Biochemical and Molecular Analysis of *Bombyx Mori* L. Strains Associated with their Host Plants

Thesis submitted in partial fulfillment of the requirements for the degree of
Doctor of Philosophy in Biotechnology

By

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CERTIFICATE

I certify that the thesis entitled **"Biochemical and molecular analysis of host plant interaction in *Bombyx mori* L. strains"** submitted to the Mizoram University for the award of a degree of Doctor of Philosophy in Biotechnology by RUTH LALFELPUII is a record of research work carried out by her during the period from 2013 to 2016 under my guidance and supervision and this work has not formed the basis for the award of any degree, diploma, associateship, fellowship or other similar titles in this University or any other University or institution of higher learning.

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Declaration of the Candidate

I, Ruth Lalfelpuii Ph. D. scholar in Biotechnology Department, Mizoram University, Aizawl do hereby solemnly declare that the subject matter of this thesis is the record of the work done by me. I have duly worked on my Ph. D. thesis under the supervision of Prof. N Senthil Kumar, Department of Biotechnology, Mizoram University and Prof. G. Gurusubramanian, Department of Zoology, Mizoram University. This is being submitted to Mizoram University for the degree of Doctor of Philosophy in Biotechnology and that I have not submitted this thesis to any other University/ Institute for any other degree.

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(RUTH LALFELPUII)

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List of Acronyms

ha	- Hectare
MT	- Metric Tonnes
Kg	- Kilogram
ROS	- Reactive oxygen species
DNA	- Deoxyribonucleic acid
SDS	- Sodium dodecyl sulphate
PAGE	- Polyacrylamide gel electrophoresis
PCR	- Polymerase chain reaction
RAPD	- Random amplified polymorphic DNA
SSR	- Simple sequence repeat
FISSR	- Fluorescent-inter simple sequence repeat - PCR
SNP	- Single nucleotide polymorphism
PMFS	- Phenylmethylsulfonyl fluoride
nm	- Nanometer
DPPH	- 1, 1-Diphenyl-2-Picrylhydrazyl
SD	- Standard deviation
%	- Percentage
Avg	- Average
GAE	- Gallic acid equivalent
QE	- Quercetin equivalent
ºC	- Degree Celsius
a.m	- Ante meridiem
p.m	- Post meridiem

h	- Hour
GR	- Growth rate
CR	- Consumption rate
CI	- Consumption index
AD	- Approximate digestibility
ECI	- Efficiency of conversion of ingested food
ECD	- Efficiency of conversion of digested food
Wt	- Weight
mL	- Milli litre
mM	- Milli molar
rpm	- Revolution per minute
M	- Molar
FC	- Folin and Ciocalteu's
O.D	- Optical density
UV- VIS	- Ultraviolet Visible
µg	- microgram
P_H	- Hydrogen ion concentration
µL	- microlitre
TCA	- Trichloroacetic acid
g	- gram
CAT	- Catalase
SOD	- Superoxide dismutase
U	- Unit
V	- Volt

NBT	- Nitro blue tetrazolium chloride
TEMED	- Tetramethylethylenediamine
RT	- Room temperature
IC_{50}	- half maximal inhibitory concentration
ANOVA	- Analysis of variance
RNA	- Ribonucleic acid
cDNA	- Complementary DNA
DNTP	- Deoxynucleotide triphosphate
RT-PCR	- Reverse transcriptase PCR
COX1	- Cytochrome oxidase 1
ND1	- NADH dehydrogenase 1
CytB	- Cytochrome b
ITS1	- Internal transcribed spacer DNA 1
SE	- Standard error
kDa	- Kilo Dalton
M	- Marker
β	- Beta
bp	- Base pair
ML	- Maximum likelihood
ME	- Minimum evolution

1. INTRODUCTION AND REVIEW OF LITERATURE

Sericulture, an agro-based industry involves rearing of silkworms for the production of raw silk, which is the yarn obtained out of cocoons spun by sericigenous caterpillar. The major activities of this industry comprises of cultivation of host plants on which silkworms feed and reeling the cocoons for extraction of the silk fiber for value added benefits such as processing and weaving. It is one of the most labour intensive sectors of economy development and is an effective tool for rural construction. Like any other agriculture allied sector, sericulture has played a critical role in rural development and employment generation. Although production costs have increased in recent times, an increased productivity witnessed through technology intervention have proved that this sector is commercially viable.

Silk, as a weavable fiber was first discovered by the Chinese empress Xi Ling Shi during 2,640 B.C. Silk was a profitable trade commodity in China, India and many other countries (Ganapathy, 2014). Till today, silk reigns supreme as an object of desire and fabric of high fashion. Being a rural based industry, the production and weaving of silk are largely carried out by relatively poor sections of the society and this aspect of sericulture has made it popular and sustainable in countries like China and India.

Systematic position

Phylum	: Arthropoda
Class	: Insecta
Order	: Lepidoptera
Family	: Bombycidae
Genus	: *Bombyx*
Species	: *mori*

Life cycle

The life cycle of silkworm represents the most advanced form of metamorphosis. Termed holometabolous, the silkworm completes life cycle (**Figure 1**) through serial progression of four distinct stages of development; egg, larva, pupa and adult. The number of life cycles (generations, which is termed as voltinism) per year depends on the silkworm strain and it varies with the environmental conditions particularly temperature. Newly hatched larva is black in colour, has large head and the body is densely covered with bristles so that it looks like a hairy caterpillar. The larva is an elongated caterpillar, the only feeding stage in the life cycle. As larva grows, it becomes smoother and lighter in color due to the rapid stretching of the cuticular skin during the different instars of the larval stage. During larval life, the larva sheds its skin (molt) 4 times to accommodate growth. The period between successive molts is called instars. At the end of final instar (fifth instar), larvae cease feeding, and their bodies become shorter, stouter, and transparent. These larvae are called mature larvae; the larva spins a silk cocoon of one continuous fiber within which it undergoes pupation. Silk cocoons are the commercial source of silk. Bred in captivity for thousands of years on trays of mulberry leaves, *B. mori* is fully domesticated and cannot survive without the assistance of man. The silk cocoon serves as protection for

the pupa. Cocoons are shades of white, cream and yellow depending on silkworm variety. After a final molt inside the cocoon, the larva develops into a brown, chitin covered structure called the pupa. Metamorphosis of the pupa results in an emerging moth or adult. The moth is covered with heavy, round, furry scales and lacks functional mouthparts, so are unable to consume food. The forewing has a hooked tip, which is a characteristic feature of this family; however it is flight-less. Wings and body are usually white, but may vary in shades of light brown. Wing span is 1.5 to 2.5 inches. (4-6 cm). It is the reproductive stage where adults mate and females lay eggs. Adult is the final stage in the life cycle of *B. mori* with short life span of 4-6 days (Savithri et al., 2013). Sericulture in India as well as in North East has a rich tradition since time immemorial and become the inseparable part of culture and custom in the society. Yet its transformation from a traditional art to a modern scientific technology is of recent origin.

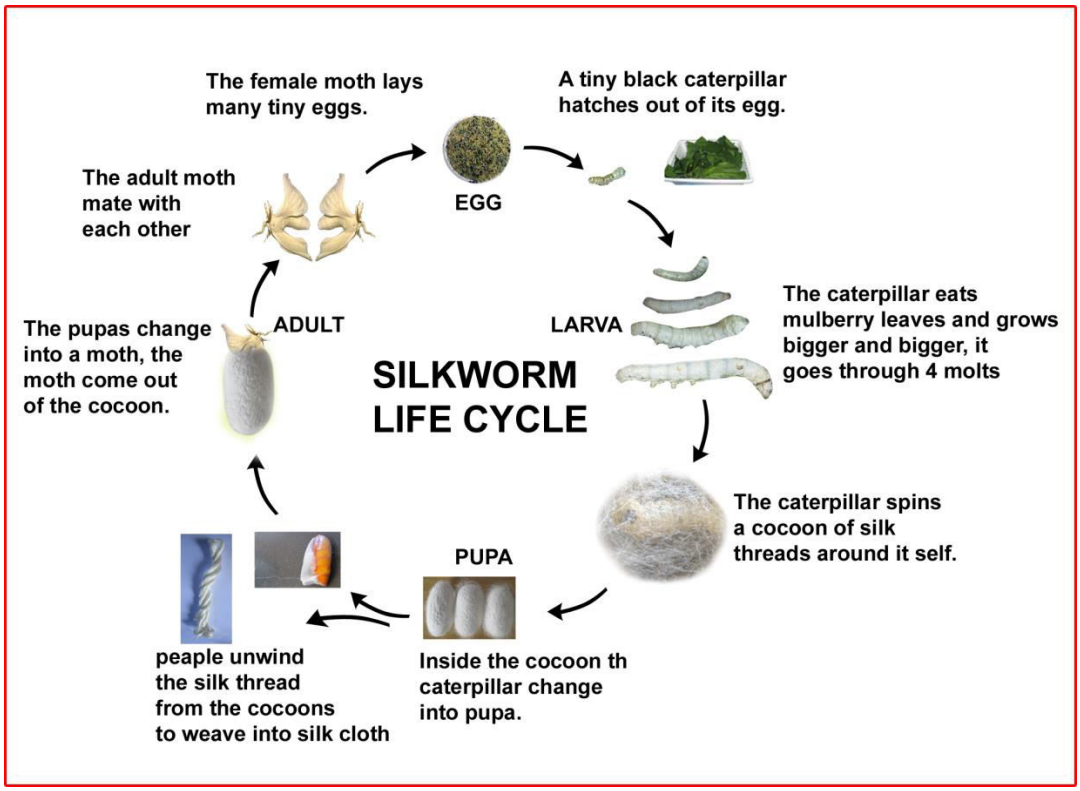


Figure 1. Life Cycle of *Bombyx mori.*

Science and technology is used as an effective instrument of growth and change. It is being brought into the mainstream of economic planning in the sectors of agriculture, industry and services (Kumar and Somashekhar, 2008). The country's resources are used to derive the maximum output for the benefit of society and improvement in the quality of life. Being a cottage industry par excellence, it is one of the most labor intensive sectors of the Indian economy combining both agriculture and industry, providing means for livelihood to a large section of the population i.e, mulberry cultivator, co-operative rearer, silkworm seed producer, farmer-cum rearer, reeler, weaver, traders etc. It is the only one cash crop in agriculture sector that gives returns within 30 days. This industry provides employment nearly to thirty five million people in our country.

In the global scenario, India is the world leader in tropical sericulture and stands second in raw silk production in the world next to China (Chinnaswamy, 2012). Silk production in temperate countries like Japan, South Korea etc., is declining steadily not only because of the high cost of labour and heavy industrialization in these countries, but also due to climatic restrictions imposed on mulberry leaf availability that allows only two cocoon crops per annum. Thus, India has a distinct advantage of practicing sericulture all through the year, yielding a stream of about 4 – 6 crops as a result of its tropical climate (Singh and Gangopadhyay, 2013). Indian domestic silk market has over the years been basically driven by multivoltine mulberry silk. Due to inferior quality of the silk produced, India could not meet the international quality standards. To produce silk of International quality, promotion of bivoltine sericulture is imperative so as to meet international demand and earn foreign exchange. Bivoltine sericulture for seed production of both cross breed layings and bivoltine hybrid layings assumes national priority. Emphasis for bivoltine sericulture was given during 1970’s only, which demand evolution of highly nutritious

and high yielding region specific mulberry varieties, silkworm breeds. The size of the cocoons of bivoltine races is bigger and silk content is more. These races require optimum climatic condition, food plants with high nutritive value (Krishnaswami, 1979).

Mizoram is located in the North Eastern part of India bordering with Myanmar and Bangla-desh. The population of Mizoram was 1,091,014 according to a 2011 census (Department of Agriculture, 2015). The state lies between 21.58º N to 23.35° N Latitude and between 92.15º E to 93.29° E Longitude. It has the agro climatic zones of humid temperate sub alpine zone, humid sub- tropical hill zone, and humid mild tropical zone (Directorate of Economics and Statistic, 2015). According to vegetations, forest of Mizoram can be classified into six types as tropical evergreen forest, sub-Himalayan semi evergreen forest, sub-tropical pine forest, mixed forest, and overlapping bamboos. The climatic condition, fertility of the soil, rainfall, etc. are most sui-table for rearing as well as for breeding of all kinds of silkworm. It may be mentioned here that India, including the state of Mizoram, is the only country where all variety of silks like Mulber-ry, Eri, Muga and Tasar are commercially exploited. Its temperature ranges during summer 22 ^{0}C to 32 ^{0}C and during winter 10 ^{0}C to 23 ^{0}C with average annual rainfall 2123 mm per year. Besides the favorable climatic condition, the land is attractive for international trade by its loca-tion. Being a biodiversity hotspot (Rai, 2009) the land of Mizoram is one of the most attractive places in the world for rearing of silkworms (both wild and domesticated silkworms). As clima-tic conditions influence the performance of sericulture, specifically the silkworm rearing depends largely on the moderate temperature and relative humidity. In this respect, the prevailing climatic condition has to be studied before the implementation of any sericultural programme. Mizoram state is known to enjoy a moderate and uniform climate all through the year. This enables the state essentially well suited to the requirements of sericulture.

Mizoram sericulture scenario

Mizoram like other states of North East are endowed with untapped natural resources. But unlike other state of North East, in Mizoram, systematic rearing of Mulberry silkworm started from the early part of the nineteenth century only. Presently, about 3700 ha land is covered under cultivation of Mulberry plantation and more than 5054 families are actively involved in sericulture in Mizoram.

Table 1: Year-wise cocoon and raw silk production of Mizoram.

Year	Cocoon production (MT)	Yarn production (MT)	Areas under plantation (Ha)
2005-06	47.00	2.00	1169
2006-07	48.00	6.00	1169
2007-08	45.00	6.00	1169
2008-09	71.00	9.00	1680
2009-10	92.00	16.00	2200
2010-11	184	26.00	2860
2011-12	190	26.00	3650
2012-13	300	29.67	2330
2013-14	340	34.00	2981
2014-15	351	40.00	3700

It can be estimated that the productivity of raw silk is only 11 kg per hectare whereas; the productivity at national level is 89 kg per hectare (**Table 1**) (Department of Sericulture, 2014; Department of Sericulture, 2015). So, there is sufficient scope to increase the productivity of the state which would be possible only by adopting scientific techniques (Choudhury et al., 2013).

Among silkworm, the bulk of commercial silk produced (92%) in the world is mulberry silk that comes from the domesticated silkworm, *B. mori* L. (Yogananda et al., 2013). *B. mori* is essentially monophagous, host specific insect which feeds solely on mulberry leaves which belongs to Family: Moracea and Genus: *Morus*. Mulberry is a member of the genus *Morus* of the family Moraceae and has many species and varieties. Different mulberry variety reacts to the climatic conditions particularly temperature which affects their quality (Lalfelpuii et al., 2014). India has rich resources of mulberry varieties which are traditionally cultivated, where a few exotic varieties have been introduced time to time. Besides environment and technology adoption, the growth and development as well as cocoon and silk production of the silkworm larvae and subsequently silk productivity entirely depends upon the quantity and quality of mulberry leaves (Nagaraju et al., 2002). Development of silkworm is greatly influenced by chemical composition of the leaves where the quality of feed is determined by its major components such as water, carbohydrates, proteins, mineral, elements, fats, amino acids and vitamins (Jyothi et al., 2014). Several scientists have established variation in biochemical components in mulberry leaves. Bose et al. (1991) revealed that different mulberry varieties differ significantly among themselves in respect of composition of nutrients. The role of soluble and crude protein in silkworm nutrition has been widely recognized (Pillai and jolly, 1985). Machii and Katagiri (1991) indicated that nutritive value of mulberry leaves depend on nitrogen content in general, and amino acids in particular. Carbohydrates are very important for growth of silkworms, especially this are required for maintaining healthy growth of young silkworm larvae. Fats or lipids are particularly the main forms of energy reserves because the stored fats are only exhausted in fasting. The role of fatty acids and fats in the developmental stages of *Anthereae assama* and its host plant were reported (Kataky and Hazarika, 1997). Silkworm larvae should fed on leaves containing balance-

ed nutrient components comprising amino acids (proteins), carbohydrates, lipids, vitamins, minerals etc. Hence, it is required to feed the silkworms with leaves containing balanced nutrient components like carbohydrates, proteins, lipids (Ito, 1978). Narayanan et al. (1967) recorded some different varietal superiority of different varieties as food for silkworm greatly affects the economy of sericulture industry. In order to expect good silk harvest, a suitable plant variety, the leaves of which are most preferred by the insect body should be selected (Murugan et al., 1998). Nutritional intake has direct impact on the overall genetic traits such as larval and cocoon weight, amount of silk production, pupation, and reproductive traits. For better understanding of any insect plant relationship, the study on quantitative aspect of nutrition in the insect is important (Waldbauer, 1968). The energy obtained from daily consumption of food by the silkworm is distributed and absorbed in the body of larvae, adult, eggs, and cocoons. A larva consumes and utilizes sufficient amount of food to grow large enough within an instar to moult into the next instar and accumulate sufficient energy reserves to perform its metabolic activities during the non-feeding moulting period. Feeding with inferior quality leaves makes the adult smaller, and less productive. As such, the amount of food consumed and the quantity digested by the silkworms has direct effect on its performance, mating success and reproduction. Deficiency of certain nutrients or imbalance of nutrient on the diet affects the digestibility and metabolic activity of larvae (Waldbauer, 1964).

One of the most rapid plant defense reactions to biotic stress is “oxygen burst” which constitutes the production of reactive oxygen species (ROS) including superoxide radical anion (O_2-), hydrogen peroxide (H_2O_2) and hydroxyl radical (-OH) (Pandhair and Sekhon, 2006). Among the generated ROS, a central role in plant defense responses is played by hydrogen per-

oxide. To avoid the hazards associated with oxidative stress many plant materials have been identified and documented as promising sources of natural antioxidants. Besides this, antioxidant attributes of these plant materials have been investigated as a function of growing location, species, and cultivation conditions etc. and noticeable differences are observed. Now, there is ample evidence regarding variation in proximate composition, phenolics and antioxidant activity with respect to different species of a plant (Iqbal et al., 2012). Antioxidant system for removing hydrogen peroxide has been detected in Lepidoptera larvae and other leaf chewing insects (Barbehenn et al., 2001). There is evidence suggesting that the fitness of insects can be influenced by the plants they feed on (Schoonhoven et al., 1998). This fitness does not just relate to the fact that a plant can be considered a suitable host because the insect can feed and develop on it, but because it gains some secondary benefits from feeding on a specific species or group of plants. Exogenous and endogenous sources, including prooxidant allelochemicals and other xenobiotics in the host orchards could severely affect herbivorous insects during host interactions and stress such as starvation may induce accumulation of reactive oxygen species such as superoxide radical's, hydroxyl radical, hydrogen peroxide, and hydrogen peroxides throughout their lifetime. Unchecked or increased levels of ROS can cause severe damage to various cellular compartments, including DNA, protein and lipids, thereby causing oxidative injury. Nonetheless, insects have evolved a complex antioxidant mechanism to overcome the toxic effects of ROS (Nagasaka et al., 2004; Krishnan and Kodrik, 2006). Antioxidant defense is primarily contributed by antioxidant enzymes such as peroxidase, superoxide dismutase and catalase (Felton and Summer, 1995). Ingestion of host plants by mulberry silkworm greatly increases mortality, a result of formation of ulcer like lesions through the midgut epithelium. The formation of midgut lesions has been attributed to the direct interaction of tannins with the midgut epithelia of insects

(Bernays et al., 1980). Another possible mode of action of ingested tannins is oxidative stress in epithelial tissues wherein some caterpillar's consumption of low molecular weight phenols causes elevated levels of oxidative damage to proteins and lipids in midgut tissues (Bi and Felton, 1995), as well as midgut lesions (Lindroth and Peterson, 1988). Ingested tannins appear to oxidize in the gut lumens of insects (Barbehenn et al., 1996), suggesting that if antioxidant defenses were inadequate, oxidative stress could produce lesions in the midgut epithelium. The antioxidant enzyme defenses of mulberry silk worm as well as in its gut lumen reared with different host plant is known less. Studies on the antioxidant enzymes of the caterpillars commonly have examined enzyme activities in the whole body homogenates, providing no information on the antioxidant defenses in gut lumen. However, it is in the gut lumen that ingested phenolic compounds may become extensively oxidized. Previous work on antioxidant enzymes in insects has been done primarily on caterpillars (Berbehenn et al., 2001), where a variety of antioxidants enzymes protects caterpillar tissues and extracellular fluids from oxidative damage. These enzymes serve as a defensive role in insect herbivores that feeds on plants containing high levels of potential pro-oxidants and their activities are high in the tissues of some insect species that feed on such host plants (Felton et al., 1992).

