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Research Article

TOTAL MIDGUT PROTEIN AND PROTEASE ACTIVITY DURING DIFFERENT STAGES OF MULTIVOLTINE BREEDS OF SILKWORM, BOMBYX MORI L AND THEIR HYBRIDS

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ABSTRACT
Protease enzyme varies in different silkworm breeds and also within a voltine group. In the present study, two multivoltine breeds viz., Madagascar (MAD) and NDV6 were utilized which are known for poor and high silk productivity respectively. The genetic phenomenon involved in the transfer of genes responsible for the production of enzyme from parents to their offspring was studied by estimating the enzyme activity in MAD x NDV6 (F1), its reciprocal, F2 and backcrosses. The findings of the study clearly indicate that the enzyme activity is maximum in NDV6 x MAD (RF1) rather than its F1 during 5^{th} instar 5^{th} day (peak eating period). The total gut protein was maximum in F1 during 3^{rd} and 5^{th} day of 5^{th} instar larvae. The protease enzyme activity in parents, F1, F2, BC1 and BC2 along with reciprocal crosses followed by the expression of hybrid vigour over their respective parents are discussed.

Key Words: *Multivoltine, Protease Enzyme, Midgut Protein, 5^{th} Instar 3^{rd} Day, 5^{th} Day Larvae And Pupae*

INTRODUCTION
In *Bombyx mori* most of the characters are polygenic in nature. Silkworm breeds within an voltine group also vary widely in many of the quantitative traits. The multivoltine breeds are known for their poor productivity and higher survival. However advancement in breeding techniques resulted in the improvement of quantitative traits with better silk yield in multivoltine breeds and resulted in the development of productive multivoltine breeds viz., BL67, ND7 and NDV6 (Ram Mohan Rao *et al.*, 2007). This can be attributed for the incorporation of bivoltine genes into the improved multivoltine breeds. The bio-chemical and Molecular Markers are the latest tools in identifying the breed for their original characters rather than from the phenotypic expression. As silkworm is known for its ability to convert mulberry protein to silk protein with the help of protease enzymes, there is a need to study the relationship of protease enzyme profiles in multivoltine breeds and total silk protein production. It is well documented that bivoltine breeds produce higher protease enzyme than multivoltines (Sarangi, 1986; Lokesh and Ananthanarayana, 2001).

The production of digestive enzymes is often dependent to a large extent upon the type of food consumed by the insets. In the silkworm, *Bombyx mori*, the presence of intestinal proteinases has been reported by Shinoda (1930) and several investigations have been done in this direction mainly in Japan (Horie *et al.*, 1963; Eguchi and Yoshitake, 1967; Hamano and Mukaiyama, 1970). Because of the importance of digestive fluid enzymes in the digestion of mulberry leaves and of higher protease activity, attention has been focused primarily on digestive fluid protease, whereas midgut tissue protease has received little attention. The proteases in the midgut tissue and digestive fluid increases with increasing feeding from 1^{st} to 5^{th} day of 5^{th} instar and the secretion of protease occurs in response to feeding (Hamano and Mukaiyama, 1970; Persaud and Davey, 1970; Lee and Anslee, 1995). The relationship between midgut proteases from larva and pharate adult is yet unclear. Keeping these in view, an attempt has been made by utilizing the exotic traditional multivoltine breed 'Madagascar' (MAD) and the productive new multivoltine breed NDV6 for estimating the total midgut protease

activity and the protein content. In addition, the total midgut protease activity and the protein content in their F1 hybrid, Reciprocal F1 (RF1), F2, Reciprocal F2 (RF2), Back crosses (BC1 and BC2) were also estimated and hybrid vigour for the activity is estimated.

