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**STUDIES ON THE CORRELATION BETWEEN DNA, RNA AND PROTEINS OF
BOMBYX MORI L.**

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ABSTRACT

Four pure mulberry silkworm breeds viz., Pure Mysore, Nistari, NB₄D₂ & CSR₂ and two hybrid (Pure Mysore x CSR₂ and Nistari x NB₄D₂) silkworms were selected for the present study. The total DNA, RNA and soluble proteins present in the mid alimentary canal was estimated during fifth instar with a regular interval of 24 h, and the average concentration during fifth instar was calculated. The average concentration of DNA, RNA and proteins were subjected to regression analysis with each other individually to know the kind and level of correlation coefficient between them. The results of regression analysis between DNA and RNA as well as between RNA and Protein exhibited very strong level of correlation coefficient between them. The average concentration of DNA and protein exhibited moderately strong positive relationship between them. The concentration of DNA, RNA and protein as well as their ratios are more in hybrid silkworms.

Keywords: Silkworm, *Bombyx mori*, Mid Gut, DNA, RNA, Protein, Correlation

INTRODUCTION

Many researchers have been studied on the morphology, anatomy, cytology, embryology and physiology of *Bombyx mori* when compared to molecular biology aspects of

silkworm [1]. The DNA content in insect tissue is an index for expressing other biochemical contents like Protein and RNA. The increase in DNA to RNA along with

protein suggests the activation of metabolic process like protein synthesis. It also expresses the protein metabolism of silkworm [2]. **Brindha et al.**, 2012, [1] reported that higher level of protein, DNA and RNA is seen by increasing from I to V instar stage. Recently, genetic markers have used in animal and plant improvement programmes for varietal and parentage identification, construction of linkage maps and evaluation of polymorphic genetic loci affecting quantitative economic traits. Development of molecular markers is important in the silkworm for construction of linkage map and fingerprinting of strains for breeding [3]. Also, PCR based techniques have been widely used to detect the polymorphic genetic markers in the silkworm [3, 4]. Random amplified polymorphic DNA (RAPD) is one the PCR based techniques used as a tool for genetic mapping and strain identification [5, 3, 6]. Because of its relative simplicity, RAPD method is being extensively used in genetic analysis [4]. The most important factor in biological genetic resource conservation regimes is to maintain pure strains of each species and establish accurate genetic relationship among species [7]. A number of reports concerning the correlation aspects of silkworm *Bombyx mori* i.e., esterase and cocoon shape [8]; esterase and

ontogeny [9]; yield and biochemical parameters [10]; amylase and larval span, cocoon weight, shell weight, filament length, cocoon color, cocoon shape [11]; commercial characters with protein [12], amylase [13], esterase [14], alkaline phosphatase [15] succinate dehydrogenase [16] were reported. However, the correlation studies between biomolecules like DNA, RNA and proteins are rather scanty. Therefore, present investigation was carried out.

MATERIALS AND METHODS

Four pure mulberry silkworm breeds viz., Pure Mysore, Nistari, NB₄D₂ & CSR₂ and two hybrid (Pure Mysore x CSR₂ and Nistari x NB₄D₂) silkworms were selected for the present investigation. The silkworm rearing was conducted in the laboratory following the method described by **Krishnaswamy**, 1979, [17]. All experimental batches were maintained in triplicate. The midgut tissues was obtained during fifth instar from five larvae daily, with a regular interval of 24 h. till the end of fifth instar by dissecting the larvae in ice cold water and the gut contents were removed. The tissues were thoroughly washed in sterile distilled water. A 10% homogenate was prepared in buffered saline (0.15 M NaCl and 0.15 M sodium citrate, pH 7.0) using mortar and pestle. The homogenate was centrifuged at 8000 rpm for

15 minutes in a cooling centrifuge at 5°C. The clear supernatant was used for the assay of total proteins, DNA and RNA.

The total proteins present in midgut tissues was determined during fifth instar daily with a regular interval of 24 h. by following the method of **Lowry et al.**, 1951, [18]. Bovine serum albumin was used as standard protein. Average total protein concentration during 5th instar was calculated. The results were expressed as µg of protein/mg tissue. The amount of DNA was estimated during fifth instar daily with a regular interval of 24 h. by diphenylamine method [19]. Calf thymus DNA was used as standard. The average DNA concentration during 5th instar was calculated. The results were expressed as µg of DNA /mg midgut tissue. The concentration of RNA was estimated during fifth instar daily with a regular interval of 24 h. by orcinol method [19]. Yeast RNA was used as standard. The average RNA concentration during 5th instar was calculated. The results were expressed as µg of RNA /mg midgut tissue.