Silk, a proteinaceous fiber is synthesized with complete metabolism of leaf protein by the silkworm. *B. mori* produces massive amount of silk proteins, stored in the middle silk gland during the final stage of larval development which are then discharged through the anterior duct and spinneret, at the end of the fifth instar (Shimizu, 2000). The major components of silk cocoon which have been distinguished are of two kinds of silk proteins, fibroin- a fibrous protein which is the main component of the silk (70-76%) forms the core of the silk thread and is

composed of High (H) - chain 350 kDa (Zhou et al., 2000), Low (L) - chain 26 kDa (Yamaguchi et al., 1989), and Glycoprotein P25 30 kDa (Chevillard et al., 1986) linked by disulfide bonds. It is the fibroin filament which is reeled and woven into silk fabric. These three types of fibroin (H-chain, L-chain and P 25) are common among different silk producing insects in Lepidoptera. Fibroin is exclusively synthesized in the posterior part of the silk gland. It is transferred by the peristalsis into the middle silk gland where it is covered with sericin and is stored until spinning. Fibroin is composed of eighteen amino acids. Out of these eighteen amino acids, glycine, alanine, tyrosine and serine are the chief constituents and constitutes about 80 percent of fibroin (Sabina et al., 2012). And, the second silk protein being sericin- is a natural macromolecular protein, serving as an adhesive to unite fibroin for making silk cocoons of silkworm, *B. mori* (Borgohain, 2015). Mulberry leaves are rich in protein and amino acids, and there is a high correlation between leaf protein levels and the production efficiency of the cocoon shell i.e., the cocoon shell weight relative to the total amount of mulberry leaves consumed by the silkworm (Machii and Katagiri, 1991). It is therefore possible that an increase in the protein level of mulberry leaves may lead to improvements in cocoon productivity. The nutritional richness of the diet influences the accumulation of storage proteins in the hemolymph of the silkworm larvae. Amounts of storage protein in silkworm larvae fed on a low protein diet were less than those fed on the standard diet, but larvae fed on optimal levels of protein showed higher levels of storage proteins (Nagata and Kobayashi, 1990). Protein quantification studies by SDS-PAGE reveal the molecular weight of the proteins in the tissues. Fibroin heavy chain and light chain were identified on the SDS-PAGE and the molecular weights were same but the expression differed in these varieties (Sabina et al., 2012). SDS-PAGE protein profile studies on tasar silkworm suggested that hemolymph proteins influence the growth and development of silkworm larvae (Barsagade

and Tembhare, 2004). Seo et al. (1985) also studied the protein patterns of the hemolymph and various tissues of final-instar larvae and pupae by electrophoresis: 15 protein bands were separated from the hemolymph during metamorphosis and were maintained at a high concentration during the final larval instar, but declined after pupation. So far, there are limited reports on the protein profile of the effects of host plant on mulberry silkworm in the silk gland protein.

Differential gene expression underlies a range of biological processes, including development, reproduction, and behavior. Intense interest in this topic and the application of new technologies has produced rapid advances in the analysis of transcriptional control of gene expression in humans and a number of other species that serve as foci for study. The impact of this area of study on various fields of biology indicates that entomology will be permeated by research on transcriptional control of gene expression. The completion of *B. mori* genome sequences has provided an opportunity to understand the silkworm and the silk gland in general (Xia et al., 2004). The effects of feeding on different varieties of mulberry host plants on different strains of *B. mori* upon the nature and gene expression of larval silk gland fibroin are less known.

For selecting a silkworm hybrid for its commercial exploitation genetic attributes like viability etc. are also to be taken into consideration. Lack of assessing genetic diversity in the available germplasm, unavailability of modern tools to know the genomes at the molecular level, environmental disturbances during the time of selection and phylogenetic control of various traits in silkworm have led to poor selection of parents in breeding programs (William et al., 1990). It is well known that the resistance to biotic and abiotic constraints is governed by polygenes with complex inheritance patterns and with lot of environmental influences (Promboon et al., 1995). Therefore, it is required to produce genotypes for particular geographical environment by utilizing the races acclimatized to that location. New tools like molecular markers can be effectively

applied with conventional breeding strategies and the genes for the resistance can be discovered (Murthy et al; 2006). For breeding or improving promising cultivars, precise determination and discrimination of the genotypes are required. A few studies employing mitochondrial DNA sequences (Hwang et al., 1999) were carried out with Bombycid and Saturniid. The most desirable or suitable silkworm race with disease tolerance or high yielding characteristics can be developed using molecular markers in selection and breeding (Reddy et al., 2009). DNA markers are known to have many advantages over morphological and biochemical markers, as they are more stable and independent of environmental influences and allow researchers to identify accessions at the taxonomic level, assess the relative diversity within and among species, and locate diverse accessions for breeding purposes. Therefore, it is imperative to develop a molecular marker system to study the genetic background of number of economically important silkworm strain. A number of DNA markers system like random amplified polymorphic DNA (RAPD), restriction fragment length polymorphism (RFLP), simple sequence repeat (SSR), fluorescent dye labeled ISSR PCR reaction (FISSR-PCR) and single nucleotide polymorphism (SNP) analysis have been developed to distinguish the genetic diversity among silkworm species (Nagaraju et al., 2002). Several cytochrome oxidase genes have been sequenced and characterized in silkmoth (Li et al., 2005). Germ plasm resources are very much required for the continuous improvement of crop plants, where genetic characterization is essential for scientific germ plasm conservation and characterization permits the estimation of genetic relatedness and diversity (Bhat and Jarret, 1995).

Sericulture by virtue of its economic importance drew maximum attention since long time, particularly, studies relating to silkworm nutrition, evolution and evaluation of new mulberry varieties and silkworm strain for high yield and quality (Lalfelpuii et al., 2014). Such

studies are very scanty in north eastern states including Mizoram, but a great deal of work has been done in other parts of India. Hence, keeping in view of the above for understanding the potential of *B. mori* strain and mulberry varieties in Mizoram, this research work proposes to assess the physiological and biochemical response of *B. mori* strain to different mulberry varieties as well as genetic diversity and expression of silk gland related traits and also to quantify the antioxidant capacity among the different mulberry varieties and *B. mori* on agro climatic conditions of Mizoram.

2. AIMS OR OBJECTIVES

The following aims are set forth to carry out the proposed work in this study:

1. To analyze the effect of the host plants on the developmental and biochemical profiles of different strains of *Bombyx mori*.
2. To analyze the transcript level of fibroin gene of *B. mori* as influenced by the host plants.
3. Assessment of genetic relatedness of *B. mori* by DNA profiling to elucidate their relatedness and to correlate with their host plant preference.

3. MATERIALS AND METHODS

3.1. Mulberry varieties used

Planting materials were collected from the mulberry plantation maintained at Research extension centre, Central Silk Board, Shillong, Meghalaya. The local variety was collected from Tanhril locality, Aizawl, Mizoram (**Figure 2A**).

Local (H1) : Locally available mulberry leaves (Hmu-te).

Jorhat (H2) : Origin not known, maintained at CSR & TI, CSB, Jorhat

BC2-59 (H3) : Variety developed at CSR & TI, CSB, Berhampore, by back crossing of hybrid of Matigare Local x Kosen (Japanese variety) with Kosen twice.

TR10 (H4) : It is a triploid selection of Berhampore S-1 variety developed at CSR & TI, Berhampore.

3.2. Study Site

The selected mulberry varieties were grown in the experimental field of Department of Biotechnology, Mizoram University, Tanhril, Aizawl, Mizoram (**Figure 2B**). Tanhril located at an altitude of 950 meters above the mean sea level, and in between 92°38' to 92°42'E longitude and 23°42' to 23°46'N latitude and situated in the north eastern part of the Indian sub-continent.

The plot was laid out in randomized block design (RBD) with three replications. Irrigation, weeding, fertilizer application measures were followed as per the package of practices for rain fed mulberry accessions (Rama Kant and Bhat, 2010).

Figure 2. Mulberry plant varieties and their cultivation. (A) Four different mulberry plant varieties used (Local, Jorhat, BC2-59, TR10). (B) Plantation of mulberry host plants.

3.3. Silkworm strains used

Disease free layings of seven bivoltine silkworm strains SK6 x SK7 (S1), SK7 x SK6 (S2), CSR2 x CSR4 (S3), CSR4 x CSR2 (S4), FC1 x FC2 (S5), FC2 x FC1 (S6) were procured from germ plasm of National silkworm seed organization, Bangalore and one pure Japanese strain J112 (S7) was procured from germ plasm of the Department of Sericulture, Aizawl, Mizoram (**Figure 3A**).

3.4. Biochemical analysis of mulberry leaves

The different host plants: Jorhat, TR10, BC2-59 and Local (Hmute) varieties were subjected to various biochemical analyses.

Total Protein

Protein content was quantitatively measured by Lowry method (1959). 100 mg of fresh mature mulberry leaves was crushed and ground with mortar and pestle in phosphate buffered saline solution. Acetone and Phenylmethylsulfonyl fluoride (PMFS) were added and vortexed until it was mixed. The solution was then centrifuged at 7000 rpm for 10 min. The supernatant was mixed with 200 µL of Na_2CO_3 and 50 µL of Lowry's reagent and allowed to stand for 30 min. The solution turned blue and color development was read at 660 nm against water blank by using UV-Vis Spectrophotometer (Molecular Devices, USA). The protein content was calculated by standard bovine serum albumin. The results were expressed in µg/mL.

Total Carbohydrates

Carbohydrate content was quantitatively measured by using a method described by Yemm and Willis (1954). For estimation of carbohydrates content, 100 mg of leaves of different mulberry varieties were ground in 2 mL of hot 80 % alcohol with the help of mortar and pestle. Then leaves samples were centrifuged at 8000 rpm for 10 min. To the supernatants, 100 µL of Anthrone reagent was added which were then heated in boiling water for 10 min where green colour developed. The absorbance of green colour was taken to estimate the carbohydrate content by using UV-Vis Spectrophotometer (Molecular Devices, USA) at 620 nm. The carbohydrates content was calculated by standard glucose. The results were expressed in µg/mL.

Total lipids

Total lipid content was quantitatively measured by using a method described by Bligh and Dyer (1959). About 100 mg of each different mulberry leaves was homogenized with 500 µL of chloroform and methanol mixture (2:1 v/v) using mortar and pestle. Samples were taken for centrifugation for 10 min at 5000 rpm. To the supernatant 500 µL of chloroform and 1% KCl were added and centrifuged at 5000 rpm for 10 min. Supernatant was vacuum concentrated and the residues of different samples were weighed for total lipids content and expressed in µg/mL.

Total amino acids

Total amino acids were estimated by modified ninhydrin method (Moore and Stein, 1948). 100 mg of different mulberry leaves were grinded with 80% methanol using mortar and pestle. Homogenate was centrifuged at 5000 rpm for 10 min. The supernatant was partitioned with an equal volume of petroleum ether to remove chloroplast pigments which was then centrifuged at

5000 rpm for 10 min. The supernatant was used for amino acid estimation. To 50 µL of sample extract, 50 µL of 80 % alcohol was mixed. 200 µL of ninhydrin reagent was added to the mixture which was then kept on boiling water for 10 min. Later, it was cooled under running water. Absorbance was read at 570 nm using UV-Vis Spectrophotometer (Molecular Devices, USA). The amino acid content was calculated by standard glycine. The results were expressed in µg/mL.

Total reducing sugar

Reducing sugars were estimated by DNS method (Miller, 1972). To 50 µL of the extract, 50 µL of water was added. 150 µL DNS reagent was mixed and boiled in a water bath for 5 min. After the development of the coloured product, 100 µL of 40 % Rochelle salt solution was added and mixed well. After cooling the mixture, absorbance was read at 510 nm using reagent blank adjusted to zero absorbance using UV-Vis Spectrophotometer (Molecular Devices, USA).

Moisture content

For estimating the moisture content of leaves, 2 g of fresh plant material was weighed and kept in an incubator at 37 ºC till a constant weight was obtained. The difference between initial and final weight was calculated and water content was represented in percentage (Shobana et al., 2010).

3.5. Minerals content

Sample preparation

To prepare the plant material for the determination of calcium, magnesium, the wet digestion was performed. An accurately weighed plant sample (0.1 - 0.3 g) was transferred to closed flask.

5 mL of 30 % H_2O_2 solution and 10 mL of concentrated H_2SO_4 solution were added and the sample was digested until the solution became clear. Then, it was transferred to the volumetric flask and the volume was made up to 100 mL with distilled water. The samples were used for analysis of minerals such as magnesium, calcium, potassium and phosphorus (Jackson, 1973).

For phosphorus estimation, plant extract was prepared with distilled water at temperature of 85 °C incubation. To the accurately weighed plant sample (1.0 g) 30 mL of hot water was added, then stirred with electromagnetic stirrer for 30 min and filtered through a paper filter with medium–sized pores (Whatman, Germany). The filtrate was collected in the volumetric flask and diluted to 50 mL with the twice-distilled water.

Calcium

Few drops of 5 % ammonium oxalate solution were added to the extract. The presence of white layer of CaC_2O_4 was noted after warming the mixture for 10 min. The amount of calcium was estimated from the white layer by the UV-Vis Spectrophotometer (Molecular Devices, USA) at 320 nm. Concentration was represented by mg/g dry wt. unit.

Magnesium

Few drops of 10 % sodium ammonium phosphate ($NaNH_4HPO_4.4H_20$) or micro-cosmic salt were added to the extract and made strongly alkaline with NH_4OH. After stirring, the presence of a flocculent layer was noted. The magnesium was estimated from the reaction mixture at 480 nm. Concentration was represented by mg/g dry wt. unit.

Phosphorous

Few drops of 5% molybdenum blue solution were added to the extract. The presence of a light blue reaction was noted after warming the mixture for 5 min. The amount of phosphorous was estimated by the UV-Vis Spectrophotometer (Molecular Devices, USA) from the white layer at 650 nm. Concentration was represented by mg/g dry wt. unit.

Potassium

Exchangeable K and Na were determined after extraction with 1N NH_4OAc, pH 7.0 (Schollenberger and Simon, 1945) by the method of flame photometric analysis. Organic matter was destroyed by $HN0_3$ oxidation. A Beckman model DU flame emission spectrophotometer was used with an oxygen-acetylene flame. Lithium was used as an internal standard (Rich, 1965). Potassium was determined at absorbance 670 nm wavelength (Dean, 1960).

3.6. Antioxidant evaluation of the different mulberry varieties

Preparation of plant extracts

Fresh leaf samples of the different mulberry species (Local- Hmute, Jorhat, BC2-59 and TR10) were collected from experimental field of Department of Biotechnology, Mizoram University. After collection, the samples were washed with running tap water followed by rinsing with distilled water. The leaves were air-dried until a constant weight was attained. The dried samples were ground to powder form by a blender followed by extraction with water and methanol using a Soxhlet apparatus. All the extracts were finally concentrated using rotary evaporator.

Total phenolic content

Total phenolic content of leaf extracts was evaluated according to Folin-Ciocalteu method (Singleton and Rossi, 1965). Folin-Ciocalteau phenol reagent was added to 0.5 mL extract and stored for 3 min. 2.5 mL of 7.5% (w/v) sodium carbonate (Na_2CO_3) solution was added and incubated at 45°C for 45 min. The absorbance was calculated at 765 nm and the total phenolic content was measured using gallic acid standard curve.

Total flavonoid content

Total flavanoid content was determined using modified method described previously (Zhishen et al., 1999). The plant extract was mixed with 1.5 mL of methanol, 0.1 mL of 10% AlCl3, and 0.1 mL of 1 M potassium acetate and 2.8 mL of distilled water. The reaction mixture was incubated for 30 min at room temp and the absorbance was measured at 415 nm and the total flavonoid content was measured using quercetin standard curve.

Scavenging of 1,1-diphenyl-2-picrylhydrazyl (DPPH)

The plant extract of different concentration was added to 3 mL of methanol DPPH (0.1 mM) and incubated at room temperature in the absence of light for 30 min. The absorbance was measured at 517 nm. The percentage of inhibition of DPPH (1%) was calculated using the following equation: $I\,\% = (A_0 - A/A_0) \times 100$

A_0 is the absorbance of the blank solution and A is the absorbance of the extract (Bruits et al., 2001).

3.7. Bioassay studies to ascertain the host plant effect on growth patterns and performance of *B. mori*

Rearing was conducted at the laboratory, Department of Biotechnology, Mizoram University, Aizawl, Mizoram. Silk worm rearing was done as per standard rearing package (Krishnaswami, 1978).

Disinfection

Prior to rearing (three days before actual hatching of the eggs), all the equipments and the rearing room were disinfected by spraying with 4% formaldehyde solution to free the rearing environment and surroundings free from pathogen. After spraying, the room is kept closed for 20 h, then the doors and windows are opened and the room kept open for approximately 24 h for all traces of formaldehyde to disappear.

Mass culture of silk worm

All the seven bivoltine races of *B. mori* were reared with locally available mulberry leaves (Hmute) up to second instar and then fed with four different mulberry varieties (**Figure 3B**) individually (temperature 25 ºC and relative humidity 72%). There were 84 experimental sets and each set was replicated thrice (7 silkworm races x 4 host plant varieties x 3 replicates = 84) (**Figure 3C**). Each experimental set comprised of a rearing

Figure 3. Rearing of *Bombyx mori* in different mulberry varieties. (A) Eggs (Disease free laying) of bivoltine hybrid silkworm; (B) *B. mori* strain CSR4 X CSR2 reared with four different host plants; (C) General view of *B.mori* silkworm rearing experiment

tray with 20 larvae. The larvae were fed *ad libitum* daily four times with known quantity of chopped mulberry leaves (**Figure 4A**) from each of the four varieties separately at an interval of 4 h. First feed was given at 07:00 h, while last feeding was at 19:00 h and continued till spinning. Nets with meshes were laid on the top of the rearing tray and fresh leaves were spread above them. The tray was cleaned off with the excreta and left over foods, when the larvae climbed on the top of the tray for feeding fresh leaves. The larvae were dusted before the first feed with (3%) bleaching powder during active stages. During moulting stage, the larvae were not fed or dusted.

Food utilization indices

The weight gain of the larvae and weight of food consumed and faeces produced were determined using an electronic balance (Mettler, USA) (**Figure 4B**). After initial weighing, the larvae were introduced into separate containers and allowed to feed on weighed quantity of different host plants for a period of 24 h. Larvae were again weighed. The difference in weight of the larvae gives the fresh weight gained during the period of study. Samples larvae were weighed, oven dried and reweighed to establish a percentage dry conversion value which was used to estimate the dry weight of the experimental caterpillars. The different host plants remaining at the end of each day were oven dried and weighed. Aliquots of the different host plants were weighed, oven dried and reweighed to establish percent dry weight conversion values to allow estimation of the dry weight of the diet given to the larvae. The quantity of the food ingested was estimated by subtracting the (measured) dry weight of diet remaining at the

end of each experiment from the (calculated) total dry weight diet initially provided. Faeces were collected daily and weighed, and oven dried and reweighed to estimate the dry weight of excreta. The experiment was continued for 6 days and observations were recorded for every 24 h. Various food utilization indices (all based on dry weight) were calculated in a traditional manner (Waldbauer, 1968) to assess the growth and feeding efficiencies of different silkworm strains feed with different host plants.

Growth rate (GR)	= P/T
Consumption rate (CR)	= E/T
Consumption index (CI)	= E/TA
Approximate digestibility (AD) (%)	= 100 (E – F)/E
Efficiency of conversion of ingested food (ECI) (%)	= 100P/E
Efficiency of conversion of digested food (ECD) (%)	= 100P/ (E–F)

Where, **A**: mean dry weight of animal during **T**; **E**: dry weight of food eaten; **F**: dry weight of faeces produced; **P**: dry weight gain of insects; **T**: duration of the experimental period.

Figure 4. Cocoon production and reeling of silk. (A) Chopping of the mulberry leaves for silkworm feed; (B) Weighing the remaining food, excreta and larva using an electronic balance accurate to ± 0.01; (C) Cocoon of J112 strain reared with different host plant; (D) Reeling of silk thread using an Eprouvette machine.