MATERIALS AND METHODS

The two multivoltine breeds 'Madagascar'(MAD) and 'NDV6' were drawn from the germplasm of CSRTI, Mysore and were reared in three replication each. The F1 hybrid viz., MAD x NDV6 and its reciprocal NDV6 x MAD (RF1) was prepared and reared.By utilizing the multivoltine breeds and their F1 and RF1, F2, RF2, BC1 i.e., Back Cross1 i.e., F1 x MAD and RF1 x MAD, BC2 i.e., Back Cross2 i.e., F1 x NDV6 and RF1 x NDV6 were prepared and reared in three replication each by following the standard rearing techniques (Kawakami, 2001). The mid gut tissue and the digestive juice were collected from all the breeds, F1,RF1, F2,RF2, BC1 and BC2 during 3rd and 5th day of 5th instar larvae and pupal stage to estimate the activity of protease by following the techniques of Eguchi and Iwamoto (1982) and the total midgut protein content by Lowry *et.al.,* (1951). The rearing was conducted in the favourable months of June~ July and August ~ September, 2010 and the mean of two seasons data for total midgut protease activity and protein content was computed. The percentage of improvement in protease activity and total midgut protein between 3rd day v/s 5th day, 5th day v/s pupal and pupal v/s 3rd day was calculated by following the formula:

$$\text{Percentage of Improvement} = \frac{(a-b)}{B} \times 100$$

Where, a= To be compared (ex:5th instar 5th day, pupal stage);
b= Compared with (ex:3rd and 5th day of 5th instar)

The hybrid vigour in the hybrids over their respective parents for the total midgut protein and Protease activity was calculated by the following formula:

$$\text{Heterosis} = \frac{(F1 - MPV)}{MPV} \times 100$$

Where MPV = Mid Parental Value

RESULTS

The total midgut protein and protease activity were highest in 3rd and 5th day of 5th instar larvae and lowest total protein during pupal stage in NDV6. Among F1 and RF1, The highest protease activity with higher protein content was recorded in F1 at all the developmental stages except for the total protein content at pupal stage and protease activity in 5th instar 5th day larval stage. The RF2 expressed maximum gut protein during 3rd day of 5th instar, while F2 showed maximum protein content in pupal stage. Marginal variation was recorded for the protease activity when F2 and RF2 was compared. Not much variation in the enzyme activity and total midgut protein was noticed when F1 x MAD v/s RF1 x MAD was compared. RF1 x NDV6 showed maximum protein content in 3rd day and F1 x NDV6 during pupal stage. The protease activity was maximum in RF1 x NDV6 during pupal stage when compared to F1 x NDV6, while marginal variation was recorded in 3rd and 5th day of 5th instar larvae for the protease activity (Table 1).

Comparative Improvement (Percentage) in the midgut protease activity and protein content in multivoltine breeds and their hybrid

Comparison between enzyme activity and protein content showed maximum expression of 50.33% increase in midgut protease activity during pupal stage in NDV6 when compared to MAD. The F1 expressed 31.09% and 22.79% higher gut protein when compared to RF1 during 3rd and 5th day of fifth instar larvae and 16.01 and 37.57% higher protease activity in 3rd day of fifth instar and pupal stage respectively. F2 exhibited maximum of 27.91% and 15.82% protein content and protease activity

respectively during pupal stage when compared over RF2. Not much improvement was recorded when F1 x MAD was compared with RF1 x MAD for protease activity and protein content during different developmental stages.

Table 1: Comparative Total Midgut Protein and Protease Enzyme Activity in Multivoltine Breeds and Their Hybrids (Mean of Two Rearings)

Breed	Total Gut Protein (mg/g)			Total Mid Gut protease (mg/g)		
	3rd day	5th day	Pupae	3rd day	5th day	Pupae
MAD	113.34	130.21	77.95	12.38	17.85	6.12
	±1.53	±2.15	±2.42	±0.68	±0.79	±0.55
NDV6	119.27	151.74	68.56	13.39	19.99	9.20
	±1.31	±1.70	±1.20	±0.55	±0.70	±0.52
% of improvement	**5.23**	**16.53**	**-12.05**	**8.16**	**11.99**	**50.33**
F1	131.38	162.88	40.89	15.94	17.59	9.52
	±2.01	±0.59	±3.26	±0.51	±0.42	±0.91
RF1	100.22	132.65	77.55	13.74	18.99	6.92
	±3.06	±2.05	±2.69	±0.59	±0.38	±1.08
% of improvement	**31.09**	**22.79**	**-47.27**	**16.01**	**-7.37**	**37.57**
F2	109.33	136.22	81.30	12.59	18.49	9.59
	±7.71	±5.52	±13.66	±0.68	±1.58	±1.15
RF2	122.93	130.94	63.56	12.72	17.45	8.28
	±9.63	±6.39	±6.37	±0.46	±0.91	±0.61
% of improvement	**-11.06**	**4.03**	**27.91**	**-1.02**	**5.96**	**15.82**
F1*MAD	95.82	126.44	68.99	14.20	19.09	9.95
	±1.15	±4.29	±2.23	±0.36	±0.54	±0.39
RF1*MAD	97.12	124.88	66.56	14.52	20.29	10.63
	±0.59	±0.99	±1.31	±1.38	±0.92	±0.93
% of improvement	**-1.34**	**1.25**	**3.65**	**-2.20**	**-5.91**	**-6.40**
F1* NDV6	100.61	133.56	68.08	14.65	22.32	6.98
	±2.02	±2.43	±0.99	±1.02	±0.36	±0.59
RF1*NDV6	110.04	138.51	50.74	14.21	21.82	11.98
	±5.97	±1.84	±1.62	±0.56	±0.54	±2.62
% of improvement	**-8.57**	**-3.57**	**34.17**	**3.10**	**2.29**	**-41.74**

