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Studies on Daba T.V. Tasar Eco-Race Tolerance to *Antheraea Mylitta* Cytoplasmic Polyhedrosis Virus (AmCPV) Under Inoculated Condition

SCIENCE

KEYWORDS : Virosis, Inoculation, Susceptible, Tolerance and Survivors

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ABSTRACT

Virosis is dangerous contiguous disease and it is estimated to an extent of 25%-30% of total crop loss. Usage of tolerant AmCPV breeds, along with the disinfection is the most suitable for the prevention of crop loss due to the virosis in the open field rearing conditions. Hence, study was conducted on Daba T.V. tasar eco-race tolerance to Antheraea mylitta Cytoplasmic Polyhedrosis Virus (AmCPV) under inoculated condition. The survival % increased from generation G_1 (2.96%) to G_2 (6.33%) when challenged with AmCPV at the concentration of 1 x 10^3 PIB/ml. In generation G_3 it came down to 6.15% and reached to 12.23% at G_4 when challenged with AmCPV at the high concentration of 1 x 10^4 PIB/ml. Further, it decreased to 12.09% in the G_5 generation and increased to 14.28% at generation G_6 when challenged with AmCPV at the higher concentration of 1 x 10^5 PIB/ml. In last generation, G_7 there was slight decrease in survival percent recorded with 13.80% when challenged with AmCPV at the high concentration of 1 x 10^6 PIB/ml. In the case of Inoculated Control, survival % was 2.96% in G_1 and decreased gradually and reached to 0.92 at the end of G_4 generation thereafter it was recorded 0% from generation G_5 to G_7. The survival % was ranged in between 21.92-28.33% in the Healthy control. Percentile improvement of survival trait in AmCPV treated batches was ranged from 10.45 (1st generation) to 62.95 (7th generation) and shown gradual linier improvement. Cocoon weight, Cocoon shell weight and shell ratio recorded lower values in AmCPV treated lots than the healthy control in initial generations and obtained almost equal values to healthy control when it reached to the generation G_7. The analysis of variance showed for all tested survival, cocoon weight, cocoon shell weight and shell ratio traits highly significant (P<0.01) values in the treatment, generation and treatment x generation.

Introduction

Among all diseases, the incidence of virosis is frequent, dangerous contiguous disease and it is estimated to an extent of 25%-30% of total crop loss. Different preventive methods using chemical disinfectants are practiced for prevention and control of the diseases. But the chemical disinfectants have limitations to be effective in open field rearing of tasar silkworms.

Hence, the usage of tolerant *AmCPV* eco-races, along with the disinfection is the most suitable for the prevention of crop loss due to the virosis in the open field conditions. All the tasar eco races are susceptible to the virus. The resistance in silkworm to virus is generally determined and is a heritable character (Watanabe, 1966, 1987; Aratake, 1973; Tanada and Kaya, 1993). Watanabe (1967) had evolved a silkworm strain resistant to cytoplasmic polyhedrosis by selecting survivors from virus fed larvae for eight generations. David and Gardiner (1960) have obtained a stock of *Pieris brassicae* resistance to the granulosis virus adopting laboratory selections. Uzigawa and Aruga (1966) have developed a strain of resistant to infectious flacherie virus after selecting survivors from infectious flacherie virus fed larvae for five consecutive generations. On the contrary, resistance of the silkworm to nuclear and cytoplasmic polyhedrosis virus (NPV and CPV) and also infectious flacherie virus (IFV) is controlled by polygenes (Watanabe, 1986). Therefore, developing a complete resistant breed against these viruses is practically impossible. However, breeding of strains with comparatively more resistance to these viruses is possible by selection of survivors after exposer to virus (Uzigawa and Aruga, 1966; Watanabe, 1967; Aratake, 1973; Watanabe, 1994). In view of above, the study was conducted on Daba T.V. tasar eco-race tolerance to *Antheraea mylitta* Cytoplasmic Polyhedrosis Virus (*AmCPV*) under inoculated condition.

MATERIALS AND METHODS

Propagation and multiplication of *AmCPV*: *AmCPV* was propagated in tasar silkworm by *per oral* inoculation and multiplied periodically for preparing the *AmCPV* inoculum.