Larval weight and cocoon characteristics of mulberry silkworm reared on different mulberry host plants

The important economic characters were recorded. Wet larval weight, cocoon weight, shell weight and filament weight were measured using electronic balance accurate to ± 0.01 g (Mettler, USA). Shell percentage and denier was calculated using the formula mention below:

Shell percentage = Wt. of cocoon shell/Wt. of cocoon x 100

Denier = (Filament Weight in grams / Filament length in meters) x 9000.

The length of the bave (silk thread) from the corresponding cocoons **(Figure 4C)** was carefully reeled employing a reeling apparatus (eprouvette) and the total length was measured in meters **(Figure 4D)**.

3.8. Biochemical estimation of *B. mori* fed with different host plants

The silkworm larva fed on different host plants were used for estimation of biochemical parameters. Larval hemolymph and silk gland protein was estimated.

Hemolymph Protein

Fifth instar larvae were collected, and the abdominal legs were punctured with sterile scissors (**Figure 5A**). The hemolymph was then collected in a clean, precooled 1.5 mL micro-centrifuge tube containing 1 mM of thiourea, centrifuged at 3000 rpm for 5 minutes in a cooling centrifuge at 5 °C (Mahesha et al., 2002) and cell free hemolymph preserved in a deep freezer at -20 °C as stock. One mL of the test solution was taken in a clean dry test tube. 5 mL of Lowry's reagent (98 mL of 2 % sodium carbonate in 0.1M NaOH + 1 mL of 1 % $CuSO_4$ + 1 mL of 2 % sodium potassium tartarate) was added.

After 15 min 0.5 mL of FC (Folin and Ciocalteu's Phenol) reagent (1:1 diluted with distilled water) was added and left for 30 min under dark condition. The O.D was measured at 660 nm in a UV-Vis spectrophotometer. Bovine serum albumin was used as standard protein. The results were expressed as µg/mL (Lowry et al., 1951).

Silk Gland Protein

Fifth instar larvae were dissected and the silk glands on both sides were extracted with the help of a forceps carefully. The middle silk glands (**Figure 5B**) were cut into small pieces with the help of scissors and 500 mg was taken and homogenated in (10%) in 0.01M Tris-HCl buffer (pH 7.0) containing 0.1% sodium dodecyl sulphate (SDS) and 0.9% NaCl. The extracts were centrifuge at 2,000 rpm for 20 min at 4^{o}C. This homogenate was used for Protein estimation (Lowry et al., 1951).

Preparation of midgut tissue extracts for enzyme assay

Silkworm was dissected and the midgut regions (25 mg) (**Figure 5C**) were taken which was then homogenized with 200 µL of phosphate buffered saline. The homogenates were centrifuged at 3000 rpm for 10 min. The supernatants were collected and used for qualitative analysis of enzymes.

Amylase

For qualitative amylase, 40 µL of gut homogenate samples were mixed with 500 µL of starch solution and incubated at room temperature for one hour. 200 µL of iodine was added to the above reaction mixture. Formation of violet color indicates the presence of amylase. Quantitative amylase activity of different midgut samples were analyzed using

standard protocol (Nagaraju et al., 1995). 50 µL of the midgut samples were mixed with 50 µL of citrate buffer and were allowed to incubate for 3 min at 37 °C. The reaction was stopped by adding 200 µL of 3, 5- DNSA. The reaction mixture was heated in a boiling water bath. Color development was read at 540 nm.

Protease

Protease activity of different midgut samples were analyzed using standard protocol (Eguchi et al., 1976). 30 µL of 1% casein was taken to centrifuge tubes. To this 30µL of 0.1M borate buffer (pH 11.0), 60 µL of 10% TCA, 50 µL of the samples were added. The mixture was centrifuged at 3000 rpm for 10 minutes. To 200 µL of the supernatant, 20 µL of 0.5N sodium hydroxide and 50 µL of folin-ciocalteau reagent were mixed and incubate for 30 minutes at room temperature. Absorbance was read at 660 nm.

Isolation of DNA and RNA from Silk gland

DNA and RNA was extracted from middle silk gland of fifth instar larvae different *B. mori* strains reared with different host plants according to the modified protocol of Co-extraction (Sambrook et al.,1989; Souvik et al., 2014). 500 µL of TRIzol solution was added 2mL centrifuge tube containing 500 mg of mid -silk gland tissue, which were covered with aluminium foil and kept at 4ºC for 24 h. The tissue was homogenized, by mortar and pestle, with the TRIzol solution and mixed with TRIzol solution by roto-

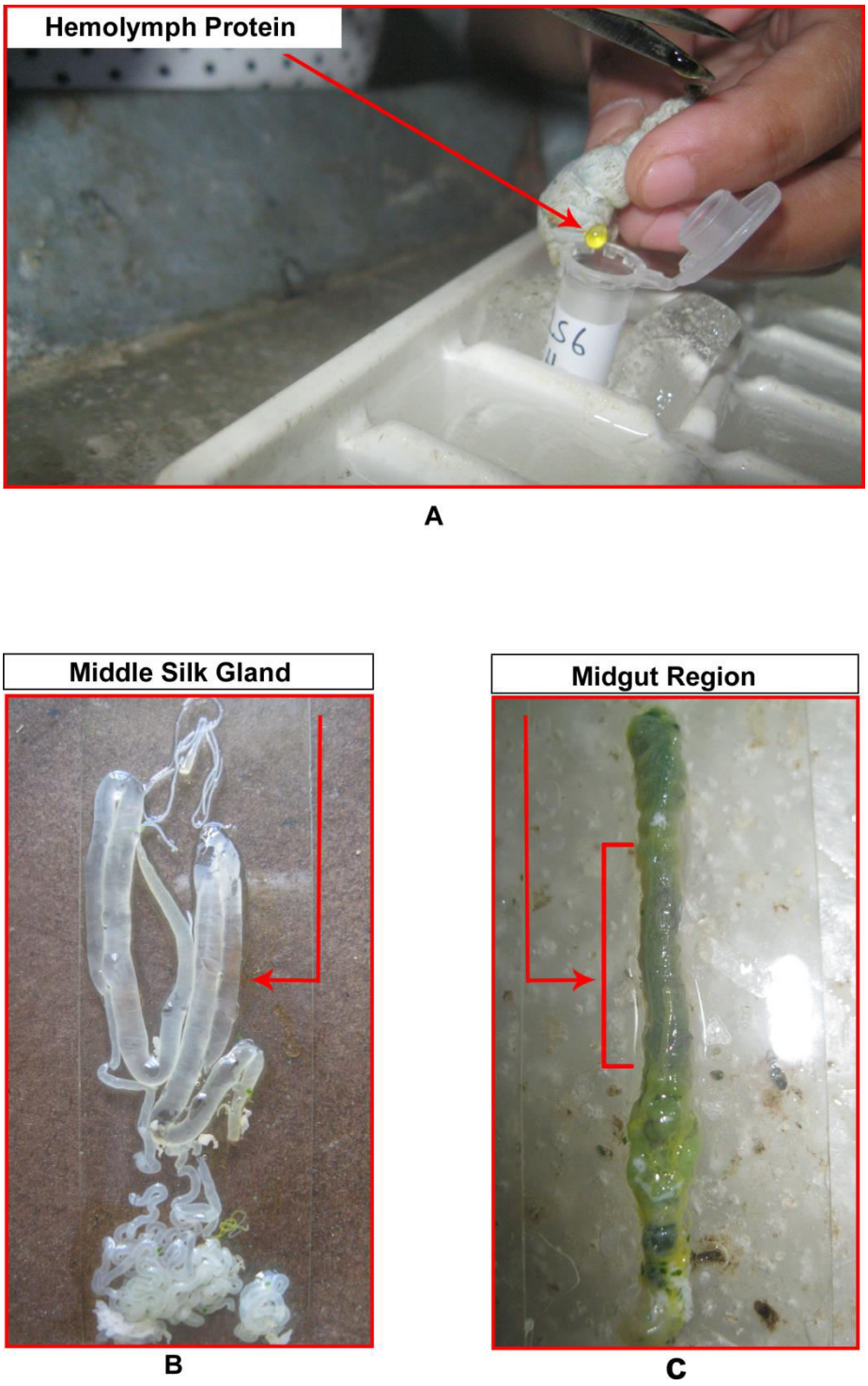


Figure 5. Collection of haemolymph and procuring silk gland and gut from larvae of *B. mori*. (A) Collection of larval hemolymph; (B) Dissected silk gland showing middle silk gland; (C) Dissected gut portion showing midgut region.

spin (Tarson, India) at room temperature for at least 5 minutes to dissociate nucleoprotein complexes.0.2 mL of chloroform was added and the tubes were vortexed vigorously for 15 sec at room temperature for 2 to 3 min. followed by centrifugation at 12,000 rpm for 10 min at 4°C. The aqueous phase was transferred to a fresh tube and the lower phase was kept at −20°C for further DNA isolation. RNA was precipitated by adding 0.6 mL of isopropyl alcohol to the aqueous phase followed by incubation at −20°C for at least 1 h and centrifuged at 12,000 rpm for 10 min at 2–8°C. The pellet was washed in 1 mL of DEPC containing 70% ethanol, air-dried at room temperature, and dissolved in RNase-free water after DNase treatment. RNA integrity checked by electrophoresis on 1.5% agarose gels stained with ethidium bromide. DNA was precipitated by adding 1,000 µL of ethanol and 30 µL of 3M sodium acetate, in the lower phase which was kept in −20ºC followed by incubation at room temperature for 15 min and centrifugation at 13,000 rpm for 10 min at 4ºC. The DNA pellet was washed with 70% ethanol, air-dried at 50ºC, resuspended in 60 µL DEPC treated water. DNA integrity was checked by electrophoresis on 1.8% agarose gels stained with ethidium bromide and quantified using UV-Vis Spectrophotometer (Molecular Devices, USA).

Fibroin and sericin percentage in cocoon

Fibroin and sericin percentage were analyzed from cocoon (Thirumalaisamy et al., 2009). The dry weight of the cocoons was recorded before treating with 2 % potassium hydroxide at 70-80 °C with constant stirring for few min. The chemically treated cocoons become fluffy and it was washed thoroughly in tap water. The fluffy washed cocoons were then treated with acetic acid solution. The dry and wet weights of chemically treated

cocoons were recorded for estimating fibroin and sericin ratio in cocoon. The percentage of fibroin and sericin in silk shell (cocoon) was calculated using the following formula:

Fibroin % = Weight of Fibroin (g)/ Weight of Shell (g) x 100

Sericin % = 100 – fibroin %

3.9. Catalase (CAT) and Superoxide dismutase (SOD) activity assay

Sample of fifth instar larval midgut content was extracted and 40 mg midgut tissue was homogenized in 70 mM potassium phosphate buffer (pH 6.5) containing 0.1% Triton X-100, and insoluble substances were centrifuged out. The supernatants were used for the assay. Tissue protein concentrations were measured according to Lowry's method (1951). Total CAT activity was spectrophotometrically measured by the method of Aebi (1984). The decrease in absorbance at 240 nm was monitored at 30°C. Total SOD activity was determined according to Marklund and Marklund (1974) method assaying the auto-oxidation and illumination of pyrogallol at 440 nm for 3 min. One unit total SOD activity was calculated as the amount of protein causing 50% inhibition of pyrogallol auto-oxidation. The total SOD activity was expressed as units per miligram of protein (U mg/L).

3.9.1. Visualization of catalase (CAT) and superoxide dismutase (SOD) activity by non-denaturing polyacrylamide gel electrophoresis (PAGE)

Fifth instar larvae of all the *B. mori* strain reared with different host plant were randomly selected from the bioassay, were immobilized by keeping at -20 °C and dissected. Midguts were removed, weighed (40mg), and homogenized with a pre-chilled mortar and

pestle in 400µL of 1X Phosphate-buffered saline. 10 mM PMSF was added and the mixture was subjected to sonication. The homogenate was centrifuged at 5000 rpm at 4°C. The supernatant was collected and divided into small aliquots and stored at -20°C until use. Protein concentration from the supernatant was estimated by the method of Lowry using bovine serum albumin as standard Lowry et al. (1951). For CAT, midgut tissue extracts containing 50 µg protein were electrophoresed on 10% native gel for 2.5 h at 80 V. Gel was incubated in 0.005% (v/v) hydrogen peroxide, rinsed with water, followed by immersion in 1% (w/v) $FeCl_3$ and 1% (w/v) $K_3Fe(CN)_6$. Achromatic catalase bands appeared against a green background (Ken et al., 2008). Visualization of separated SOD activity isoforms was performed was performed using Chen and Pan (1996). For SOD, midgut tissue extracts containing 50 µg protein was loaded on 10% native gel, electrophoresed at constant current of 80 V. Gel was washed with distilled water distilled water and equilibrated in 0.1 M phosphate buffer (pH 8) which was then soaked in 1.23 mM NBT solution in light. Excess NBT was removed, by washing the gel with distilled water. The gels were soaked in 0.1 M Tris–HCl buffer (pH 8) containing 2.8 x 10^{-2} mM riboflavin and 28 mM tetramethylethylenediamine (TEMED) and incubated for 10 minutes in dark. The gel was thoroughly rinsed with distilled water to remove excess TEMED and riboflavin, which was further, transferred into distilled water and developed under white-light box for 10-15 minutes at room temperature (photochemical reaction) until activity bands were observed. Gels for both CAT and SOD were analyzed using gel image analysis software-Syngene G-Box and Image J.

3.10. Differential expression of silk gland protein by SDS-PAGE

Fibroin protein isolated from the middle silk glands of each of the silkworm strains reared on different host plants were subjected to sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) according to the method of Vasudha et al. (2006) in 10% resolving gel (80 V).

3.11. Densitometric Analysis

Protein gels were analyzed to compare the banding pattern, intensity and their molecular mass against protein molecular markers run parallely with samples using gel image analysis software Syngen G-Box (Sacramento, CA, USA) and Image J. The protein expressions of all the *B. mori* strains reared with different host plant were compared by HEMI 1.0.1-Heatmap Illustrator software.

3.12. Statistical Analysis

To identify the significance of difference between *B. mori* strains reared on different mulberry host plant and to identify the nature of biochemical simulation in insect larva, all data obtained were expressed as mean ± SD using statistical software Origin Pro 8 SRO v8.0724 (B724), Northampton, MA, USA. We also made use of ANOVA and class prediction statistical tools by R statistical package. To identify differentially biochemical alteration among all the strain and host plant, the ANOVA analysis on normalized data generated a list of differentially parameter (at $P < 0.05$) (Duncan, 1955).

3.13. Analysis of transcript level of fibroin gene using RT-PCR

cDNA synthesis from total RNA

For semiquantitative RT-PCR analysis, RNA was reverse transcribed using superscript II reverse transcriptase (Invitrogen) and random hexamer primers and cDNA was used in PCR according to manufacturer's instructions (Invitrogen). With superscript II reverse transcriptase, 20 µL reaction tube containing 0.5 µg of random hexamer primers, 0.4 mM of each dNTP, 1 µL of RiboLock and 1 µg of RNA were set following the instructions. The reaction conditions were as follows: 10 min. at 25°C, 30 min. at 36°C, and 10 min at 72°C, using vepa-protect Mastercycler (Eppendorf, Germany).

Semi-quantitative Reverse Transcription-PCR

100 ng of cDNA was used in a 25 µl PCR reaction, with 1X Taq buffer, 0.2 mM dNTPs, 1.5 mM $MgCl_2$, 0.2 µM primers and 1 U of Taq DNA polymerase. The primers used were Fibroin F-5´-ACAGCGGCATCTACTTACG- 3´ and Fibroin R-5´-CGTCAATGG CACCGACTATC- 3´ with an expected product size of 400 bp. β-Actin (350 bp), a housekeeping gene was used for RT-PCR for the study of expression of fibroin. The primers β- actin AF-5´-ATGTACGTCGCCATCCAGGC- 3´ and β- actin AR-5´ - CGAT GGTGATGACCTGTCCGT- 3´ (Kumar et al., 2008) were obtained from eurofins ™ (Operon). The reaction conditions for both primer pairs using oligo d(T) primers for the RT step were as follows: primary denaturation 94 °C for 7 min; 94 °C for 30 s, 60 °C for 30 s, and 72 °C for 1 min (35 cycles); and final extension at 72 °C for 10 minutes. The PCR products were analyzed on 2% agarose gel for a better resolution.

3.14. Analysis of genetic relationship by DNA profiling

Sequences of three mitochondrial DNA region such as COX1, ND1, CytB, and one nuclear DNA region, ITS1 gene of the seven *B. mori* strains were amplified using Polymerase chain reaction (PCR). For amplification of COX1 gene, universal primers as described by Hwang et al. (1999) were used having the sequence of Forward Primer: 5′-TGATCAAATTTATAATAC-3′ and Reverse Primer: 5′-GTAAAATTAAAATATAAA C-3′. For amplification of ND1 gene, specific primers as described by Rach et al. (2008) were used having the sequence of forward Primer: 5′-TTCAAACCGGTGTAAGCCAG G-3′and Reverse Primer 5′-TAGAATTAG AAGATCAACCAG-3′. The CytB region was amplified using PCR procedure as described by Li et al. (2005). The primer sequences were forward Primer: 5′-TATGGACCATTACGATCATCAA-3′ and 5′-TGGTACTTTA CCTCGTTATCGT-3′. For amplification of ITS1 region, genomic DNA of each of the strain was amplified with ITS1 primer (Mahendran et al., 2006) having the sequence of forward 5'- GCGTTCGAAGT GTCGATG-3' and reverse 5' GTAGCGA CGGGCGGTG T- 3'. Phylogenetic reconstruction was done for seven different strains of *B. mori* using the four markers. For this, maximum parsimony and minimum evolution tree building methods were employed using MEGA 5.0 version software. A standard bootstrapping method of sampling with replacement with 1000 replicates was employed.

4. RESULTS

4.1. Biochemical parameters of mulberry leaf

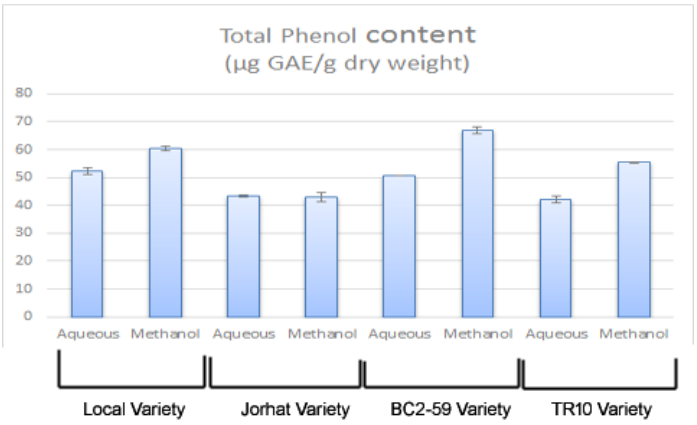
There were significant differences in the levels of biochemical components among the tested mulberry cultivars. Quantitative determinations of various biochemical constituents as well as minerals of different varieties in leaves of mulberry plants are shown in **Table 2**. Protein content varied significantly between the plants, which could be placed in the order Local (hmute) > TR10 > BC2-59 > Jorhat mulberry varieties. Lipid content was highest in Jorhat mulberry variety and lowest in BC2-59 mulberry variety. Carbohydrate content was greatest in Local (hmute) variety and least in BC2-59 variety. Reducing sugar was highest in Local (hmute) mulberry plant variety and lowest in Jorhat mulberry plant variety. Total amino acid, water content, and calcium was highest in TR10 and lowest in BC2-59 mulberry plant variety. Potassium was also highest in TR10 mulberry plant variety but lowest in Local (hmute) mulberry variety. Magnesium was highest in Jorhat mulberry plant variety and lowest in BC2-59. And phosphorus content was highest in Jorhat followed by TR10 and was lowest in Local (hmute) mulberry plant variety. Total phenolic, flavonoid and DPPH content was estimated using aqueous and methanol extract of the four different mulberry varieties. We have observed that methanol extract contains significant higher phenolic content when compared with aqueous extract in most of the host plant except Jorhat mulberry plant variety. The highest concentration of phenol was found in methanol extract of BC2-59 mulberry plant variety followed by Local > TR10 > Jorhat mulberry plant variety (**Figure 6A**). For flavonoid content also we have observed a significant higher value for methanol extract when compared with aqueous extract. Methanol extract of

Local mulberry plant variety was highest followed by aqueous extract of BC2-59> Jorhat (methanol) > TR10 (aqueous) mulberry plant variety (**Figure 6B**). Methanol extract of Jorhat mulberry plant variety exhibited the highest radical scavenging potential followed by TR10>Local>BC2-59 mulberry plant variety (**Table 3**).