F1 x NDV6 exhibited 34.17% higher total midgut protein at pupal stage than RF1 x NDV6 (Table 1).
Comparative Improvement (Percentage) in the midgut protease activity and protein content between stages
Comparative midgut protease activity and total midgut protein between different stages in multivoltine breeds and hybrids revealed improvement when 5th instar 5th day larvae was compared with 3rd day of 5th

Table 2: Comparative Total Midgut Protease Enzyme Activity in Percentage Between Different Developmental Stages of Multivoltine Breeds and Their Hybrids

Stage/Breed and hybrids	MAD	NDV6	F1	RF1	F2	RF2	F1*MAD	RF1*MAD	F1* NDV6	RF1* NDV6
5thv/s 3rd	44.14	49.24	10.34	38.16	46.85	37.27	34.46	39.74	52.32	53.56
Pupalv/s5th	-65.72	-53.97	-45.89	-63.57	48.13	-52.54	-47.88	-47.52	68.71	-45.07
Pupalv/s3rd	-50.59	-31.31	-40.29	-49.67	-23.83	-34.85	-29.92	-26.76	52.34	-15.65

Table 3: Comparative Total Midgut Protein Content in Percentage Between Different Developmental Stages of Multivoltine Breeds and Their Hybrids

Stage/Breed and hybrids	MAD	NDV6	F1	RF1	F2	RF2	F1*MAD	RF1*MAD	F1* NDV6	RF1*NDV6
5thv/s 3rd	14.85	27.22	23.97	32.36	24.40	6.03	31.96	28.59	32.76	25.88
Pupalv/s5th	-40.14	-54.81	-74.90	-41.54	-40.23	-51.24	-45.43	-46.70	-49.04	-63.37
Pupalv/s3rd	-31.25	-42.51	-68.88	-22.63	25.64	-48.30	-27.99	-31.46	32.34	-53.89

Table 4 Hybrid vigour For The Total Midgut Protein and Protease Activity in RF1,RF1,F2,RF2 and Their Back Crosses

	Total Mid Gut Protein			Total Mid Gut Protease		
	3rd day	5th day	Pupae	3rd day	5th day	Pupae
F1	12.95	15.54	-44.18	23.68	-7.03	24.27
F2	-16.8	-16.5	98.8	-21.0	5.1	0.8
F1*MAD	-21.7	-13.7	16.13	0.28	7.77	27.3
F1*NDV6	-19.7	-15.1	24.4	-0.1	18.8	-25.4
RF1	-13.84	-5.90	5.86	6.63	0.36	-9.68
RF2	9.09	2.53	4.84	-7.47	-8.07	19.76
RF1*MAD	-9.07	-4.99	-14.38	11.16	10.17	63.17
RF1*NDV6	0.26	-2.59	-30.55	4.71	11.75	48.71

instar larvae for both protease activity and total midgut protein. When the pupal protease activity is compared with 5th day of fifth instar larvae, only F2 (48.13%) and F1 x NDV6 (68.71%) expressed improvement, while for the total midgut protein there was no improvement. Comparison between the pupal stage v/s 3rd day of fifth instar larvae, improvement in the protease activity was noticed in F1 x NDV6(52.34%) and for the protein content improvement was recorded in F2 (25.64%) and F1 x NDV6 (32.34%) (Table 2 and 3).