The experimental data were statistically analyzed through SPSS by one way ANOVA [20], Scheffe's post hoc test [21] and linear regression analysis [22] wherever they were applicable

RESULTS

The concentration of DNA, RNA and total protein in midgut tissue is shown in the **Table 1**. From the Table it is clear that the average concentration of DNA was high in Pure Mysore x CSR₂ (17.1 µg/mg) followed by Nistari x NB₄D₂ (17 µg/mg), Pure Mysore (16.6 µg/mg), NB₄D₂ (15.70 µg/mg), CSR₂ (15.20 µg/µl), and Nistari (13.51 µg/mg). In case of RNA, almost same trend was observed i.e., the highest average concentration of RNA was observed in Pure Mysore x CSR₂ (30.60 µg/mg) followed by Pure Mysore (28.30 µg/mg), Nistari x NB₄D₂ (27.60 µg/mg), CSR₂ (23.24 µg/µl), NB₄D₂ (21.64 µg/mg) and Nistari (24.20 µg/mg). Further, in the case of midgut total proteins the highest average concentration of protein was observed in Pure Mysore x CSR₂ (33.82 µg/mg) followed by Nistari x NB₄D₂ (29.99 µg/mg), Pure Mysore (23.99 µg/mg), CSR₂ (26.90 µg/µl), NB₄D₂ (26.60 µg/mg) and Nistari (20.39 µg/mg). The concentration of DNA, RNA and total proteins in midgut tissue samples showed significant changes in their levels among the experimental sets. The results of one way ANOVA revealed that the variation among the experimental batches are all found to be significant at 0.1 % (P<0.001). Further, the results of regression analysis between midgut DNA and RNA, RNA and total proteins, as well as DNA and total

proteins are presented in the **Figures 1-3**. From the results of regression analysis it is clearly showed that DNA exhibited highly strong positive correlation ($R^2=0.661$) with RNA. The RNA also revealed highly strong positive ($R^2=0.713$) relationship with protein. However, The DNA showed moderately strong positive ($R^2=0.357$) correlation with proteins. In the case of the DNA, RNA and protein ratio the hybrid silkworms exhibited highest position followed by pure strains of both multivoltine and bivoltines.

DISCUSSION

In the present investigation two multivoltines, two bivoltines and two hybrid silkworm strains were used. The multivoltines are known for poor productivity and good resistance against fluctuating environmental conditions. The bivoltines are known as good yielder with poor resistance. The hybrids silkworms exhibit mid parental values for the commercial characters analyzed in the present investigation. The concentration of DNA, RNA and proteins was high in case of hybrids when compared to their parents except RNA concentration in Pure Mysore silkworms. The ratios of DNA, RNA and protein showed statistically significantly variation in their level among the experimental sets. The regression analysis between DNA and RNA, RNA and proteins

as well as DNA and proteins clearly indicated the positive correlation coefficient (**Figures 1 - 3**) with each other. The deoxyribonucleic acids and ribonucleic acid are most important biomolecules of the cell as they controls overall metabolism. Such biochemical growth-rate indicators, such as RNA concentration or the RNA/DNA ratio, are routinely used for estimating growth rates and nutritional condition of larval fish [23]. Also, studies on the concentration of DNA are of paramount importance in the breeding program, because the DNA content in insect tissue is an index for expressing other biochemical contents like RNA, Protein, cell division, growth and development. The increase in RNA to DNA along with protein suggests the activation of metabolic process like protein synthesis. Our results also correlation with this assumption as hybrids exhibited more protein ratio with both DNA and RNA when compared to their parents. Of the hybrids Pure Mysore x CSR₂ silkworms revealed increment in overall ratios. **Singh and Saratchandra**, 2004, [24] reported that RNA plays a major role in protein metabolism and morphogenesis. **Brindha et al.**, 2012, [1] in their studies reported that the DNA and RNA content were increased proportionately in the larva as the age progressed as well as in silk gland. Also they reported that higher

level of protein, DNA is seen by increasing from first to fifth instar stage. Our results are also in agreement with the above researchers.

CONCLUSION

The concentration of DNA, RNA and proteins was found to be high in the case of hybrid silkworms when compared to their parents. Such a basic knowledge about these molecular aspects is essential to plan detailed studies at the molecular level for identification and exploitation of biochemical markers during hybridization experiments. In addition, the correlation between various biomolecules during the breeding of new strains of silkworm *Bombyx mori* or any other crop varieties with improved traits. Also, the information gathered from this research work contributes a lot to basic molecular biology of insects in general.