4.2. Food utilization efficiency measures

The data given in **Table 4** shows food utilization measures of fifth instar larvae of different strain of *B. mori* reared on Local (hmute), Jorhat, BC2-59 and TR10 mulberry plant variety. For *B. mori* strain SK6 x SK7, GR was greatest in larvae fed with Local (hmute) followed by Jorhat, TR10 mulberry plant variety. GR values were lower when fed with BC2-59 mulberry plant variety. CR was higher on TR10 and lower on Jorhat mulberry plant variety. A higher value of CI was evident on TR10 and lower value on BC2-59 mulberry plant variety. AD was higher when the larvae were fed with TR10, while a lower value of AD was recorded when fed with Local (hmute) mulberry plant variety. ECI and ECD were higher on Local (hmute) but lower value was observed on BC2-59 and TR10 mulberry plant variety, respectively.

For SK7 x SK6 *B. mori* strain, GR was greatest in larvae fed with Local (hmute) followed by Jorhat, TR10 mulberry plant variety. GR values were lower when fed with BC2-59 mulberry plant variety. CR, CI and AD were higher on TR10 and lower on Local (hmute) plant varieties. A higher value of ECI was evident on Local (hmute) and lower value on Jorhat plant variety. ECD was higher when the larvae were fed with Local



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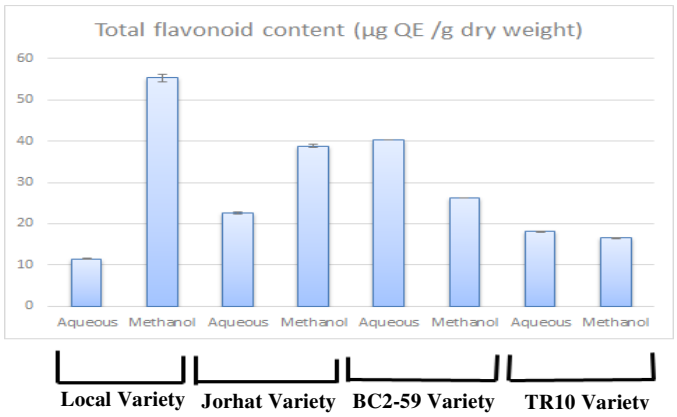


B

Figure 6. Estimation of total (A) phenol content (B) flavonoid contents in Mulberry varieties

Table 2: Biochemical parameters of four different mulberry plant varieties.

Host plant	Protein (µg/mL)	Amino acid (µg/mL)	Lipid (µg/mL)	Carbohydrate (µg/mL)	Reducing sugar (µg/mL)	Water content	Magnesium (mM/L)	Calcium (mM/L)	Potassium (mM/L)	Phosphorous (mM/L)
Local	1766.2 ± 4.72[a]	34.99 ± 1.28[b]	930 ± 2.96[b]	3842.83 ± 4.97[a]	193.23 ± 5.49[a]	1.268 ± 0.73[a]	0.562± 0.082[a]	1.362± 0.074[b]	1.752± 0.086[b]	0.352± 0.049[b]
Jorhat	449.4 ± 3.91[d]	34.07 ± 1.03[b]	970 ± 3.61[a]	3839.06 ± 7.36[b]	131.05 ± 9.31[c]	1.244 ± 0.39[a]	0.678± 0.079[a]	1.582± 0.115[a]	1.793± 0.095[b]	1.672 ±0.064[a]
BC2-59	473.7 ± 3.82[c]	31.47 ± 1.95[b]	880 ± 3.72[c]	3746.09 ± 7.48[c]	164.91 ± 4.47[b]	1.215 ± 0.41[a]	0.529± 0.161[a]	1.322± 0.108[b]	1.872± 0.061[ab]	0.363± 0.029[b]
TR10	909.8 ± 5.49[b]	40.58 ± 1.68[a]	930 ± 3.29[b]	3841.56 ± 5.72[a]	168.53 ± 6.28[b]	1.272 ± 0.86[a]	0.645 ± 0.038[a]	1.583± 0.093[a]	1.952 ± 0.072[a]	0.371± 0.037[b]
F value 3,11	18391	6.314	116.96	53.995	14.834	0.001732	0.4809	2.004	1.237	197.12
P value	<0.0001	<0.0167	<0.0001	<0.0001	<0.0012	<0.9999	<0.7046	<0.1921	<0.3583	<0.0001

Mean ± SE of 3 observations. Within the row means followed by same letter (s) are not significantly different at P<0.05 level by Tukey's test

Table 3: Estimation of Antioxidant activity of aqueous and methanol extract of four different mulberry plant varieties

Mulberry Variety	Fraction	Total phenol content (µg GAE/g dry weight)	Total flavonoid content (µg QE /g dry weight)	DPPH Antioxidant activity IC50 (µg/mL)
Local	Aqueous	52.2ab ± 1.21	11.45b ± 0.06	342a ± 1.20
	Methanol	60.38ab ± 0.92	55.33a ± 0.15	523a ± 1.24
Jorhat	Aqueous	43.19b ± 0.38	22.62ab ± 0.18	342a ± 1.23
	Methanol	42.87b ± 1.55	38.83ab ± 0.41	674a ± 1.22
BC2-59	Aqueous	50.58ab ± 0.04	40.34ab ± 0.08	300a ± 1.31
	Methanol	66.82a ± 1.17	26.16ab ± 0.04	453a ± 1.13
TR10	Aqueous	42.19b ± 1.17	18.02b ± 0.17	653a ±1.30
	Methanol	55.45ab ± 0.18	16.48b ± 0.10	328a ± 1.28

(hmute), while a lower value of ECD was recorded when fed with TR10 mulberry plant variety.

For *B. mori* strain CSR2 x CSR4, GR was greatest in larvae fed with Local (hmute) followed by TR10, BC2-59 mulberry plant variety. GR values were lower when fed with Jorhat mulberry plant variety. CR was higher on TR10 and lower on Local (hmute) plant variety. A higher value of CI was evident on TR10 and lower value on Jorhat mulberry plant variety. AD was higher when the larvae were fed with Jorhat mulberry plant variety, while a lower value of AD was recorded when fed with BC2-59 mulberry plant variety. A higher value of ECI was evident on Local (hmute) and lower value on Jorhat mulberry plant variety. Both ECI and ECD were higher on Local (hmute) but lower value was observed on Jorhat plant variety.

For CSR4 x CSR2 *B. mori* strain, GR was greatest in larvae fed with Jorhat followed by BC2-59, Local (hmute) mulberry plant variety. GR mulberry plant variety values were lower when fed with TR10 mulberry plant variety. CR was higher on TR10 and lower on Local (hmute) mulberry plant variety. AD was higher when the larvae were fed with Jorhat mulberry plant variety, while a lower value of AD was recorded when fed with Local (hmute) mulberry plant variety. Both ECI and ECD were higher on BC2-59 but lower value was observed on Jorhat plant variety.

For FC1 x FC2 *B. mori* strain, GR was greatest in larvae fed with TR10 followed by BC2-59, Local (hmute) mulberry plant variety. GR values were lower when fed with Jorhat mulberry plant variety. CR, CI, AD was higher on TR10 and lower on Local

(hmute) mulberry plant variety. Both ECI and ECD were higher on TR10 but lower value was observed on Jorhat plant variety.

For *B. mori* strain FC2 x FC1, GR was greatest in larvae fed with Jorhat followed by BC2-59, Local (hmute) mulberry plant variety. GR values were lower when fed with TR10 mulberry plant variety. CR was higher on TR10 and lower on Local (hmute) plant varieties. A higher value of CI was evident on Jorhat and lower value on Local (hmute) plant variety. AD was higher when the larvae were fed with TR10, while a lower value of AD was recorded when fed with Local (hmute) mulberry plant variety. Both ECI and ECD were higher on Local (hmute) but lower value was observed on Jorhat plant variety.

For J112 *B. mori* strain, GR was greatest in larvae fed with Jorhat mulberry plant variety followed by TR10, BC2-59 and Local (hmute) mulberry plant variety. CR was higher on TR10 and lower on BC2-59 mulberry plant variety. A higher value of CI was evident on Jorhat and lower value on Local (hmute) mulberry plant variety. AD was higher when the larvae were fed with TR10 mulberry plant variety, while a lower value of AD was recorded when fed with BC2-59 mulberry plant variety. Both ECI and ECD were higher on Jorhat but lower value was observed on Local (hmute) mulberry plant variety.

4.3. Rearing performance of different mulberry silkworm on different mulberry variety

Silkworm rearing performance results of 5^{th} instar larva of different mulberry silkworm reared with different mulberry variety was presented in **Table 5**. Regarding the larval weight and economic parameters for SK6 x SK7 *B. mori*, the host plant BC2-59 mulberry plant variety fed larvae gives highest larval weight followed by Jorhat, TR10 and Local (hmute) mulberry plant variety. Cocoon weight, shell weight, filament weight

was highest on Jorhat and lowest on Local (hmute) mulberry plant variety. A higher value of Shell percentage was evident on TR10 and lower value on Local (hmute) mulberry plant variety. Filament length was higher when the larvae were reared with Jorhat mulberry plant variety, while a lower value was recorded when reared with TR10. Denier was higher on TR10 but lower value was observed on Local (hmute) mulberry plant variety.

The larval weight for SK7 x SK6 *B.mori* strain was highest on BC2-59 and lowest on Local (hmute) mulberry plant variety. Local (hmute) mulberry plant variety reared larvae gives the highest cocoon weight where BC2-59 mulberry plant variety gives the lowest. Maximum shell weight and shell percentage was found on TR10 mulberry plant variety and minimum was observed on Local (hmute) host plant. Filament length and filament weight was higher when the larvae were reared with Jorhat mulberry plant variety, while a lower value was recorded when reared with Local (hmute) mulberry plant variety. Denier was higher on Jorhat mulberry plant variety but lower value was observed on TR10 mulberry plant variety.

The host plant Jorhat gives highest larval weight followed by Jorhat, TR10 and Local (hmute) mulberry plant variety for the *B. mori* strain CSR2 X CSR4. Cocoon weight was highest on BC2-59 and lowest on TR10 mulberry plant variety. Shell weight was greater on Jorhat mulberry plant variety where it was lower on Local mulberry plant variety. A higher value of Shell percentage was evident on Jorhat and lower value on BC2-59 mulberry plant variety. Filament length and filament weight was higher when the larvae were reared with Jorhat mulberry plant variety, while a lower value was recorded

when reared with TR10 mulberry plant variety. Denier was higher on TR10 but lower value was observed on Local (hmute) mulberry plant variety.

In CSR4 x CSR2 *B. mori* strain, larval weight was greatest in larvae reared with TR10 followed by Jorhat, BC2-59, Local (hmute) mulberry plant variety. Cocoon weight was higher on larva reared with Jorhat mulberry plant variety whereas, lower values were observed when reared with TR10 mulberry plant variety. Shell weight and shell percentage were higher on Jorhat and lower on BC2-59 mulberry plant variety. A higher value of filament length was evident on Jorhat mulberry plant variety and lower value on TR10 mulberry plant variety. Filament weight was higher when the larvae were reared with Jorhat mulberry plant variety, while a lowest value was recorded when reared with BC2-59 mulberry plant variety. Denier was highest on Local (hmute) but lower value was observed on Jorhat mulberry plant variety.

The larval weight for FC1 x FC2 was highest on Jorhat followed by TR10, BC2-59 mulberry plant variety, and lowest on Local (hmute) mulberry plant variety. Jorhat mulberry plant variety fed larvae gives the highest cocoon weight and shell weight where BC2-59 mulberry plant variety gives the lowest. Maximum shell percentage was found on TR10 mulberry plant variety and minimum was observed on Local host plant. Filament length was higher in Jorhat host plant reared larvae and lower on local (hmute) mulberry plant variety reared larvae. Higher value of filament weight and denier was observed when the larvae were reared with Jorhat mulberry plant variety, while a lower value was recorded when reared with BC2-59 mulberry plant variety.

In FC2 x FC1 *B. mori* strain, larval weight was greatest in larvae reared with Jorhat mulberry plant variety followed by BC2-59, TR10 and Local (hmute) mulberry plant variety. Cocoon weight and shell weight was higher on larva reared with Jorhat mulberry plant variety whereas, lower values were observed when reared with Local (hmute) mulberry plant variety. Shell percentage was higher on TR10 and lower on Jorhat mulberry plant variety. A higher value of filament length was evident on Jorhat mulberry plant variety and lower value on BC2-59 mulberry plant variety. Filament weight was higher when the larvae were reared with Jorhat mulberry plant variety, while a lowest value was recorded when reared with Local (hmute) mulberry plant variety. Denier was highest on TR10 mulberry plant variety but lower value was observed on Local mulberry plant variety.

The mulberry plant variety TR10 mulberry plant variety gives highest larval weight followed by Jorhat, BC2-59 and Local (Hmute) mulberry plant variety for the *B. mori* strain J112. Cocoon weight was highest on Local (Hmute) mulberry plant variety and lowest on BC2-59 mulberry plant variety. Shell weight was greater on Jorhat mulberry plant variety where it was lower on BC2-59 mulberry plant variety. A higher value of Shell percentage was evident on TR10 mulberry plant variety and lower value on BC2-59 mulberry plant variety. A higher value of filament length was observed on Jorhat and lower value on TR10 mulberry plant variety. Filament weight and denier were higher when the larvae were reared with Local (Hmute), while a lowest value was recorded when reared with BC2-59 mulberry plant variety.

Table 4: Food utilization efficiency measures of fifth instars larvae of seven *B. mori* strains reared on different mulberry varieties.

Strain	Host plant	GR (g/insect/day)	CR (g/insect/day)	CI (g/insect/day)	AD (%)	ECI (%)	ECD (%)
SK6 X SK7	Local	0.076 ± 0.016[a]	0.345 ± 0.086[a]	0.196 ± 0.025[a]	91.58 ± 1.90[a]	22.49 ± 0.42[a]	24.55 ± 0.68[a]
	Jorhat	0.067 ± 0.017[b]	0.331 ± 0.125[a]	0.172 ± 0.045[a]	92.03 ± 2.01[a]	20.24 ± 0.66[b]	21.99 ± 0.78[b]
	BC2-59	0.049 ± 0.015[b]	0.390 ± 0.149[a]	0.164 ± 0.024[a]	91.88 ± 1.59[a]	12.55 ± 0.54[d]	13.66 ± 0.91[c]
	TR10	0.060 ± 0.017[b]	0.521 ± 0.135[a]	0.251 ± 0.040[a]	93.76 ± 1.85[a]	15.43 ± 0.77[c]	12.26 ± 0.64[c]
P value		<0.6982	<0.7099	<0.3460	<0.8356	<0.0001	<0.0001
F Value $_{3,11}$		0.4910	0.4725	1.278	0.2842	54.411	63.853
SK7 X SK6	Local	0.058 ± 0.015[a]	0.348 ± 0.129[a]	0.162 ± 0.028[a]	90.79 ± 1.84[a]	16.88 ± 0.54[a]	18.59 ± 0.70[a]
	Jorhat	0.067 ± 0.019[b]	0.466 ± 0.137[a]	0.246 ± 0.030[a]	93.20 ± 1.89[a]	14.49 ± 0.78[b]	15.55 ± 0.87[b]
	BC2-59	0.061 ± 0.019[b]	0.388 ± 0.125[a]	0.194 ± 0.060[a]	92.07 ± 1.69[a]	15.77 ± 0.49[ab]	17.13 ± 0.85[ab]
	TR10	0.064 ± 0.016[b]	0.520 ± 0.133[a]	0.263 ± 0.026[a]	94.07 ± 1.34[a]	16.18 ± 0.89[ab]	13.15 ± 0.72[c]
P value		<0.9842	<0.7925	<0.2976	<0.5801	<0.1804	<0.0067
F Value $_{3,11}$		0.04988	0.3470	1.456	0.6960	2.087	8.718
CSR2X CSR4	Local	0.096 ± 0.018[a]	0.335 ± 0.147[a]	0.219 ± 0.036[ab]	92.83 ± 1.76[a]	28.65 ± 0.63[a]	30.87 ± 0.82[a]
	Jorhat	0.046 ± 0.006[ab]	0.458 ± 0.168[a]	0.164 ± 0.062[b]	94.65 ± 1.76[a]	10.17 ± 0.59[d]	10.74 ± 0.65[c]
	BC2-59	0.059 ± 0.008[ab]	0.391 ± 0.128[a]	0.184 ± 0.025[b]	91.83 ± 1.64[a]	15.28 ± 0.65[c]	16.64 ± 0.68[b]
	TR10	0.083 ± 0.019[b]	0.525 ± 0.136[a]	0.325 ± 0.036[a]	93.57 ± 1.22[a]	19.63 ± 0.69[b]	16.88 ± 0.96[b]
P value		<0.1234	<0.8110	<0.1008	<0.6643	<0.0001	<0.0001
F Value $_{3,11}$		2.612	0.3199	2.912	0.5465	149.30	117.79
CSR4X CSR2	Local	0.068 ± 0.016[b]	0.348 ± 0.125[a]	0.192 ± 0.024[a]	90.58 ± 1.67[a]	19.72 ± 0.87[ab]	21.77 ± 0.67[a]
	Jorhat	0.241 ± 0.018[a]	0.452 ± 0.126[a]	0.171 ± 0.029[a]	94.82 ± 1.97[a]	5.33 ± 0.88[c]	5.62 ± 0.95[c]
	BC2-59	0.078 ± 0.015[b]	0.384 ± 0.127[a]	0.224 ± 0.046[a]	92.17 ± 1.77[a]	20.48 ± 0.49[a]	22.22 ± 0.73[a]
	TR10	0.068 ± 0.015[b]	0.514 ± 0.129[a]	0.285 ± 0.052[a]	94.47 ± 1.67[a]	17.93 ± 0.58[b]	14.15 ± 0.63[b]
P value		<0.0001	<0.7991	<0.2677	<0.3476	<0.0001	<0.0001
F Value $_{3,11}$		28.035	0.3373	1.584	1.273	95.777	107.04

Contd…….Table 4

Strain	Host plant	GR (g/insect/day)	CR (g/insect/day)	CI (g/insect/day)	AD (%)	ECI (%)	ECD (%)
FC1X FC2	Local	0.063 ± 0.017^{a}	0.355 ± 0.149^{a}	0.176 ± 0.043^{b}	89.90 ± 2.01^{a}	17.91 ± 0.84^{b}	19.93 ± 0.91^{b}
	Jorhat	0.049 ± 0.007^{a}	0.471 ± 0.137^{a}	0.279 ± 0.026^{ab}	92.68 ± 1.56^{a}	10.45 ± 0.76^{c}	11.28 ± 0.73^{c}
	BC2-59	0.078 ± 0.016^{a}	0.395 ± 0.126^{a}	0.268 ± 0.038^{ab}	91.86 ± 1.95^{a}	19.75 ± 0.57^{ab}	21.50 ± 0.96^{b}
	TR10	0.081 ± 0.016^{a}	0.523 ± 0.157^{a}	0.334 ± 0.039^{a}	93.92 ± 1.94^{a}	20.29 ± 0.85^{a}	39.37 ± 0.89^{a}
P value		<0.4306	<0.8394	<0.0875	<0.5223	<0.0001	<0.0001
F Value $_{3,11}$		1.027	0.2787	3.129	0.8116	35.514	180.83
FC2 X FC1	Local	0.071 ± 0.016^{a}	0.357 ± 0.126^{a}	0.195 ± 0.061^{a}	89.92 ± 1.98^{b}	20.00 ± 0.85^{a}	22.25 ± 0.69^{a}
	Jorhat	0.078 ± 0.013^{a}	0.471 ± 0.129^{a}	0.282 ± 0.050^{a}	92.90 ± 1.94^{ab}	16.72 ± 0.67^{b}	18.00 ± 0.89^{b}
	BC2-59	0.076 ± 0.017^{a}	0.395 ± 0.129^{a}	0.240 ± 0.025^{a}	91.27 ± 1.89^{b}	19.34 ± 0.78^{a}	21.19 ± 0.80^{a}
	TR10	0.062 ± 0.015^{a}	0.525 ± 0.128^{a}	0.279 ± 0.027^{a}	93.69 ± 1.44^{a}	16.83 ± 0.69^{b}	12.58 ± 0.90^{c}
P value		<0.8820	<0.7924	<0.4921	<0.5038	<0.0293	<0.0001
F Value $_{3,11}$		0.2169	0.3471	0.8776	0.8516	5.086	27.761
J112	LOCAL	0.067 ± 0.018^{c}	0.406 ± 0.128^{a}	0.215 ± 0.028^{a}	61.47 ± 1.23^{c}	16.58 ± 0.86^{c}	26.98± 0.98^{d}
	JORHAT	0.131 ± 0.015^{a}	0.492 ± 0.126^{a}	0.447 ± 0.139^{a}	66.38 ± 1.88^{b}	26.62 ± 0.85^{a}	40.10 ± 0.67^{a}
	BC2-59	0.077 ± 0.019^{bc}	0.400 ± 0.087^{a}	0.236 ± 0.034^{a}	59.08 ± 1.45^{c}	19.42 ± 0.69^{b}	32.88 ± 0.79^{b}
	TR10	0.118 ± 0.019^{ab}	0.552 ± 0.066^{a}	0.461 ± 0.135^{a}	70.69 ± 1.01^{a}	25.23 ± 0.75^{a}	30.39 ± 0.95^{c}
P value		<0.0931	<0.0931	<0.2298	<0.0019	<0.0019	<0.0001
F Value $_{3,11}$		3.033	0.7038	1.774	13.117	36.152	42.143

Mean ± SE of 3 observations. Within the row means followed by same letter (s) are not significantly different at P<0.05 level by Tukey's test

4.4. Biochemical and enzymatic activities of different strains of *B. mori* reared on different host plants

Biochemical as well as enzymatic analyses of seven different strains of *B. mori* reared on four different host plants (Local- Hmute, Jorhat, BC2-59 and TR10) varieties of mulberry leaves was presented in Table 6.