Isolation and Purification of *AmCPV*: Completely whitened midgut obtained from polyhedrosised silkworms at an advanced stage of infection are placed in to a beaker and stirred vigorously in distilled water with glass rod or homogenized with a blender. The polyhedral suspension is then filtered through a cheese cloth and filtrate is centrifuged at 3000 rpm for 10 to 15 minutes. The sediment is re-suspended washed with distilled water by repeated centrifugation and filtration. The purity of polyhedral suspension was examined with a light microscope. The preparation thus obtained contains purified polyhedra which was stored in refrigerator at 5° C (Aizwa, 1971).

Induction of tolerance to *AmCPV* in Daba T.V: The Daba T.V is per orally inoculated with *AmCPV* and reared in indoor conditions for seven generations. *AmCPV* concentration was started with the 1 x 10^3 PIB/ml in the first generation and ended with 1 x 10^6 PIB/ml in the seventh generation. In every two generations, the Daba T.V progenies were exposed to higher concentration of *AmCPV* (10 times) in order to enhance the selection pressure and families having higher *AmCPV* tolerance in term of survival rate was selected for further programme and assessed for important economic traits such as survival rate (%), cocoon weight (g), cocoon shell weight (g) and cocoon shell ratio (%). Two controls i.e. Inoculated control and Healthy control were maintained for the comparison.

Statistical analysis: Collected data was analyzed in CRD design with two factors (Generation and Treatment) for transformed values and tabulated.

RESULTS AND DISCUSSION

To study Daba T.V. tasar eco-race tolerance to *Antheraea mylitta* Cytoplasmic Polyhedrosis Virus (*AmCPV*), exposer of progenies to *AmCPV* and selection of survivors from the segregating population was done.

***AmCPV* tolerant Daba T.V generation wise rearing performance:** The survival % increased from G_1 (2.96%) to G_2 (6.33%) when challenged with *AmCPV* at the concentration of 1 x 10^3 PIB/ml. In generation G_3 it came down to 6.15% and reached to 12.23% at G_4 when challenged with *AmCPV* at the high concentration of 1 x 10^4 PIB/ml. Further, it decreased to 12.09% in the G_5 generation and increased to 14.28% at generation G_6 when

challenged with *AmCPV* at the high concentration of 1 x 10^5 PIB/ml. In last generation, G_7 there was slight decrease in survival % recorded with 13.80% when challenged with *AmCPV* at the high concentration of 1 x 10^6 PIB/ml. In the case of Inoculated Control, survival % was 2.96% in G_1 and decreased gradually and reached to 0.92% at the end of G_4 generation thereafter it was recorded 0% from generation G5 to G7. The survival percent was ranged in between 21.92-28.33% in the Healthy control. The C.D values of this trait recorded for Treatment 0.66, Generation 0.43 and Treatment X Generation 1.14 at 5% (Table 1). Cocoon weight decreased from G_1 (9.96g) to G_2 (9.77g). In G_3 generation it decreased to 9.63g and thereafter increased to 9.70g at G_4. Further, it recorded 9.85g in the G_5 generation and reached to 9.88g at G_6 generation. In the last generation, G_7 it further increased to 10.23g which was almost equal to healthy control. In the case of Inoculated Control, cocoon weight was 9.77g in G_1 and decreased gradually and reached to 8.10g at the end of G_4 generation thereafter no cocoons were produced from generation G_5 to G_7. The cocoon weight was ranged in between 10.23-10.46g in the Healthy control. The C.D values of this trait recorded for Treatment 0.18, Generation 0.12 and Treatment X Generation 0.31at 5% (Table 1).

Cocoon shell weight was recorded 1.08g in G_1 and reached to 1.11g at the end of G_7 which is almost equal to the value of the Healthy control. In the case of Inoculated Control, shell weight was 9.77g in G_1 and decreased gradually and reached to 8.10g at the end of G_4 generation thereafter no cocoons were produced from generation G_5 to G_7. The cocoon shell weight was ranged in between 1.09-1.21g in the Healthy control. The C.D values of this trait recorded for Treatment 0.09, Generation 0.06 and Treatment X Generation 0.15 at 5% (Table 1). Cocoon shell ratio was recorded 10.84% in G_1 and reached to 10.85% at the end of G7 In the case of Inoculated Control, shell weight was 10.50% in G_1 and decreased gradually and reached to 8.31% at the end of G_4 generation. The cocoon shell weight was ranged in between 10.57-11.60% in the healthy control. The C.D values of this trait recorded for Treatment 0.26, Generation 0.17 and Treatment X Generation 0.45 at 5% (Table 1).