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Table 1: Average Concentration of DNA, RNA and Total Proteins (µg/mg) in Midgut Tissue During Fifth Instar and Their Ratios

Silkworm Breeds	Average concentration of DNA	Average concentration of RNA	RNA ratio to DNA	Average concentration of total proteins	Protein Ratio to RNA	Protein Ratio to DNA	Ratio of DNA, RNA and Protein
PURE MYSORE	16.60	28.30	1.70	23.99	0.85	1.44	1:1.70:1.44
NISTARI	13.51	24.23	1.79	20.39	0.84	1.51	1:1.79:1.50
CSR ₂	15.20	26.90	1.77	23.24	0.86	1.53	1:1.77:1.52
NB ₄ D ₂	15.70	26.60	1.67	21.64	0.81	1.38	1:1.67:1.38
PURE MYSORE x CSR ₂	17.10	30.60	1.79	33.82	1.10	1.97	1:1.79:1.97
NISTARI x NB ₄ D ₂	17.00	27.60	1.62	29.99	1.09	1.76	1:1.62:1.76

NOTE: The Variation Between the Races is Statistically Significant at 0.1 % (P<0.001)

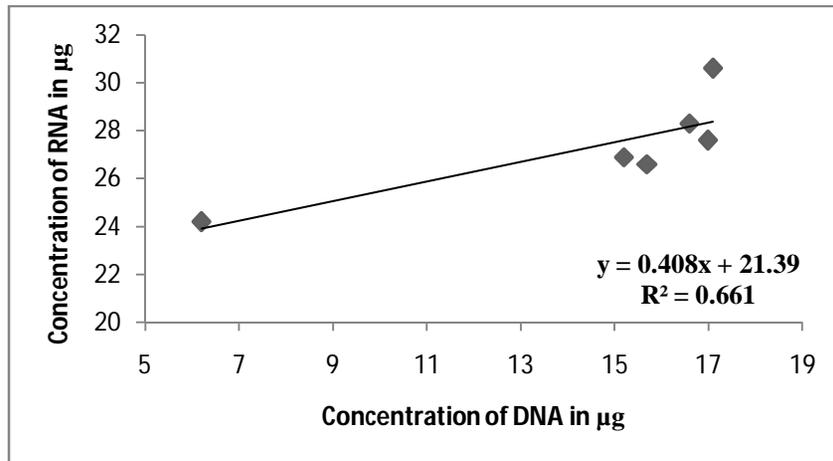


Figure 1: Correlation Between DNA and RNA

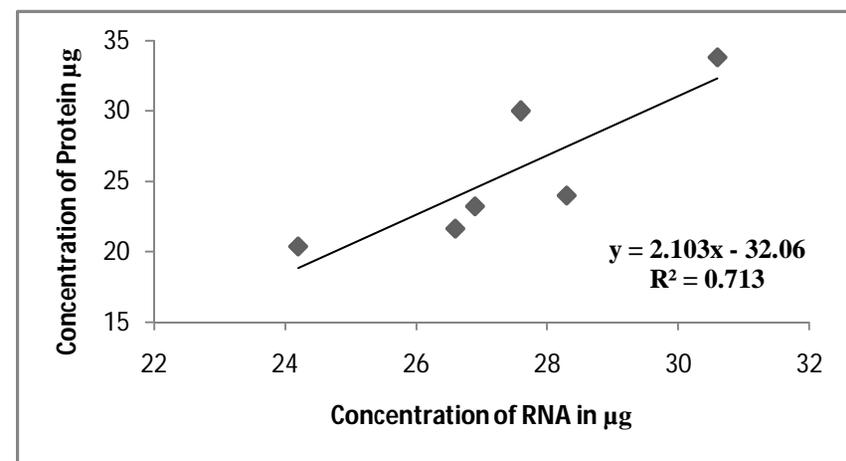


Figure 2: Correlation Between RNA and Proteins

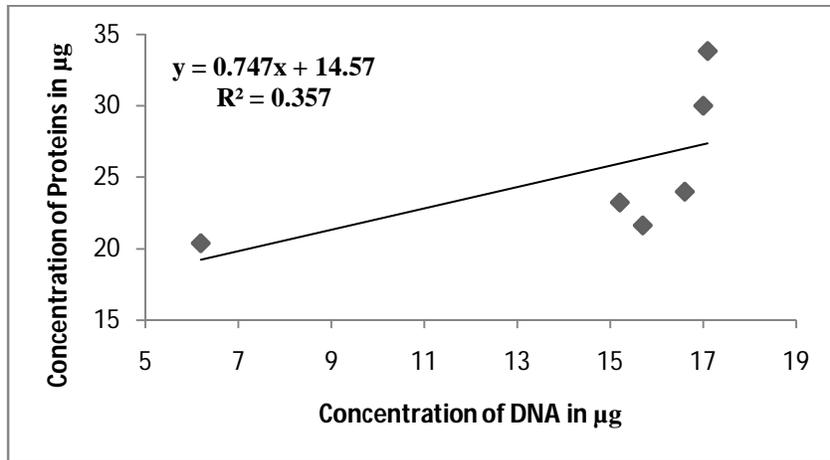


Figure 3: Correlation Between DNA and Proteins