The *B. mori* strain SK6 x SK7 cocoon has fibroin: sericin percentage of 70.30%: 29.70% when reared with local (Hmute) mulberry plant variety, 74.00%: 26.00% when reared with Jorhat mulberry plant variety, 72.32%: 27.68% when reared with BC2-59 mulberry plant variety and 66.37%: 33.63% when reared with TR10 mulberry plant variety. Regarding the hemolymph protein, silk gland protein and RNA content, Jorhat mulberry plant variety was highest and TR-10 mulberry plant variety was lowest for all. Maximum amylase activity was found in those larval midgut sample reared with Jorhat mulberry plant variety. The least amylase activity was found in those larval midgut sample reared with BC2-59 mulberry plant variety whereas, for protease maximum activity was observed in those larvae reared with Local (Hmute) mulberry plant variety and minimum activity was on those larvae reared with BC2-59 mulberry plant variety. DNA content was high on TR10 and low on BC2-59 mulberry plant variety.

The *B. mori* strain SK7 x SK6 cocoon has fibroin: sericin percentage of 74.47%: 25.53% when reared with Local (Hmute) mulberry plant variety, 65.62.00%: 34.38% when reared with Jorhat mulberry plant variety, 74.29%: 25.71% when reared with BC2-59 mulberry plant variety and 76.04%: 23.96% when reared with TR10 mulberry plant variety. Regarding the hemolymph protein TR10 mulberry plant variety was observed highest and Local (Hmute) mulberry plant variety was observed lowest. In silk gland protein greater value was found in BC-259 mulberry plant variety and lower value was found on TR10 mulberry plant variety. Maximum amylase

activity was found in those larval midgut sample reared with Jorhat mulberry plant variety. The least amylase activity was found in those larval midgut sample reared with TR10 mulberry plant variety whereas, for protease, maximum activity was also observed in Jorhat mulberry plant variety and Local (Hmute) mulberry plant variety but minimum activity was observed on those larvae reared with BC2-59 mulberry plant variety. DNA and RNA content were high on TR10, BC2-59 and low on Jorhat and TR10 mulberry plant variety respectively.

The *B. mori* strain CSR2 x CSR4 cocoon has fibroin: sericin percentage of 62.74%: 37.26% when reared with Local (Hmute) mulberry plant variety, 71.01%: 28.99% when reared with Jorhat mulberry plant variety, 68.60%: 31.40% when reared with BC2-59 mulberry plant variety and 67.08%: 32.92% when reared with TR10 mulberry plant variety. TR10 mulberry plant variety gives highest hemolymph protein and BC2-59 mulberry plant variety gives lowest hemolymph protein among all the host plant use.

For silk gland protein, BC2-59 mulberry plant variety is the one with the highest score whereas, TR-10 mulberry plant variety is the one with the lowest scores. Maximum amylase activity was found in those larval midgut sample reared with Jorhat mulberry plant variety. The least amylase activity was found in those larval midgut sample reared with TR10 mulberry plant variety whereas, for protease, maximum activity was also observed in Jorhat mulberry plant variety but minimum activity was observed on those larvae reared with BC2-59 mulberry plant variety. DNA and RNA content were high on BC2-59 mulberry plant variety and low on Local (Hmute) and TR10 mulberry plant variety, respectively.

Table 5: Economic parameters of fifth instar larvae of seven *B. mori* strains reared on different mulberry varieties

Strain	Host plant	Larval weight (g/insect)	Cocoon weight (g/insect)	Shell weight (g/insect)	Shell %	Filament length (m)	Filament weight (g/cocoon)	Denier (g)
SK6 X SK7	Local	2.46 ± 0.93[a]	1.23 ± 0.03[b]	0.22 ± 0.01[a]	18.52 ± 0.11[d]	840 ± 1.01[c]	0.219 ± 0.02[a]	2.35 ± 0.02[c]
	Jorhat	3.13 ± 0.82[a]	1.40 ± 0.04[a]	0.27 ± 0.03[a]	19.32 ± 0.05[b]	962 ± 1.06[a]	0.267 ± 0.06[a]	2.49 ± 0.05[bc]
	BC2-59	3.39 ± 1.52[a]	1.39 ± 0.04[a]	0.26 ± 0.04[a]	19.11 ± 0.05[c]	892 ± 1.04[b]	0.255 ± 0.04[a]	2.57 ± 0.04[ab]
	TR10	3.02 ± 0.84[a]	1.38 ± 0.05[a]	0.27 ± 0.02 [a]	20.21 ± 0.02[a]	836 ± 1.03[d]	0.248 ± 0.01[a]	2.67 ± 0.08[a]
P value		<0.9364	<0.0543	<0.5495	<0.0001	<0.0001	<0.8301	<0.0142
F Value $_{3,11}$		0.1350	3.919	0.7556	112.20	3228.7	0.2921	6.703
SK7 X SK6	Local	2.25 ± 3.08[a]	1.54 ± 0.12[a]	0.25 ± 0.04[a]	16.17 ± 0.02[d]	877 ± 1.08[d]	0.273 ± 0.01[a]	2.80 ± 0.03[a]
	Jorhat	3.62 ± 3.22[a]	1.53 ± 0.15[a]	0.29 ± 0.04[a]	18.93 ± 0.03[c]	1054 ± 1.08[a]	0.331 ± 0.08[a]	2.82 ± 0.03[a]
	BC2-59	3.36 ± 1.33[a]	1.52 ± 0.05[a]	0.29 ± 0.01[a]	19.16 ± 0.12[b]	922 ± 1.02[c]	0.283 ± 0.05[a]	2.76 ± 0.06[a]
	TR10	3.38 ± 3.49[a]	1.52 ± 0.11[a]	0.30 ± 0.03[a]	20.35 ± 0.11[a]	1049 ± 1.07[b]	0.273 ± 0.05[a]	2.34 ± 0.05[b]
P value		<0.9866	<0.991	<0.7126	<0.0001	<0.0001	<0.8470	<0.0002
F Value $_{3,11}$		0.04449	0.007120	0.4683	449.83	7120.7	0.2676	26.329
CSR2X CSR4	Local	2.93 ± 2.32[a]	1.46 ± 0.13[a]	0.31 ± 0.02[a]	21.59 ± 0.04[c]	1035 ± 1.03[b]	0.270 ± 0.04[a]	2.35 ± 0.01[c]
	Jorhat	3.68 ± 1.27[a]	1.43 ± 0.10[a]	0.34 ± 0.07[a]	23.85 ± 0.02[a]	1060 ± 1.03[a]	0.292 ± 0.09[a]	2.48 ± 0.02[b]
	BC2-59	3.17 ± 3.01[a]	1.56± 0.10[a]	0.33 ± 0.06[a]	21.47 ± 0.02[d]	984 ± 2.03[c]	0.272 ± 0.03[a]	2.48 ± 0.03[b]
	TR10	3.57 ± 1.94[a]	1.37 ± 0.15[a]	0.31 ± 0.04[a]	22.68 ± 0.04[b]	800 ± 1.06[d]	0.259 ± 0.07[a]	2.86 ± 0.06[a]
P value		<0.9444	<0.7409	<0.9659	<0.0001	<0.0001	<0.9847	<0.0001
F Value $_{3,11}$		0.02459	0.4242	0.08571	1234.0	7497.3	0.04875	38.847
CSR4X CSR2	Local	2.84 ± 0.80[a]	1.54 ± 0.02[a]	0.35 ± 0.06[a]	22.85 ± 0.03[b]	973 ± 1.04[b]	0.273 ± 0.03[a]	2.52 ± 0.04[b]
	Jorhat	3.79 ± 1.26[a]	1.57 ± 0.11[a]	0.39 ± 0.02[a]	24.94 ± 0.06[a]	998 ± 2.03[a]	0.278 ± 0.02[a]	2.50 ± 0.04[b]
	BC2-59	3.72 ± 0.74[a]	1.52 ± 0.03[a]	0.30 ± 0.03[a]	19.93 ± 0.05[d]	915 ± 1.03[c]	0.234 ± 0.05[a]	2.30 ± 0.02[c]
	TR10	3.79 ± 1.38[a]	1.50 ± 0.02[a]	0.32 ± 0.06[a]	21.58 ± 0.07[c]	811 ± 1.08[d]	0.254 ± 0.04[a]	2.82 ± 0.03[a]
P value		<0.9444	<0.8534	<0.5668	<0.0001	<0.0001	<0.8264	<0.0001
F Value $_{3,11}$		0.02459	0.2585	0.7216	1501.9	3719.6	0.2975	40.859

Contd…Table 5

Strain	Host plant	Larval weight (g/insect)	Cocoon weight (g/insect)	Shell weight (g/insect)	Shell %	Filament length (m)	Filament weight (g/cocoon)	Denier (g)
FC1X FC2	Local	3.64 ± 3.32[a]	1.58 ± 0.14[ab]	0.32 ± 0.03[a]	20.26 ± 0.01[c]	1020 ± 1.05[d]	0.297 ± 0.05[a]	2.62 ± 0.05[b]
	Jorhat	4.20 ± 0.82[a]	1.71 ± 0.06[a]	0.37 ± 0.08[a]	22.09 ± 0.01[d]	1135 ± 1.05[a]	0.353 ± 0.04[a]	2.79 ± 0.07[a]
	BC2-59	3.78 ± 1.51[a]	1.41 ± 0.06[b]	0.31 ± 0.04[a]	22.39 ± 0.03[b]	1056 ± 1.04[c]	0.287 ± 0.03[a]	2.44 ± 0.05[c]
	TR10	4.08 ± 1.51[a]	1.57 ± 0.05[ab]	0.35 ± 0.03[a]	22.82 ± 0.13[a]	1127 ±2.04[b]	0.345 ± 0.03[a]	2.75 ± 0.02[ab]
P value		<0.9969	<0.1840	<0.8181	<0.0001	<0.0001	<0.5509	<0.0049
F Value $_{3,11}$		0.01656	2.060	0.3095	282.36	1670.0	0.7528	9.657
FC2X FC1	Local	3.21 ± 0.70[a]	1.38 ± 0.01[a]	0.30 ± 0.07[a]	22.23 ± 0.06[b]	1069 ± 1.02[b]	0.263 ±0.03[a]	2.22 ± 0.04[b]
	Jorhat	3.85 ± 1.12[a]	1.60 ± 0.08[a]	0.34 ± 0.01[a]	21.34 ± 0.02[d]	1166 ± 1.04[a]	0.297 ± 0.05[a]	2.29 ± 0.05[b]
	BC2-59	3.63 ± 0.31[a]	1.53 ± 0.12[a]	0.33 ± 0.01[a]	22.03 ± 0.06[c]	950 ± 1.05[d]	0.297 ± 0.02[a]	2.81 ± 0.07[a]
	TR10	3.50 ± 1.44[a]	1.43 ± 0.10[a]	0.33 ± 0.05[a]	23.03 ± 0.02[a]	1052 ± 1.0[c]	0.292 ± 0.07[a]	2.89 ± 0.04[a]
P value		<0.9728	<0.3501	<0.9216	<0.0001	<0.0001	<0.9441	<0.1059
F Value $_{3,11}$		0.07306	1.264	0.1579	76.013	7410.3	0.1227	2.838
J112	Local	2.26 ± 0.60[a]	1.29 ± 0.05[a]	0.17 ± 0.02[a]	13.47 ± 0.04[c]	820 ± 2.01[b]	0.180 ± 0.04[a]	1.97 ± 0.06[a]
	Jorhat	2.38 ± 0.70[a]	1.28 ± 0.07[a]	0.17 ± 0.05[a]	13.95 ± 0.07[b]	908 ± 1.08[a]	0.161 ± 0.03[a]	1.59 ± 0.09[b]
	BC2-59	2.28 ± 1.27[a]	1.08 ± 0.04[b]	0.12 ± 0.05[a]	11.75 ±0.01[d]	784 ± 1.09[c]	0.109 ± 0.06[a]	1.25 ± 0.03[c]
	TR10	2.40 ± 0.92[a]	1.18 ± 0.03[ab]	0.17 ± 0.04[a]	14.38 ± 0.04[a]	726 ± 1.03[d]	0.118 ± 0.06[a]	1.46 ± 0.08[b]
P value		<0.9993	<0.0544	<0.7856	<0.0001	<0.0001	<0.7070	<0.0005
F Value $_{3,11}$		0.005963	3.916	0.3571	648.73	3118.1	0.4770	19.289

Mean ± SE of 3 observations. Within the row means followed by same letter (s) are not significantly different at P<0.05 level by Tukey's test

The fibroin: sericin percentage for the *B. mori* strain CSR4 X CSR2 cocoon has 68.83%: 31.17% when reared with Local (Hmute) mulberry plant variety, 94.97%: 5.03% when reared with Jorhat mulberry plant variety, 67.47%: 32.53% when reared with BC2-59 mulberry plant variety and 69.35%: 30.65% when reared with TR10 mulberry plant variety. The mulberry plant variety TR10 scores the highest and Jorhat mulberry plant variety scores the lowest for hemolymph protein. The silk gland protein was greater on BC2-59 mulberry plant variety and lower on Jorhat mulberry plant variety. Amylase activity was found highest on those larval midgut sample reared with Local (Hmute) mulberry plant variety. The least amylase activity was found in those larval midgut sample reared with BC2-59 mulberry plant variety whereas, for protease, maximum activity was also observed in Local (Hmute) mulberry plant variety but minimum activity was observed on those larvae reared with TR10 mulberry plant variety. DNA and RNA content was high on Local (Hmute), BC2-59 respectively and low on Jorhat mulberry plant variety for both.

The *B. mori* strain FC1 x FC2 cocoon has fibroin: sericin ratio of 68.83%: 31.17% when reared with local mulberry plant variety, 79.25%: 20.75% when reared with Jorhat mulberry plant variety, 68.16%: 31.84% when reared with BC2-59 mulberry plant variety and 83.91%: 16.09% when reared with TR10 mulberry plant variety. Regarding the hemolymph protein, Jorhat mulberry plant variety was observed highest and BC2-59 mulberry plant variety was observed lowest. In silk gland protein greater value was found in BC-259 mulberry plant variety and lower value was found on TR10 mulberry plant variety. Maximum amylase activity was found in those larval midgut sample reared with

TR10 mulberry plant variety. The least amylase activity was found in those larval midgut sample reared with BC2-59 mulberry plant variety whereas, for protease, maximum activity was observed in Local mulberry plant variety but minimum activity was observed on those larvae reared with TR10 mulberry plant variety. DNA and RNA content were high on Local (Hmute), BC2-59 and low on Jorhat and TR10 mulberry plant variety respectively.

The fibroin: sericin percentage for the *B. mori* strain FC2 x FC1 cocoon has 71.20%: 28.80% when reared with Local (Hmute) mulberry plant variety, 71.92%: 28.08% when reared with Jorhat mulberry plant variety, 74.00%: 26.00% when reared with BC2-59 and 66.15%: 33.85% when reared with TR10 mulberry plant variety. The mulberry plant variety TR10 and Local (Hmute) scores the highest for hemolymph and silk gland protein respectively, whereas, BC2-59 mulberry plant variety was lowest for both. Amylase activity and protease activity was found highest on those larval midgut sample reared with Jorhat mulberry plant variety. The least amylase as well as protease activity was found in those larval midgut sample reared with Local (Hmute) mulberry plant variety. DNA and RNA content were highest on Jorhat mulberry plant variety but lowest value was observed on BC2-59 and TR10 mulberry plant variety, respectively.

The *B. mori* strain J112 cocoon has fibroin: sericin percentage of 73.76%: 26.24% when reared with local mulberry plant variety, 71.30%: 28.70% when reared with Jorhat mulberry plant variety, 59.40%: 40.60% when reared with BC2-59 mulberry plant variety and 84.17%: 15.83% when reared with TR10 mulberry plant variety. Regarding the

hemolymph protein and silk gland protein were high on TR10 mulberry plant variety. Lowest value for hemolymph protein was observed on Jorhat mulberry plant variety whereas, for silk gland protein lowest value was observed on BC2-59 mulberry plant variety. Maximum amylase and protease activity was found in those larval midgut sample reared with TR10 and Local (Hmute) mulberry plant variety respectively. Whereas, the least amylase and protease activity was found in those larval midgut sample reared with Jorhat mulberry plant variety. DNA and RNA content were highest on BC2-59 and Jorhat mulberry plant variety respectively whereas, lowest value was observed on Local (Hmute) and TR10 mulberry plant variety respectively.

4.5. Catalase (CAT) and Superoxide dismutase (SOD) activity assay

Catalase

There was a great difference ($P<0.001$) in amount of CAT activity (**Figure 7A**) in the seven different *B. mori* strain reared on different mulberry plant variety and was observed relatively high activity in the CSR4 X CSR2 *B. mori* strain, followed by SK7 x SK6 *B. mori*, respectively. BC2-59 mulberry plant variety showing highest activity relatively followed by Local (Hmute) mulberry plant variety.

Superoxide dismutase

There was a great difference ($P<0.001$) in amount of SOD activity (**Figure 7B**) in the seven different strain reared on different mulberry plant variety and was observed relatively high activity in the FC1 x FC2 strain, followed by FC2 x FC1, respectively.

Over all Jorhat mulberry plant variety showing highest activity followed by TR10 mulberry plant variety.

4.6. Visualization of catalase (CAT) and superoxide dismutase (SOD) activity by non-denaturing polyacrylamide gel electrophoresis (PAGE)

Catalase

CAT activity pattern varied among the different strains of *B. mori* reared with different host plants. Catalase expression was observed to be highest *B. mori* larvae reared with BC2-59 mulberry plant variety followed by Jorhat, TR10 mulberry plant variety and was lowest in Local (Hmute) mulberry plant variety. Among the *B. mori* strains used SK7 x SK6 shows high expression followed by CSR2 x CSR4, CSR4 x CSR2, FC1 x FC2 (**Figure 7C**).

Superoxide dismutase

SOD activity pattern varied among the different strains of *B. mori* reared with different host plants. SOD expression was observed to be highest in Jorhat mulberry plant variety for all the *B. mori* strain used followed by TR10 mulberry plant variety where expression was high in all the *B. mori* strain used except for the strain SK6 x SK7. BC2-59 mulberry plant variety reared *B. mori* strain also showed high expression except for the *B. mori* strain SK6 x SK7, SK7 x SK6 and J112. Expression was lowest in Local mulberry plant variety reared *B. mori* strains (**Figure 7D**).