Survival rate is an important parameter which reflects the viability of the breed. Fluctuations in survival rate were observed among the generations studied. As the viability and productivity are negatively correlated and after reaching a threshold level, shell weight may lead to decrease in survival rate (Mahadevappa, 2006; Guruswamy, 2006). In the present investigation, the balance between these two traits was maintained in the last generations.

An increase in the average survival % in F_2 on challenge with the same concentration of *AmCPV* indicates the positive response of the tolerance. When the progenies were exposed to higher concentration of *AmCPV* (10 times) in every two generations the survival % and cocoon characters decreased in G1, G3, G5 and G7 generations when compared with G2, G4 and G7 generations. The concentration of *AmCPV* was increased gradually in order to exert more selection pressure during breeding so that the population could be made homozygous with more tolerant individuals. Watanabe (1967) has emphasized that a selection pressure causing > 60 % mortality is effective in inducing and retaining the tolerance in the population. Despite continuous selection, there was no further considerable increase in survival % on challenge with *AmCPV* from G_6 onwards. This gives hint towards attaining a plateau.

Table 1. AmCPV tolerant Daba T. V generation wise rearing performance

Generation & AmCPV dose	Treatment	Survival (%)	Single Cocoon Weight (gm)	Single Shell weight (gm)	Shell Ratio (%)
G1 (1 x 10^3 PIB/ml)	AmCPV	2.96	9.96	1.08	10.84
		(9.89 ± 0.39)	(18.40 ± 0.08)	(5.97 ± 0.05)	(19.22 ± 0.08)
	Inoculated Control	3.06	9.77	1.03	10.50
		(10.07 ± 0.12)	(18.21 ± 0.10)	(5.81 ± 0.08)	(18.91 ± 0.21)
	Healthy Control	28.33	10.46	1.21	11.60
		(32.15 ± 0.27)	(18.87 ± 0.05)	(6.32 ± 0.05)	(19.91 ± 0.10)
G2 (1 x 10^3 PIB/ml)	AmCPV	6.33	9.72	1.05	10.78
		(14.47 ± 1.30)	(18.17 ± 0.18)	(5.88 ± 0.10)	(19.16 ± 0.22)
	Inoculated Control	3.07	9.70	1.06	10.89
		(10.09 ± 0.09)	(18.15 ± 0.12)	(5.90 ± 0.07)	(19.27 ± 0.12)
	Healthy Control	27.76	10.38	1.18	11.33
		(31.80 ± 0.21)	(18.80 ± 0.02)	(6.23 ± 0.02)	(19.67 ± 0.04)
G3 (1 x 10^4 PIB/ml)	AmCPV	6.15	9.63	1.02	10.63
		(14.30 ± 0.97)	(18.07 ± 0.20)	(5.81 ± 0.05)	(19.02 ± 0.30)
	Inoculated Control	1.21	8.05	0.63	7.76
		(6.31 ± 0.32)	(16.48 ± 0.02)	(4.54 ± 0.05)	(16.18 ± 0.21)
	Healthy Control	23.26	10.29	1.11	10.78
		(28.80 ± 0.13)	(18.71 ± 0.03)	(6.05 ± 0.03)	(19.17 ± 0.09)
G4 (1 x 10^4 PIB/ml)	AmCPV	12.23	9.70	1.00	10.36
		(20.50 ± 0.16)	(18.14 ± 0.25)	(5.75 ± 0.04)	(18.78 ± 0.23)
	Inoculated Control	0.92	8.10	0.67	8.31
		(5.51 ± 0.04)	(16.54 ± 0.01)	(4.71 ± 0.04)	(16.75 ± 0.16)
	Healthy Control	23.31	10.29	1.11	10.78
		(28.87 ± 0.14)	(18.71 ± 0.02)	(6.05 ± 0.03)	(19.17 ± 0.10)
G5 (1 x 10^5 PIB/ml)	AmCPV	12.09	9.85	1.01	10.25
		(20.35 ± 0.13)	(18.29 ± 0.20)	(5.77 ± 0.08)	(18.67 ± 0.19)
	Inoculated Control	0.00	0.00	0.00	0.00
		(4.06 ± 0.04)	(4.06 ± 0.01)	(4.06 ± 0.04)	(4.06 ± 0.16)
	Healthy Control	22.55	10.28	1.09	10.57
		(28.35 ± 0.07)	(18.70 ± 0.03)	(5.98 ± 0.06)	(18.97 ± 0.22)
G6 (1 x 10^5 PIB/ml)	AmCPV	14.28	9.88	1.02	10.32
		(22.20 ± 0.10)	(18.32 ± 0.19)	(5.80 ± 0.06)	(18.74 ± 0.16)
	Inoculated Control	0.00	0.00	0.00	0.00
		(4.06 ± 0.04)	(4.06 ± 0.01)	(4.06 ± 0.04)	(4.06 ± 0.16)
	Healthy Control	22.16	10.23	1.10	10.71
		(28.08 ± 0.37)	(18.66 ± 0.05)	(6.01 ± 0.08)	(19.10 ± 0.23)