Hybrid Vigour

The positive hybrid vigour for the protease activity was recorded in F1 (3rd day of fifth instar and pupal stage), F2 (5th day of fifth instar and pupal stage),F1 x NDV6 (5th instar fifth day), RF1(in 3rd and 5th day of 5th instar), RF2 (in pupal stage), F1 x MAD, RF1 x MAD and RF1 x NDV6 in all the three stages (Table 4). For the total midgut protein, positive hybrid vigour was noticed during 3rd and 5th day of fifth instar in F1, pupal stage in F2, F1 x MAD, F1 x NDV6 and RF1. RF2 expressed positive heterosis for all the three stages taken for the study. RF1 x MAD expressed negative heterosis for the midgut protein in all the three stages, while RF1 x NDV6 showed positive heterosis during 3rd day of fifth instar larval stage (Table 4).

DISCUSSION

The biochemical studies are highly valuable to understand the genetic variation in natural population and have been used as useful indicator in plant and animal breeding programmes (Tanksley *et al.,* 1982). Among many digestive enzymes, protease enzyme in silkworm plays a key role in converting the mulberry protein to silk protein. It has been reported that the Protease enzyme activity was observed to be higher in the midgut tissues of the multivoltine breeds and their hybrids (Eguchi *et al.*,1972; Eguchi and Arai, 1983; Chatterjee *et al*., 1989; Tanaka, 1964; Sarangi, 1985) and the same has been confirmed in the present study.

The higher protease activity has been recorded in the 5th instar 5th day in the multivoltine breeds and their hybrids and agrees with the earlier findings where it has been well documented that the activity of midgut protease increases with age of the silkworm larvae (Maribashetty *et al.,* 2001; Horie *et al*., 1963; Mukayama *et al.*,1964*;* Chatterjee *et al.,* 1989; Lakshmikumari, *et al.,* 1997). Between the two breeds, NDV6 expressed higher midgut gut protease activity as it is a productive multivoltine breed compared to MAD and parallels the findings of Sarangi (1986) who reported higher activity of protease in bivoltine (NB7) over the multivoltine (PM) breed as bivoltines are productive. The higher percentage of increase in the protease enzyme activity during 5th instar 5th day can be attributed for the voracious eating habit during 5th day and higher consumption of mulberry protein (Tables 1~3). The productive multivoltine breed NDV6 by higher midgut protease enzyme with better response to feed supplement shows increased capability of protein digestion. The contribution of productivity (protease enzyme and midgut protein) from parents to their offspring does not follow Mendelian pattern of inheritance which has been clearly depicted in F1, F2,RF1, RF2, BC1 and BC2 indicating that the genes responsible for enhancement of protease activity is not under the control of single gene but it is polygenic.

The maximum hybrid vigour for protein content in F1 (3rd and 5th day of fifth instar) and RF1(pupal stage) clearly shows that the protein content is very less in MAD resulting in lower Mid Parental value. During 5th day of 5th instar stage, only F1 x NDV6 showed positive vigour and at pupal stage F2,F1 x MAD,F1 x NDV6 and RF2 expressed positive vigour indicating the contribution of NDV6 over generation for the midgut protein (Table 4). For the protease activity, the positive hybrid vigour was recorded in F1 x MAD, RF1 x MAD and RF1 x NDV6 during larval stages, while at pupal stage F1, RF1, RF2 and RF1 x NDV6 exhibited positive vigour clearly indicating the higher contribution from NDV6 (Table 4).

From the present findings it is very much clear that to enhance productivity in multivoltine breeds, it is better to cross highly productive multivoltine breed with low productivity breed so that the hybrid

express better for productivity as the genes received from the productive breed express fully in F1. To conclude, the breed with lower potentiality for the conversion of mulberry protein to silk protein through protease enzymes as female parent when crossed with breed with higher silk potentiality as male parent gives better silk output in its F1 rather than the reciprocal hybrids. The hybrid MAD x NDV6 has been adjudicated as the productive multivoltine hybrid than NDV6 x MAD.

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