Table 6: Biochemical and Enzymatic activity for different Strain reared on different host

Strain	Host plant	Fibroin % (Cocoon)	Sericin % (Cocoon)	Hemolymph protein (µg/mL)	Silk gland protein (µg/mL)	Amylase (µg/mL)	Protease (µg/mL)	DNA (ng/µL)	RNA (ng/µL)
SK6 X SK7	Local	70.30 ± 0.05^{c}	29.70 ± 0.04^{b}	416.44 ± 0.88^{b}	365 ± 1.20^{b}	1.01 ± 0.08^{b}	0.20 ± 0.08^{a}	1042 ± 0.02^{b}	162 ± 0.03^{c}
	Jorhat	74.00 ± 0.12^{a}	26.00 ± 0.07^{d}	472.61 ± 0.90^{a}	525 ± 2.23^{a}	1.38 ± 0.06^{a}	0.20 ± 0.04^{a}	892 ± 0.05^{c}	1032 ± 0.01^{a}
	BC2-59	72.32 ± 0.09^{b}	27.68 ± 0.06^{c}	416.20 ± 1.30^{b}	332 ± 1.85^{c}	0.60 ± 0.04^{c}	0.06 ± 0.07^{a}	727 ± 0.07^{d}	528 ± 0.05^{b}
	TR10	66.37 ± 0.05^{d}	33.63 ± 0.07^{a}	414.91 ± 0.96^{b}	282 ± 1.78^{d}	0.61 ± 0.03^{c}	0.20 ± 0.05^{a}	1327 ± 0.05^{a}	126 ± 0.10^{d}
P value		<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.3475	<0.0001	<0.0001
F Value $_{3,11}$		1571.6	2881.3	768.23	3393.3	44.437	1.273	2.522-E	5.254E
SK7 X SK6	Local	74.47 ± 0.08^{c}	25.53 ± 0.06^{c}	407.37 ± 1.20^{b}	354 ± 1.30^{b}	0.93 ± 0.03^{a}	0.19 ± 0.01^{b}	1125 ± 0.03^{b}	324 ± 0.02^{b}
	Jorhat	65.62 ± 0.07^{d}	34.38 ± 0.04^{a}	363.17 ± 0.81^{c}	425 ± 1.85^{a}	1.02 ± 0.06^{a}	0.28 ± 0.02^{a}	855 ± 0.04^{d}	186 ± 0.03^{c}
	BC2-59	74.29 ± 0.06^{b}	25.71 ± 0.03^{b}	336.83 ± 1.57^{d}	348 ± 1.42^{c}	0.62 ± 0.08^{b}	0.05 ± 0.03^{c}	1035 ± 0.02^{c}	1464± 0.04^{a}
	TR10	76.04 ± 0.03^{a}	23.96 ± 0.04^{d}	410.61 ± 0.75^{a}	256 ± 1.56^{d}	1.05 ± 0.02^{a}	0.26 ± 0.02^{a}	1432 ± 0.07^{a}	108 ± 0.03^{d}
P value		<0.0001	<0.0001	<0.0001	<0.0001	<0.0016	<0.0016	<0.0001	<0.0001
F Value $_{3,11}$		5646.2	11586	997.23	2009.1	13.699	24.074	2.984E	4.249E
CSR2 X CSR4	Local	62.74 ± 0.10^{d}	37.26 ± 0.01^{a}	329.57 ± 0.89^{d}	380 ± 3.01^{b}	0.72 ± 0.06^{b}	0.19 ± 0.04^{a}	720 ± 0.09^{d}	168 ± 0.04^{c}
	Jorhat	71.01 ± 0.04^{a}	28.99 ± 0.14^{d}	376.17 ± 0.78^{c}	345 ± 1.03^{c}	0.95 ± 0.05^{a}	0.19 ± 0.05^{a}	855 ± 0.03^{c}	354 ± 0.10^{b}
	BC2-59	68.60 ± 0.03^{b}	31.40 ± 0.07^{c}	395.54 ± 0.85^{b}	511 ± 1.29^{a}	0.66 ± 0.09^{b}	0.07 ± 0.07^{a}	1110 ± 0.05^{a}	1848 ± 0.01^{a}
	TR10	67.08 ± 0.10^{c}	32.92 ± 0.08^{b}	484.79 ± 0.63^{a}	272 ± 2.05^{d}	0.62 ± 0.09^{b}	0.09 ± 0.06^{a}	937 ± 0.02^{b}	108 ± 0.06^{d}
P value		<0.0001	<0.0001	<0.0001	<0.0001	<0.0548	<0.3390	<0.0001	<0.0001
F Value $_{3,11}$		2150.1	1560.6	6714.0	2503.1	3.903	1.302	8938151	1.782E
CSR4 X CSR2	Local	68.83 ± 0.09^{c}	31.17 ± 0.07^{b}	425.98 ± 1.76^{c}	425 ± 1.05^{b}	0.74 ± 0.03^{a}	0.20 ± 0.03^{a}	1732 ± 0.03^{a}	456 ± 0.05^{b}
	Jorhat	94.97 ± 0.08^{a}	5.03 ± 0.06^{d}	287.49 ± 0.84^{d}	348 ± 1.68^{c}	0.67 ± 0.05^{ab}	0.08 ± 0.02^{b}	735 ± 0.06^{d}	192 ± 0.06^{d}
	BC2-59	67.47 ± 0.13^{d}	32.53 ± 0.16^{a}	438.57 ± 0.64^{b}	611 ± 2.54^{a}	0.61 ± 0.03^{b}	0.09 ± 0.04^{b}	1545 ± 0.04^{b}	1374 ± 0.04^{a}
	TR10	69.35 ± 0.05^{b}	30.65 ± 0.10^{c}	488.10 ± 0.59^{a}	427 ± 1.89^{b}	0.71 ± 0.04^{ab}	0.06 ± 0.02^{b}	847 ± 0.05^{c}	300 ± 0.03^{c}
P value		<0.0001	<0.0001	<0.0001	<0.0001	<0.1732	<0.0338	<0.0001	<0.0001
F Value $_{3,11}$		20665	15885	6484.1	3579.7	2.141	4.798	1.150E	1.356E

Contd…Table 6

Strain	Host plant	Fibroin % (Cocoon)	Sericin % (Cocoon)	Hemolymph protein (µg/mL)	Silk gland protein (µg/mL)	Amylase (µg/mL)	Protease (µg/mL)	DNA (ng/µL)	RNA (ng/µL)
FC1 X FC2	Local	68.83 ± 0.09^{b}	31.17 ± 0.07^{b}	424.98 ± 1.76^{b}	425 ± 1.05^{b}	0.71 ± 0.01^{a}	0.20 ± 0.03^{a}	1732 ± 0.03^{a}	984 ± 0.03^{b}
	Jorhat	79.25 ± 0.07^{d}	20.75 ± 0.05^{c}	482.41 ± 0.90^{a}	422 ± 1.09^{b}	0.73 ± 0.02^{a}	0.08 ± 0.05^{b}	757 ± 0.08^{d}	822 ± 0.04^{c}
	BC2-59	68.16 ± 0.04^{c}	31.84 ± 0.05^{a}	393.81 ± 0.95^{c}	624 ± 1.65^{a}	0.70 ± 0.03^{a}	0.17 ± 0.04^{ab}	1042 ± 0.11^{c}	1002 ± 0.03^{a}
	TR10	83.91 ± 0.07^{a}	16.09 ± 0.04^{d}	425.27 ± 0.79^{b}	282 ± 2.06^{c}	0.74 ± 0.04^{a}	0.07 ± 0.09^{b}	1342 ± 0.02^{b}	192 ± 0.01^{d}
P value		<0.0001	<0.0001	<0.0001	<0.0001	<0.7278	<0.3446	<0.0001	<0.0001
F Value $_{3,11}$		12465	21136	1004.4	8549.7	0.4444	1.282	3.522E	1.656E
FC2X FC1	LOCAL	71.20 ± 0.04^{c}	28.80 ± 0.10^{b}	385.21 ± 2.64^{b}	480 ± 2.20^{b}	0.61 ± 0.13^{a}	0.08 ± 0.02^{b}	1072 ± 0.04^{b}	792 ± 0.11^{b}
	JORHAT	71.92 ± 0.02^{b}	28.08 ± 0.03^{c}	389.47 ± 0.73^{b}	478 ± 1.07^{b}	1.27 ± 0.48^{a}	0.27 ± 0.05^{a}	1222 ± 0.12^{a}	1284± 0.03^{a}
	BC2-59	74.00 ± 0.05^{a}	26.00 ± 0.02^{d}	263.04 ± 2.00^{c}	619 ± 3.09^{a}	0.61 ± 0.18^{a}	0.09 ± 0.08^{b}	892 ± 0.03^{d}	720 ± 0.06^{c}
	TR10	66.15 ± 0.06^{d}	33.85 ± 0.03^{a}	434.90 ± 1.57^{a}	478 ± 1.61^{b}	1.26 ± 0.14^{a}	0.22 ± 0.02^{ab}	1065 ± 0.05^{c}	540 ± 0.02^{d}
P value		<0.0001	<0.0001	<0.0001	<0.0001	<0.2062	<0.0618	<0.0001	<0.0001
F Value $_{3,11}$		5477.5	3636.7	1551.2	1086.7	1.911	3.698	3753041	2.382E
J112	Local	73.76 ± 0.06^{b}	26.24 ± 0.03^{c}	325.98 ± 1.76^{c}	128 ± 1.09^{c}	0.85 ± 0.04^{a}	0.07 ± 0.02^{a}	780 ± 0.10^{d}	816 ± 0.02^{b}
	Jorhat	71.30 ± 0.16^{c}	28.70 ± 0.02^{b}	225.49 ± 0.84^{d}	311 ± 2.06^{b}	0.67 ± 0.02^{b}	0.05 ± 0.03^{a}	982 ± 0.04^{b}	1050 ± 0.05^{a}
	BC2-59	59.40 ± 0.02^{d}	40.60 ± 0.13^{a}	429.10 ± 0.64^{b}	123 ± 2.12^{c}	0.71 ± 0.02^{b}	0.06 ± 0.02^{a}	1095 ± 0.14^{a}	252 ± 0.01^{d}
	TR10	84.17 ± 0.04^{a}	15.83 ± 0.06^{d}	476.57 ± 0.59^{a}	325 ± 3.00^{a}	0.86 ± 0.04^{c}	0.07 ± 0.01^{a}	847 ± 0.07^{c}	240 ± 0.07^{c}
P value		<0.0001	<0.0001	<0.0001	<0.0001	<0.0054	<0.8910	<0.8910	<0.0001
F Value $_{3,11}$		13263	18982	10975	2618.4	9.358	0.2037	21885	8.429E

Mean ± SE of 3 observations. Within the row means followed by same letter (s) are not significantly different at P<0.05 level by Tukey's test

4.7. Differential expression of silk gland protein by SDS-PAGE

The total proteins from the middle silk gland were extracted and resolved on the SDS-PAGE for identification of fibroin protein. The heavy chain fibroin proteins from these varieties were 350 kDa and the light chain fibroin proteins were 25 kDa on the gel when compared against the known protein marker (M). There was no change in the molecular weight of these proteins but the intensity of bands varied among different *B. mori* strains reared with different mulberry varieties. Similarly, fibroin heavy chain and light chain were identified on the SDS-PAGE and the molecular weights were same but the expression differed in these varieties. A known protein marker was used as control for comparing the silk proteins and the intensity of the bands was observed as per our estimated protein concentration (**Figure 8A**). According to the densitometric analysis, FC2 x FC1 strain has highest expression for fibroin for all the four different host plants. Strain SK6 x SK7, SK7 x SK6, FC1 x FC2 and FC2 x FC1 showed highest expression level for Jorhat mulberry plant variety followed by strain CSR2 x CSR4, CSR4 x CSR2, FC2 x FC1 showed moderate level of expression for TR10 mulberry plant variety and strain CSR2 x CSR4, CSR4 x CSR2, FC1 x FC2 and FC2 x FC1 showed moderate level of expression for BC2-59 mulberry plant variety.

4.8. Analysis of transcript level of fibroin gene

The formaldehyde agarose gel used for the separation of RNA of seven different strain of *B. mori* reared with different host plant was shown in **Figure 8B.** The expression of the Fibroin and β-actin (housekeeping) partial genes for fifth instar *B. mori silk* gland was investigated by semi-quantitative Reverse Transcriptase PCR analysis. All the seven strains of *B. mori* reared on four different mulberry host plant varieties show significant differences for the level of fibroin gene expression. According to the densitometry analysis, FC2 x FC1 strain has highest

expression for fibroin for all the four different host plants. Strain SK6 x SK7, SK7 x SK6, FC1 x FC2, FC2 x FC1, showed highest expression for Jorhat mulberry plant variety, and followed by strain CSR2 x CSR4, CSR4 x CSR2, FC1 x FC2, and FC2 x FC1 for BC2-59 variety. Strain CSR2 x CSR4, CSR4 x CSR2, and FC2 x FC1 showed moderate level of expression for TR10 mulberry plant variety (**Figure 8C**).

4.9. Analysis of genetic relationship by DNA profiling (COX1, ND1, CytB, ITS1)

Optimization of the PCR was performed using four primers amplifying fragments of the mitochondrial genomes such as COX1-700 bp (**Figure 9A**), ND1-580 bp (**Figure 9B**), CytB - 1200 bp (**Figure 9C**), and nuclear genes ITS1-1000 bp (**Figure 9D**).

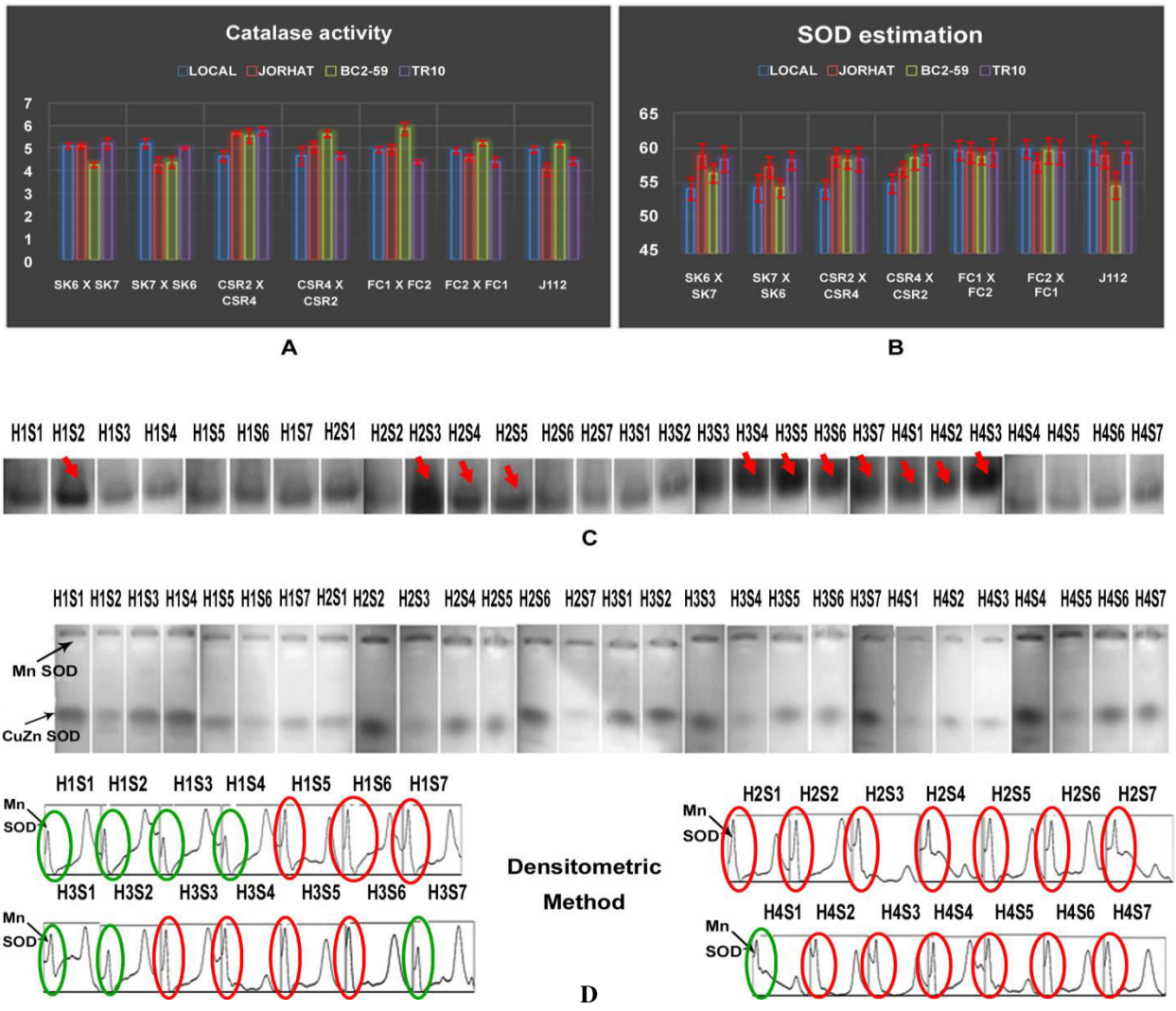


Figure 7. Quantification of CAT (A), SOD (B) activities in midgut in different silworm strains reared on different host plant regimes (Units/mg protein), Each value is the mean ± SD of 6 separate replications, Detection of Catalase (C) and Superoxide dismutase (D). *The crude protein was loaded onto 10% native polyacrylamide gel with protein molecular weight standard (14.3-97.4 kb) Electrophoresis was carried out at constant current of 20 mA.*

H1S1 – Local (SK6 X SK7), H1S2 – Local (SK7 X SK6), H1S3 – Local (CSR2 X CSR4), H1S4 – Local (CSR4 X CSR2), H1S5 – Local (FC1 X FC2), H1S6 – Local (FC2 X FC1), H1S7 – Local (J112), H2S1 – Jorhat (SK6 X SK7), H2S2 – Jorhat (SK7 X SK6), H2S3 – Jorhat (CSR2 X CSR4), H2S4 – Jorhat (CSR4 X CSR2), H2S5 – Jorhat (FC1 X FC2), H2S6 – Jorhat (FC2 X FC1), H2S7 – Jorhat (J112), H3S1 – BC2-59 (SK6 X SK7), H3S2 – BC2-59 (SK7 X SK6), H3S3 – BC2-59 (CSR2 X CSR4), H3S4 – BC2-59 (CSR4 X CSR2), H3S5 – BC2-59 (FC1 X FC2), H3S6 – BC2-59 (FC2 X FC1), H3S7 – BC2-59 (J112), H4S1 – TR10 (SK6 X SK7), H4S2 – TR10 (SK7 X SK6), H4S3 – TR10 (CSR2 X CSR4), H4S4 – TR10 (CSR4 X CSR2), H4S5 – TR10 (FC1 X FC2), H4S6 – TR10 (FC2 X FC1), H4S7 – TR10 (J112)

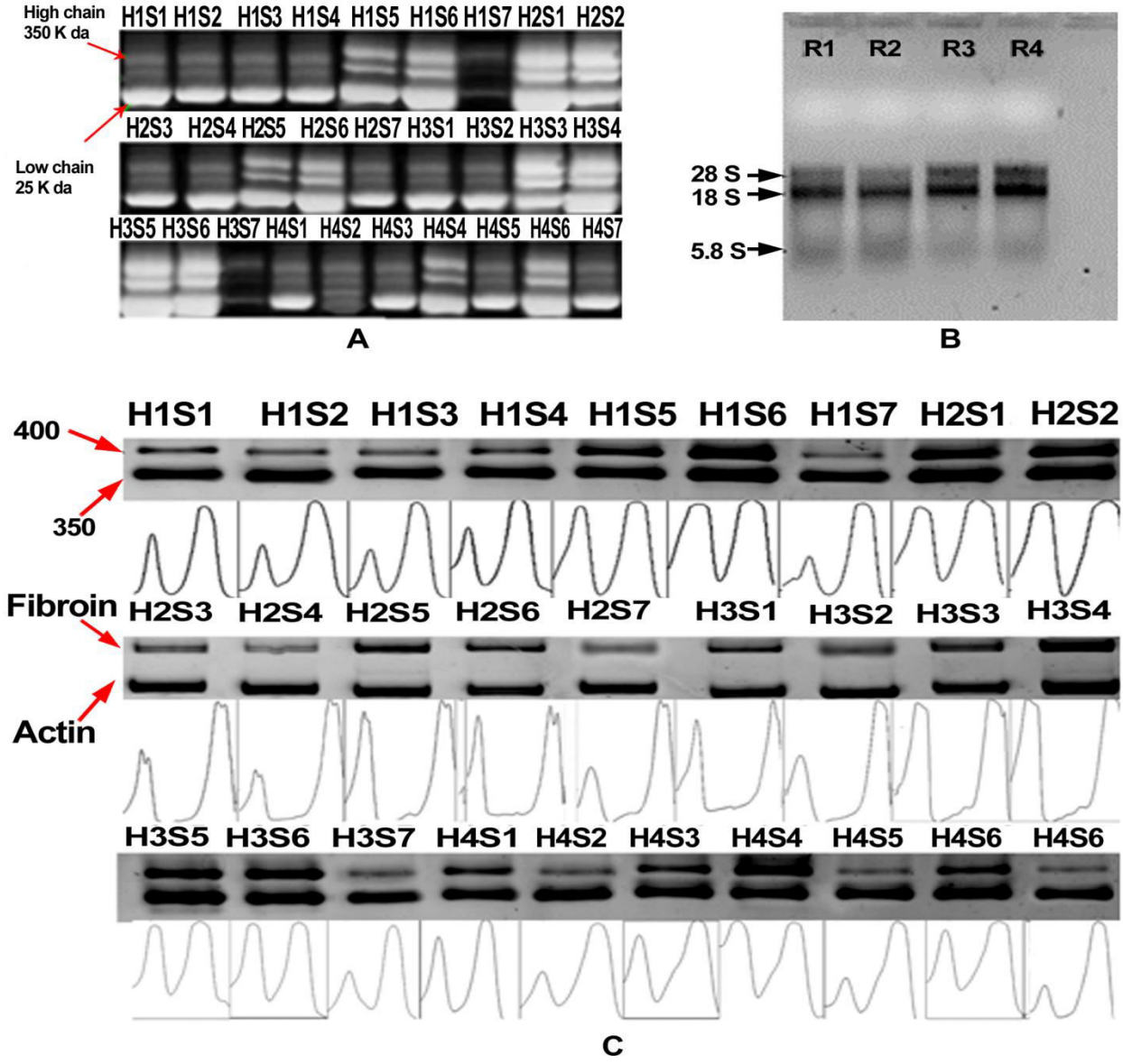


Figure 8. (A) Effects of different mulberry plant varieties on the expression of Fibroin protein on different commercially available strains of silkworm *B. mori.* (B) Extracted RNA from *B. mori* strains. (C) Gene expression of fibroin gene (400 bp) and β-Actin gene (350 bp) through Semi-quanti tative RT-PCR analysis.