G7 (1 x 10^6 PIB/ml)	AmCPV	13.80	10.23	1.11	10.85
		(21.81 ± 0.10)	(18.66 ± 0.02)	(6.05 ± 0.04)	(19.23 ± 0.12)
	Inoculated Control	0.00	0.00	0.00	0.00
		(4.06 ± 0.04)	(4.06 ± 0.01)	(4.06 ± 0.04)	(4.06 ± 0.16)
	Healthy Control	21.92	10.26	1.13	11.04
		(27.92 ± 0.32)	(18.69 ± 0.03)	(6.11 ± 0.02)	(19.41 ± 0.10)
CD at 5%:					
Treatment (T)		0.66	0.18	0.09	0.26
Generation (G)		0.43	0.12	0.06	0.17
T x G		1.14	0.31	0.15	0.45

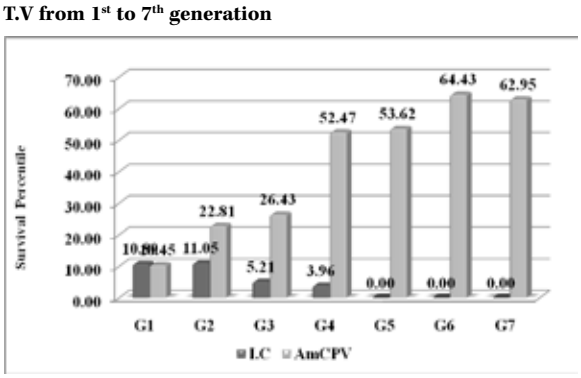
Statistical Analysis: Analysis of variance was conducted to study the effect of *AmCPV* in Daba TV among seven generations. The analysis of variance (Table 2) showed highly significant (P<0.001) values in the treatment, generation and treatment x generation for all tested traits i.e. survival, cocoon weight, cocoon shell weight and shell ratio.

Table 2. Analysis of Variance to study the effect of AmCPV in Daba TV among seven generation

Source of variation	DF	Mean Sum of Square			
		SUR	SCW	SSW	SR
Generation (G)	6	4.931 ***	50.124 ***	0.925 ***	59.847 ***
Treatment (T)	2	2807.458 ***	330.830 ***	11.275 ***	370.039 ***
G*T	12	47.054 ***	51.394 ***	0.566 ***	52.809 ***
Error	42	0.481	0.035	0.008	0.076
Total	62	100.474	25.494	0.569	28.001
Note: *** indicate highly significant at p<0.001 level					

Percentile improvement: Percentile improvement of Survival trait in *AmCPV* treated batches was increased from 10.45 (1st generation) to 62.95 (7th generation) and shown gradual linier improvement (Fig 1) where as in the inoculated control percentile improvement was decreased from generation G_1 to G_4 and reached to zero from generation G_5 to G_7.

Fig 1. Percentile improvement of AmCPV tolerance in Daba T.V from 1st to 7th generation



Note: Survival percentile of I.C and AmCPV is calculated by treating Healthy control as 100%

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