1 in its economic characters (Table 4).

Discussion

The F1 population was found to have a survival percent (50.37 ± 8.92) which is the average of resistance Zhenon1 (16.55 ± 3.27) and susceptible SU12 (81.60 ± 8.92) parent indicating the involvement of additive gene action. Since F2 generation comprised of a segregating population, a wide range of survival percent (65 85%) was observed. In order to improve the cocoon characters, the selected F2 progeny was back crossed with the susceptible parent Zhenon1 which has better cocoon characters as compared to SU12 (Table 1). Despite the selection of F2 progeny from the batches which had survival ratio of 8 : 10 (80%) or above, the same decreased to 62.26% in BF2 progeny, probably because thay were back crossed with susceptible breed Zhenon1 and concentration of BmDNV-2 was increased 10 times (0.06 to 0.6%) in order to enhance the selection pressure.

Further increase in the average survival percent in G4 generation (80.38%) in response with same concentration of BmDNV-2 indicates the positive response of selection rendered in 3rd generation. Again increase in concentration of BmDNV-2 (6.0%) has reflected the survival percent and it was slightly decreased at G5 generation (75.28 ± 11.12%). The repeated progeny selection followed by family rearing challenged by higher concentration of BmDNV-2 and interfamily mating has resulted in increase of average survival percent of the population to above 97% from G6 onwards.

The concentration of BmDNV-2 was increased gradually in succeeding generations in order to exert more selection pressure during breeding so that the population could be made homozygous with more resistant individuals. Watanabe (1967) has emphasized that a selection pressure causing > 60% mortality is effective in inducing and retaining the resistance in the population.

Population mean of the survival percent on challenge with BmDNV-2 was variable in the different generations probably due to heterozygosity of population in earlier generations and change in the concentration in the BmDNV-2 inoculum after every two succeeding generations. The effert was made to select the individuals which were well above the average of the population in every generation.

G6 onwards the resistance to BmDNV-2 was developed almost 100% as the survival percent was above 97.5% Eguchi *et al.* (1998) has also evolved a silkworm hybrid "Taisei" which is completely resistant to BmDNV-1.

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