H1S1 – Local (SK6 X SK7), H1S2 – Local (SK7 X SK6), H1S3 – Local (CSR2 X CSR4), H1S4 – Local (CSR4 X CSR2), H1S5 – Local (FC1 X FC2), H1S6 – Local (FC2 X FC1), H1S7 – Local (J112), H2S1 – Jorhat (SK6 X SK7), H2S2 – Jorhat (SK7 X SK6), H2S3 – Jorhat (CSR2 X CSR4), H2S4 – Jorhat (CSR4 X CSR2), H2S5 – Jorhat (FC1 X FC2), H2S6 – Jorhat (FC2 X FC1), H2S7 – Jorhat (J112), H3S1 – BC2-59 (SK6 X SK7), H3S2 – BC2-59 (SK7 X SK6), H3S3 – BC2-59 (CSR2 X CSR4), H3S4 – BC2-59 (CSR4 X CSR2), H3S5 – BC2-59 (FC1 X FC2), H3S6 – BC2-59 (FC2 X FC1), H3S7 – BC2-59 (J112), H4S1 – TR10 (SK6 X SK7), H4S2 – TR10 (SK7 X SK6), H4S3 – TR10 (CSR2 X CSR4), H4S4 – TR10 (CSR4 X CSR2), H4S5 – TR10 (FC1 X FC2), H4S6 – TR10 (FC2 X FC1), H4S7 – TR10 (J112)

The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test is shown next to the branches. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. Phylogenetic analysis of COX1, ND1, CytB and ITS1 region involved seven sequences. According to the phylogenetic tree analysis, all the *B. mori* strains are clustering together in a monophyletic tree. Genetic distances were also calculated using Tamura 3-parameter model where we have observed very less variation among the seven strains used.

COX1

In COX1 gene, the strain FC1 x FC2 was branching out from the main cluster (ML and ME method) in the phylogenetic tree (**Figure 10A & B**). But the genetic distance between all the strain showing nil except for FC1 x FC2 (0.002) (**Table 7A**) due to the single nucleotide change.

ND1

In ND1 gene, the strain CSR2 x CSR4 was branching out from the main cluster (ML and ME method) in the phylogenetic tree (**Figure 10C & D**) But the genetic distance between all the strain showing nil except for CSR2 x CSR4 (0.002) (**Table 7B**) due to the single nucleotide change.

ITS1

For ITS1 region phylogenetic tree revealed single cluster (**Figure 10E & F**) for all the strain as a unique monophyletic tree (ML and ME). There was no genetic distance observed between all the strain for ITS1 (**Table 7C**).

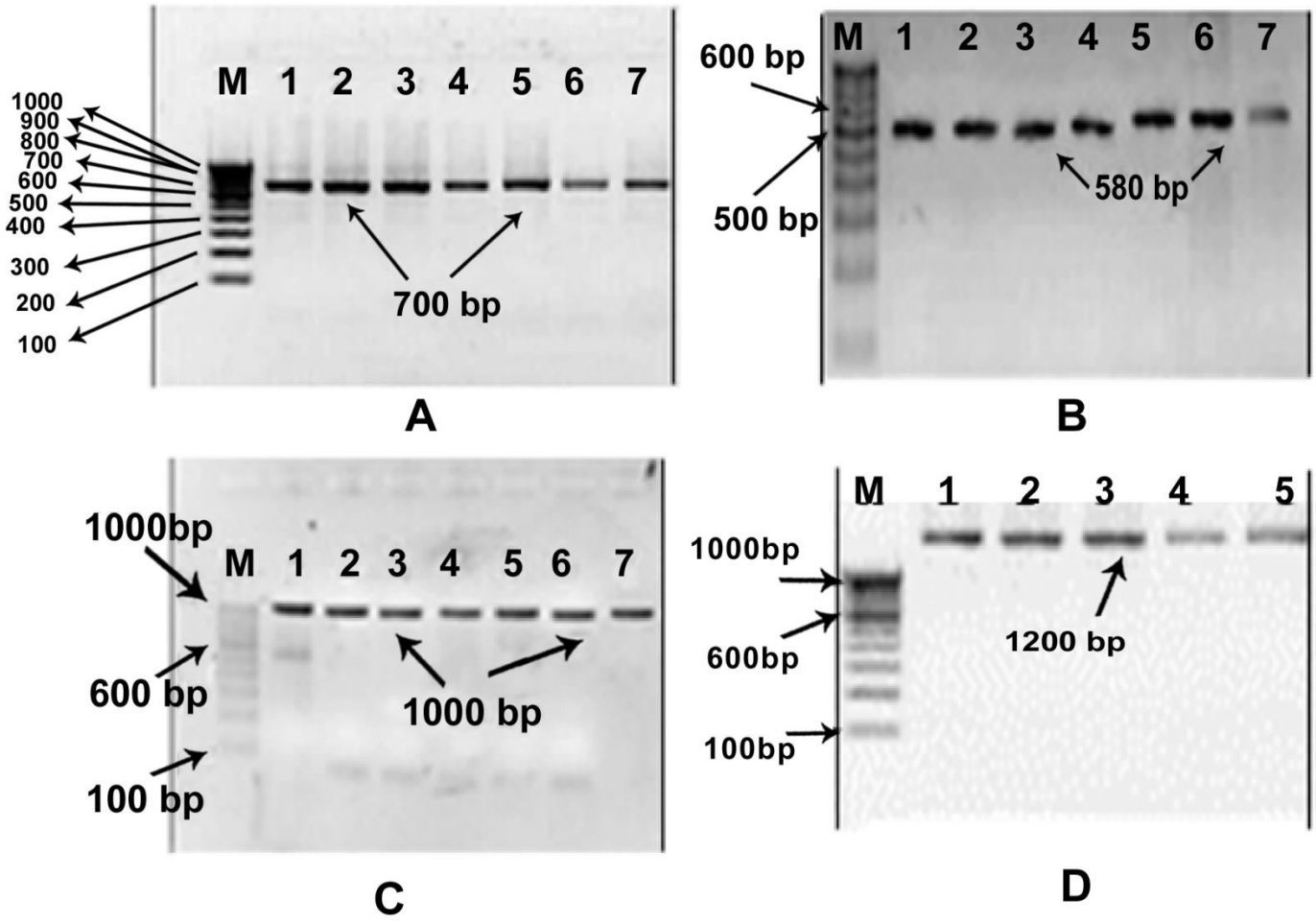
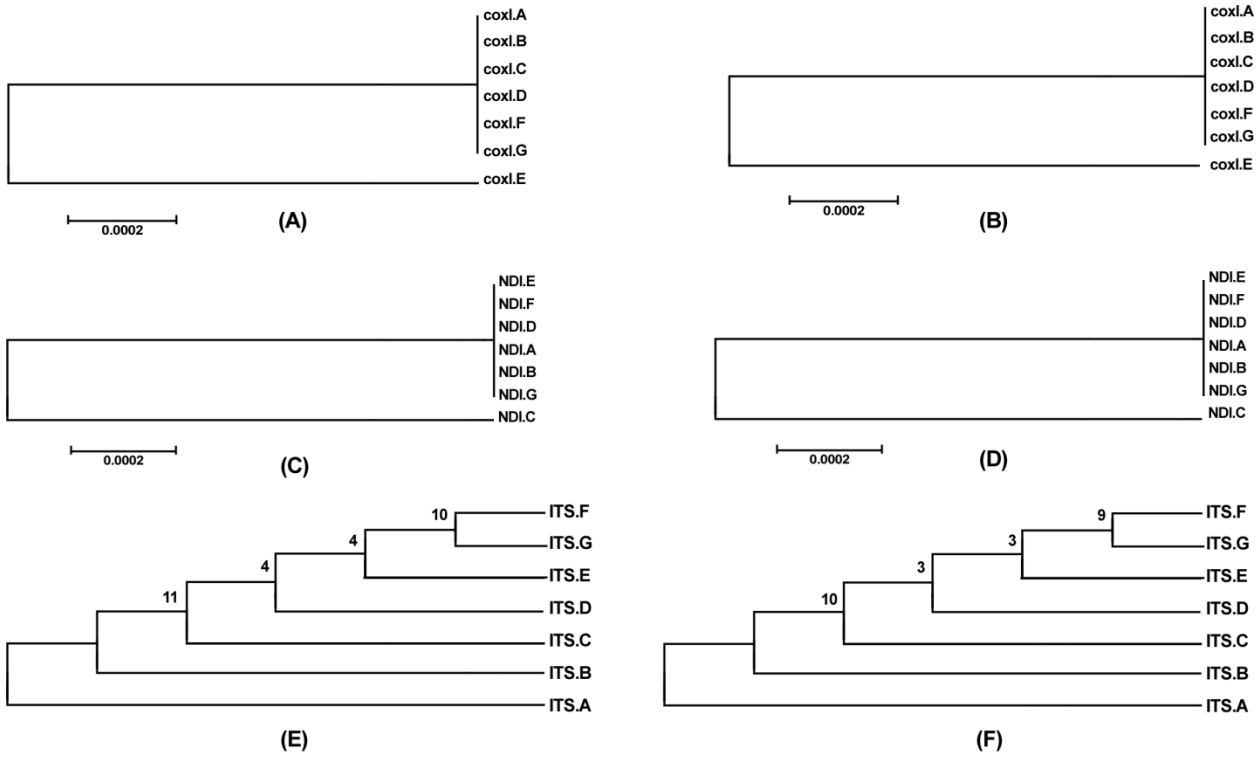


Figure 9. Amplified product for COX1- 700 bp (A), ND1- 580 bp (B), CytB- 1000 bp (C), ITS1- 1200 bp (D) for seven different strains of *B. mori* fifth instar larva.



A –SK6 X SK7, B– SK7 X SK6, C– CSR2 X CSR4, D– CSR4 X CSR2, E– FC1 X FC2, F– FC2 X FC1, G– J112

Figure 10. Phylogenetic tree constructed from COX1, ND1, CytB, ITS1 sequences of seven strains of *B. mori* using ML method and ME method of Mega 5.0 version. The ME tree was searched using the Close- Neighbor Interchange (CNI) algorithm at a search level of 1.

COX1- (A) Evolutionary history inferred by using Maximum Likelihood method (Tamura 3-parameter model) .The tree with the highest log likelihood (-761.4605) is shown. (B) Evolutionary distances computed using Tamura 3- parameter method and are in the units of the number of base substitutions per site.

ND1- (C) Evolutionary history inferred by using the Maximum Likelihood method (Hasegawa-Kishino-Yano model) . The tree with the highest log likelihood (-634.5061) is shown. (D) The evolutionary distances were computed using the Tamura 3- parameter method and are in the units of the number of base substitutions per site.

ITS1- (E) Evolutionary history inferred by using the Maximum Likelihood method (Jukes-Cantor model) . The tree with the highest log likelihood (890.0010) is shown. (F) The evolutionary distances were computed using the Maximum Composite Likelihood method and are in the units of the number of base substitutions per site.

COX1.A		**0.000**	**0.000**	**0.000**	**0.002**	**0.000**	**0.000**
COX1.B	**0.000**		**0.000**	**0.000**	**0.002**	**0.000**	**0.000**
COX1.C	**0.000**	**0.000**		**0.000**	**0.002**	**0.000**	**0.000**
COX1.D	**0.000**	**0.000**	**0.000**		**0.002**	**0.000**	**0.000**
COX1.E	**0.002**	**0.002**	**0.002**	**0.002**		**0.002**	**0.002**
COX1.F	**0.000**	**0.000**	**0.000**	**0.000**	**0.002**		**0.000**
COX1.G	**0.000**	**0.000**	**0.000**	**0.000**	**0.002**	**0.000**	
ND1.A		**0.000**	**0.002**	**0.000**	**0.000**	**0.000**	**0.000**
ND1.B	**0.000**		**0.002**	**0.000**	**0.000**	**0.000**	**0.000**
ND1.C	**0.002**	**0.000**		**0.002**	**0.002**	**0.002**	**0.002**
ND1.D	**0.000**	**0.002**	**0.002**		**0.000**	**0.000**	**0.000**
ND1.E	**0.000**	**0.000**	**0.002**	**0.000**		**0.000**	**0.000**
ND1.F	**0.000**	**0.000**	**0.002**	**0.000**	**0.000**		**0.000**
ND1.G	**0.000**	**0.000**	**0.002**	**0.000**	**0.000**	**0.000**	
ITS1.A		**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**
ITS1.B	**0.000**		**0.000**	**0.000**	**0.000**	**0.000**	**0.000**
ITS1.C	**0.000**	**0.000**		**0.000**	**0.000**	**0.000**	**0.000**
ITS1.D	**0.000**	**0.000**	**0.000**		**0.000**	**0.000**	**0.000**
ITS 1.E	**0.000**	**0.000**	**0.000**	**0.000**		**0.000**	**0.000**
ITS1.F	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**		**0.000**
ITS1.G	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	

Table7: Pairwise distance matrix of COX1- The number of base substitutions per site from between sequen- ces are shown. Standard error estimate(s) are shown above the diagonal. Analyses were conducted using the Tamura 3-parameter model, ND1 - The number of base substitutions per site from between sequences are shown. Standard error estimate(s) are shown above the diagonal. Analyses were conducted using the Tamura 3-parameter model, ITS1 - The number of base substitutions per site from between sequences are shown. Standard error estimate(s) are shown above the diagonal. Analyses were conducted using the Jukes-Cantor model.

A –SK6 X SK7, B– SK7 X SK6, C– CSR2 X CSR4, D– CSR4 X CSR2, E– FC1 X FC2, F– FC2 X FC1, G– J112

5. DISCUSSION

In the present study four mulberry genotypes i.e. Local (Hmute), Jorhat, BC2-59 and TR10 were selected to evaluate difference in their nutritive values. Shinde et al. (2014) stated that mulberry leaf quality depends on the plant variety, availability of plant nutrients and agro-ecological conditions, which reflects on the quality of silk production. In *B. mori,* the nutritional elements of mulberry leaves determine the growth and development of the larvae and cocoon production (Seidavi et al., 2005). The quality of feed plays a remarkable role and therefore is an important parameter used for evaluation for selecting best varieties for silkworm rearing and biochemical composition of mulberry leaves varies which in turn affects the economic traits of cocoons (Das et al., 2001; Kumar and Vadamalai, 2010). From the nutritional analysis of four different varieties of mulberry leaves, we have found high amount of protein, carbohydrate, reducing sugar for Local (Hmute) mulberry plant variety than any other hybrid variety but TR10 mulberry plant variety showed significant amount of amino acid, calcium, potassium, water content whereas, Jorhat mulberry plant variety showed significant amount of lipid, magnesium and phosphorus.

Antioxidant has a crucial role on larval growth and silk production (Jha, 2014). Many plant materials have been identified and documented as promising sources of natural antioxidants (Wada and Ou, 2002). Besides this, antioxidant attributes of these plant materials have been investigated as a function of growing location, species, and cultivation conditions etc., and noticeable differences were observed. Now, there is ample evidence regarding variation in

phenolics and flavonoid contents and as well as antioxidant activity with respect to different species of a plant (Zia-ul-haq et al., 2008; Perez-Lamela et al., 2007). Epidemiological studies have confirmed the disease preventive role and antioxidant activities of phenolics and many reports have highlighted variation in phenolic compounds as function of plant species (Gonzalez, 2012). The content of total phenolics was estimated as µg GAE/g of dried leaves. Flavonoids, the largest subgroup of plant phenolics, constitute almost half of the reported phenolic compounds (Rodrigues et al., 2011). Many biological effects including free radical scavenging activity have been reported for flavonoids, which are generally attributed to their structural features (Anagnostopoulou et al., 2006). The flavonoid content in mulberry leaves was estimated as µg QE /g dry weight of dried leaf samples. In our study, we have observed high amount of phenolic and flavonoid contents on Local (Hmute) and BC2-59 mulberry plant variety. Jorhat and TR10 mulberry variety showed optimum level of phenolic and flavonoid contents. DPPH assay is widely used for the evaluation of antioxidant activity of biological samples. The working principle of this assay is based on discoloration of DPPH free radical upon reacting with hydrogen donating species *i.e.*, antioxidants present in plant extracts (Krishnaiah et al., 2011). The results of DPPH assay for the leave extracts from three varieties of mulberry were calculated as IC50 (µg/mL). Jorhat exhibited the highest radical scavenging potential followed by TR10, Local (Hmute) and BC2-59 respectively. Girgih et al. (2011) reported that the reducing power activity was improved with the increase in molecular weight of peptide(s) in hemp seed protein. Biological activity of peptide depends on their amino acid composition (Korhonen and Pihlanto, 2003). Peptide containing aromatic amino acid (*Try* and *Phe*) had strong antioxidant activity (Iqbal and Khan, 2010). Some amino acids in presence of theiraromatic side chain like *Trp* and *Tyr* (indolic and phenolic group respectively) acts as strong free radical scavenger (Sharma et al.,

2010). Saiga et al. (2003) stated that the presence of hydrophobic amino acids like *Phe, Ala*, in peptide shows higher free radical scavenging activity. Thus, it can be stated that the existence of increase specific amino acids in peptides of Jorhat and TR10 mulberry variety are responsible for enhanced antioxidant activity in the selected silkworms.

Food quality influences the growth rate, consumption rate, utilization efficiency and developmental time of herbivorous insects (Levesque et al., 2002). The feed utilization efficiency of silkworm was also utilized as a tool to evaluate the quality of the mulberry varieties (Ramesha et al., 2010). In silkworm, the food consumption has a direct relevance on growth and development as well as the economic parameters. However, these interdependentparameters of consumption and productivity vary depending on the silkworm breeds. Ueda and Suzuki (1967) reported that silkworm larvae take up nutrients from mulberry leaf, digest, absorb, assimilate and convert it to silk fibre differs from race to race. Also, digestion in different insects is appropriately adapted to the nutritional composition of host upon which the specific insect feeds (Applebaum, 1985), and so we would expect to find that different silkworm strains performed best on its most preferred host plants (Slansky and Scriber, 1985). The influence of mulberry varieties on the efficiency of converting ingested and digested food into bodyweight, cocoon and cocoon shell have been emphasized by Rahmathulla et al. (2005). Hence, four mulberry varieties namely Local, Jorhat, BC2-59 and TR10 mulberry plant varieties which are known to be different in their nutritive composition were utilized in the present study.

In our study, significant difference in growth rate was observed for the SK6 x SK7 *B.mori* strain. Mean larval growth rate of SK6 x SK7 strain of *B. mori* was highest when fed with Local (Hmute) mulberry plant variety which was then followed by Jorhat, TR10, and BC2-59

mulberry plant variety. Higher CR and CI values were found in TR10 mulberry plant variety. Based on the value of the AD index, the host plants can be arranged in order of food quality as TR10, Jorhat, BC2-59, and Local (Hmute) mulberry plant varieties. ECI and ECD values of Local plant variety fed larva were greater than those of the larva fed on other host plants. In SK7 x SK6 strain of *B.mori*, we observed a higher GR for larvae fed on Jorhat and TR10 mulberry plant variety. GR values were lower on BC2-59 and Local (Hmute) mulberry plant variety. Insects fed with TR10 and Jorhat mulberry plant variety showed a higher rate of dry food consumption (CR), whereas, insects fed with BC2-59 and Local (Hmute) mulberry plant varieties showed a lower value of this index. Higher CI values were recorded on TR10 and Jorhat mulberry plant variety than for BC2-59 and Local mulberry plant variety. AD values were higher for insects fed TR10 and Jorhat mulberry plant variety and lower for those given Local (Hmute) and BC2-59 mulberry plant variety. Higher ECI and ECD values were evident on Local (Hmute) mulberry plant variety.

Greater values of GR were recorded for *B.mori* strain CSR2 x CSR4 fed on Local (Hmute) and in TR10 mulberry plant variety, and were lower in Jorhat and BC2-59 mulberry plant variety. Food consumption (CR) was greatest when larvae were fed with TR10 mulberry plant variety and least when fed with Local (Hmute) mulberry plant variety. A higher value of CI was observed in TR10 mulberry plant variety; whereas, Jorhat mulberry plant variety fed larvae showed lower CI values. A higher value of AD was found in Jorhat mulberry plant variety, while AD was lower when the larvae were fed with BC2-59 mulberry plant variety. Higher ECI and ECD values were shown by Local (Hmute) mulberry plant variety fed larvae. CSR4 x CSR2 *B.mori* strain reared on BC2-59 mulberry plant variety showed higher values of GR. The highest CR was observed on larvae fed with TR10 mulberry plant variety, while the value of this index

was very much reduced in insects fed on Local (Hmute) mulberry plant variety. TR10 mulberry plant variety fed larvae showed higher CI. Larvae reared on Jorhat mulberry plant variety showed higher values of AD, whereas; the value of this index was lower in Local (Hmute) mulberry plant variety given larvae. The value of ECI and ECD was greater in larvae fed BC2-59 mulberry plant variety.

FC1 x FC2 strain reared on TR10 mulberry plant variety showed higher values of GR. GR values were lower when fed with Jorhat mulberry plant variety. CR was higheronTR10 and lower on Local (Hmute) mulberry plant variety. A higher value of CI was evident on TR10 mulberry plant variety. AD was higher when the Larvae were fed with TR10 mulberry plant variety, while a lower value of AD was recorded when fed with Local (Hmute) mulberry plant variety. Based on ECI and ECD values host plant quality could be arranged as TR10, BC2-59, Local, Jorhat mulberry plant variety. Greater values of GR were recorded for larvae of FC2x FC1 strain fed on Jorhat mulberry plant variety, and were lower in TR10 mulberry plant variety. Food consumption (CR) was greatest when insects were fed with TR10 and least when fed with Local (Hmute) mulberry plant variety. A higher value of CI was observed in Jorhat mulberry plant variety, whereas; Local (Hmute) mulberry plant variety fed larvae showed lower CI values. A higher value of AD was found in TR10 mulberry plant variety while AD was lower when the larvae were fed with Local (Hmute) mulberry plant variety. Higher ECI and ECD values were shown by Local mulberry plant variety fed larvae.

B.mori strain J112 reared on Jorhat mulberry plant variety showed higher values of both GR. The highest CR was observed on larvae fed with TR10 mulberry plant variety while the value of this index was very much reduced in insects fed on BC2-59 mulberry plant variety. TR10 mulberry plant variety fed larvae showed higher CI. Larvae reared on TR10 mulberry

plant variety showed higher values of AD, whereas; the value of this index was lower in larvae given BC2-59 mulberry plant variety. The value of ECI and ECD was greater in larvae fed on Jorhat mulberry plant variety whereas the value of this index was reduced in case of Local (Hmute) mulberry plant variety fed larvae.

Assal et al. (1994) studied the nutritional behavior of three different strains of mulberry silkworm in relation to Insegar (Fenoxycarb) and reported that the most important effect was on the consumption index, growth rate and approximate digestibility, while efficiency of conversion of ingested or digested food was not significantly affected. So, taking into consideration of the above mentioned important effects which was on the consumption index, growth rate and approximate digestibility for estimating the nutritional efficiency of different strain of *B.mori* as well as different host plant, we have found that for SK6 x SK7 *B.mori* strain, higher GR was observed in Local (Hmute) mulberry plant variety fed larvae whereas; AD and CI was high in TR10 fed larvae. *B.mori* strain SK7 x SK6 larvae fed on Jorhat mulberry variety leaves showed improved GR in comparison to other varieties. Regarding CI and AD, TR10 mulberry plant variety showed the highest values in comparison with other plant varieties.

For CSR2 x CSR4 silkworm strain, the Local (Hmute) mulberry plant variety is the best among the host plants regarding growth rate. Certainly, a racial trait in silkworm and higher food intake does not necessarily result in higher digestibility (Ogunbanwo and Okanlawon, 2009). CI for this silkworm strain was highest on TR10 mulberry plant variety; whereas, AD was highest on Jorhat mulberry plant variety fed larvae. However, for CSR4 x CSR2 *B.mori* strain, BC2-59 mulberry plant variety is the best host plant among all the host plant evaluated for GR. AD is high in Jorhat mulberry plant variety and CI is high inTR10 mulberry plant variety. For FC1x

FC2 strain, TR10 mulberry plant variety is high in GR, CI and AD. The silkworm strain FC2 x FC1 performed best when fed with Jorhat mulberry plant variety giving highest score on GR and CI whereas; AD was highest on TR10 mulberry plant variety. The Strain of J112 when fed with Jorhat mulberry plant variety gives highest GR among other host plant. Regarding AD and CI, TR10 mulberry plant variety is highest. The study on consumption and utilization of food is of great importance to identify promising silkworm breeds (Kumara and Roy, 2011). The silkworm growth is manifested by the accumulation of organic matter resulting from the balance between anabolic and catabolic reactions fuelled by the nutritive substances absorbed after digestion of food. The silkworms from the same genetic stock responded variedly when fed on the leaves of different nutritional quality, which is an indicator of efficient utilization and conversion of food into silk substance. Variation of the ingesta and digesta values among the different breeds and same breed in different seasons have been reported (Yamamoto and Fujimaki, 1982; Sabhat et al., 2011).

Nutritional efficiency study of all the bivoltine silkworms strains used in our study revealed variation in the nutritional requirements when reared with different host plants. Significant differences in GR were observed between most of the strains reared on four different host plants, except FC1 x FC2 and FC2 xFC1 *B.mori* strain. GR was higher in insects fed with Local (Hmute) mulberry plant variety followed by TR10 and Jorhat mulberry plant variety leaf. CR was significantly higher in TR10 mulberry plant variety for all the silkworm strain except FC2 x FC1 where CI value was highest for larvae fed with Jorhat mulberry plant variety. Approximate digestibility depends on a number of factors like rate and quantity of food intake, retention time in the midgut, nature and efficiency of digestive enzymes and digestibility of the

complex nutritive components in the diet. Based on the value of the AD, it was observed that TR10 mulberry plant variety is the best plant for the entire strain digestibility, where Jorhat mulberry plant variety stands second. The ECD measures the efficiency of conversion of assimilated food and the ECI measures the overall efficiency of conversion of ingested food into insect biomass. The mean ECI were relatively higher on local mulberry plant variety fed larvae followed by Jorhat mulberry plant variety fed larvae. Whereas, ECD was significantly higher for Jorhat mulberry plant variety fed larvae followed by TR10 mulberry plant variety. Higher ECD values suggest higher food efficiency and lower cost of maintenance. Significant improvement in larval growth characters were observed in high amino acid containing mulberry reared group of insects, as compared to less amino acids containing mulberry leaves (Das et al., 1996; Radjabi 2010). We also found similar kind of result from our experiment- Local (Hmute) and TR10 mulberry plant variety reared insects are showing high growth rate due to the presence of high amino acids content. Relatively high AD might result from high water content. The present study shows that the AD values increase with the increases in leaf water content (Radha, 2013) and so in our study, most of the strains performed best on its preferred host plant TR10 mulberry plant variety except for the strain CSR2 x CSR4 and CSR4 x CSR2 which performed best on Jorhat mulberry plant variety. The high contents of most of the measured nutrients of TR10 mulberry plant variety might have been responsible for the relatively high consumption indices shown by all the *B. mori* strain feeding on these plants. Next to TR10 mulberry plant variety, Jorhat mulberry plant variety fed larvae shows high consumption indices.

The most preferred plant or plant part not only provided the nutritive requirements but also capable of being assimilated and converted into energy and structural substances required

for the normal activities and development which have been found for Local (Hmute), Jorhat and TR10 mulberry plant variety reared larva. Larvae fed with diet containing more minerals divert minimum energy for maintenance by which the larvae can channel maximum energy for seed and silk production where we also found similar kind of result in our study in which mineral contents was high in TR10 and Jorhat mulberry plant variety. Present study revealed that Local (Hmute), Jorhat and TR10 mulberry plant variety were suitable for silkworm rearing in Mizoram as was evident from having higher growth rate, consumption indices as well as approximate digestibility which are related to the optimal nutritional ability and feeding activity of silkworms.

Dietary efficiency of silkworm and its nutrition of leaves play a major role in converting the mulberry leaves to silk as well as silkworm growth (Soo-Hoo and Fraenkal, 1966; Adolkar et al., 2007). Any improvement in the leaf silk conversion ability of a given mulberry genotype or silkworm race will add to its economics (Trivedi and Nair, 1998). Studies on the influence of different mulberry varieties on silkworm's behavior and cocoon traits were studied in tropical conditions of India (Gangwar, 2011). Plants contain all nutrients required by herbivorous insects but the concentrations and proportions of these nutrients vary greatly among species (Roy and Barik, 2012). Besides environment and technology adoption, nutritive value of mulberry leaf is a key factor for better growth and development of the silkworms and cocoon production (Yogananda, 2013). Several reports are available on the evaluation of mulberry varieties through silkworm rearing performances (Iwanari and Ohno, 1969; Das and Vijayaraghavan, 1990). Thangamani and Vivekananda (1984) reported the significant influence of different mulberry genotypes on the growth and development of silkworm and cocoon production. Sujathamma et al. (2001) evaluated mulberry genotypes in Andhra Pradesh and recommended two varieties (TR10 and Mr2) for commercial cultivation. Bohidar et al. (2007) reported effect of different

mulberry genotypes on the economic parameters of silkworm in Orissa climate and made suggestion for use of mulberry variety (V1, S36, and DD) for more silk production. In our study, significant differences were observed in larval parameters and commercial cocoon characters for all the silkworm strain reared with different mulberry varieties.

For the *B.mori* strain SK6 x SK7 larval weight, the nutritional superiority of the host plant can be written accordingly as BC2-59>Jorhat>TR-10>Local (Hmute). In case of cocoon weight, filament length and filament weight Jorhat mulberry plant variety shows superiority when compared with other host plant. For shell weight, Jorhat and TR10 mulberry plant varieties were highest. Regarding shell percentage and Denier, higher value was recorded in TR-10 mulberry plant varieties than other host plant used. SK7 x SK6 strain which fed on Jorhat mulberry plant variety shows higher value of larval weight, filament length, filament weight and denier than other host plant whereas, cocoon weight is highest in Local (Hmute) mulberry plant variety fed larvae. However, the Shell weight and shell percentage of TR-10 mulberry plant variety fed larvae bear's higher value when compared to other host plant used. The host plant Jorhat mulberry plant variety shows nutritional superiority over other host plants to the *B.mori* Strain CSR2 x CSR4 regarding larval weight, shell weight, shell percentage, filament weight, filament length. Cocoon weight is highest in BC2-59 fed larvae whereas, denier value is highest in TR10 fed larvae. For the silkworm strain CSR4 x CSR2, TR-10 mulberry plant variety reared larvae showed highest larval weight whereas, in case of cocoon weight, shell weight, shell percentage, filament length and filament weight highest value was observed in Jorhat treated larvae. However, highest denier value was observed in TR10 mulberry variety. In case of FC1 x FC2 *B.mori* strain, the weight of larval, cocoon, shell, and filament, filament length and denier

was observed to be superior in Jorhat mulberry variety in its nutritional quality than other host plant used. Whereas, shell percentage value was observed to be highest in TR10 mulberry plant variety reared larvae. FC2 x FC1 *B.mori* strain showed higher larval weight, cocoon weight, shell weight, filament weight, and filament length when reared with Jorhat mulberry plant variety. Whereas, TR10 mulberry plant variety reared larvae showed highest shell percentage as well as denier value. J112 *B.mori* strain when reared with TR10 mulberry plant variety gives highest larval weight, shell weight and shell percentage. However, Local (Hmute) plant variety holds the highest value for cocoon weight, filament weight and denier. For shell weight and filament length, Jorhat mulberry plant variety showed nutritional superiority over other host plants used.

According to Fonseca et al. (1990) and Seidavi (2011) rearing performance of silkworm races differed significantly when they are subjected to same conditions, some of them being better performer whereas, some races shows poor performance. Present study also confirms the same where different host plant was evaluated for different silkworm strain. Gangawar (2010) reported that among eight mulberry varieties i.e; S1, S146, S1635, AR12, AR14, TR10, BR2 and K2 evaluated for nutritional potential by silkworm rearing experiments, silkworm larvae fed on BR2 variety leaves showed higher larval weight and improved economic traits like cocoon weight, shell weight and silk percentage in comparison to other varieties. Cocoon weight and shell weight are the most important characters evaluated for productivity (Gaviria et al., 2006). Shell weight percentage indicates the amount of raw silk can be reeled from the given quantity of fresh cocoons and shell weight percentage varies according to age and breed of silkworm. So, taking into consideration the economic significance of the above mentioned parameters for productivity, we have found that for SK6 x SK7 *B.mori* strain showed higher cocoon weight and

shell weight when fed on Jorhat mulberry variety. However, TR10 also shows its nutritional superiority with regards to shell weight and shell percentage.

According to Food and Agricultural Organization, (1999) total silk filament length is ranging from 600 m-1500 m out of which only 80 percentage is reelable. In the present study, silk filament length of cocoons recovered from silkworms reared on different mulberry varieties falls within this range and cocoons recovered from silkworms reared on Jorhat mulberry variety leaves produced longest filament length. Thus, we can conclude that Jorhat mulberry plant variety is the best host plant for the silkworm strains- SK6 x SK7. *B. mori* strain SK7 x SK6 larvae fed on TR10 mulberry variety leaves showed improved economic traits like shell weight and silk percentage in comparison to other varieties. Regarding the cocoon weight there is not much significant difference and for filament length, TR10 falls next to Jorhat plant variety. So, we can conclude that TR10 is the best host plant for this strain. For CSR2 x CSR4 silkworm strain, there is no significant difference regarding the cocoon weight. Jorhat is the best for the strain among the host plants in both Shell Weight, shell percentage as well filament length which proves it is the most recommendable host plant for this strain. However, for CSR4 x CSR2 *B.mori* strain, Jorhat mulberry plant variety is the best host plant among all the host plant evaluated for nutritional superiority regarding cocoon weight, shell weight and shell percentage as well as filament length. For FC1 x FC2 strain, Jorhat mulberry plant variety holds the highest value in case of cocoon weight and Shell Weight. TR10 mulberry plant variety fed larvae showed the highest value for shell percentage, and stand next to BC2-59 mulberry plant variety regarding the filament length. So, we can say that, both Jorhat and TR10 mulberry plant variety is likely to be the best host plant for this silkworm strain. The silkworm strain, FC2 x FC1

performed best when fed with Jorhat mulberry plant variety when compared with other host plant with regards to economic traits such as cocoon weight, shell weight and filament length. Strain J112 when fed with Jorhat mulberry plant variety gives highest shell weight, filament length among other host plant. Regarding cocoon weight and shell percentage it stands next to Local (Hmute) and TR10 plant varieties, respectively. So, we can assume Jorhat to be the most recommendable host plant for J112 *B.mori* strain. However, Local (Hmute) as well TR10 also performed well for this strain. Feeding with high content of potassium, magnesium and calcium in mulberry leaves significantly increased the larval weight, cocoon weight, shell weight, shell percentage (Mane et al., 1998) which have been found for the TR10 and Jorhat plant variety reared larva. The shell percentage, shell weight and cocoon weight is high for Jorhat and TR10 plant variety reared larva, may be due to the high amount of amino acids, potassium, magnesium, calcium present in the leave which have been previously noticed by the Verma and Atwal (1963). Rearing performance of all the bivoltine silkworms strains used in our study proved to be better when fed with Jorhat mulberry variety leaves followed by TR10. This might be because the higher antioxidant present in Jorhat and TR10 mulberry varieties acts as reducing agents for free radical ions presents in the silkworm larval body such that they produce more silk proteins, the oxidative stress being mitigated through antioxidant rich peptides. Cocoon shell weight depends on the weight of raw silk. Economic attributes like cocoon weight and single shell weight might be increased with the elicitation of free-radical scavenging properties in mulberry leaves by absorbing these peptides. Other possibility may be that the antioxidants directly or indirectly affect silk protein synthesis in the larval body and subsequently increase shell weight (Jha et al., 2015). Among the different silkworm strain used CSR4 x CSR2 strain fed with Jorhat mulberry variety exhibited the best performance. FC2 x FC1 strain fed with Jorhat mulberry

variety proved promising. Besides Jorhat mulberry variety, FC1 x FC2 strain fed with TR10 mulberry plant variety also showed good performance. Leaves of these varieties supported good growth and development of silkworm larvae, which is reflected in better commercial cocoon characteristic features. Local (Hmute) mulberry varieties occupied last place in bioassay results. From the results, it is reported that, *B.mori* strain CSR4 x CSR2 and FC2 x FC1 fed with Jorhat mulberry plant variety and *B.mori* strain FC1 xFC2 fed with TR10 mulberry plant variety turns out to be superior in among other *B. mori* strains as well as host plant used in this experiment.

Bombyx mori strain SK6 x SK7 shows high fibroin percentage, hemolymph protein, silk gland protein, amylase and protease for Jorhat mulberry plant variety and high sericin percentage for TR10 and Jorhat mulberry plant variety. SK7 x SK6 strain shows high fibroin percentage, hemolymph protein, amylase for TR10 mulberry plant variety. Sericin percentage, silk gland protein, protease for Jorhat mulberry plant variety. CSR2 x CSR4 strain shows high fibroin percentage, amylase, protease for Jorhat mulberry plant variety, high hemolymph protein for TR10 variety and high silk gland protein for BC2-59 mulberry plant variety. CSR4 x CSR2 strain shows high fibroin percentage for Jorhat mulberry plant variety, high hemolymph protein for TR10 variety and high sericin percentage, silk gland protein for BC2-59 mulberry plant variety and amylase and protease for Local mulberry plant variety. FC1x FC2 strain shows high fibroin percentage and amylase for TR10 mulberry plant variety, high hemolymph protein for Jorhat mulberry plant variety and high sericin percentage, silk gland protein for BC2-59 mulberry plant variety. FC2 x FCstrain shows high fibroin percentage and silk gland protein for BC2-59 mulberry plant variety; high hemolymph protein, sericin percentage for TR10 mulberry plant variety and high amylase and protease for Jorhat plant variety. J112 strain shows high

fibroin, hemolymph protein, silk gland protein, amylase, protease for TR10 mulberry plant variety, high sericin percentage, for BC2-59 mulberry plant variety. The conversion of leaf nutrition into the silk protein mainly takes place during larval stage. The various aspects of protein metabolism including quantitative changes in the midgut and haemolymph protein and synthesis and metabolic activity of specific enzymes have attracted the interest of many insect biochemists. The available results from these biochemical studies indicate that protein metabolism is of considerable importance in characterizing different stages of insect development (Chen, 1966). Nutrition relates to the physiology of digestion. The silkworm digests albumin, fat and carbohydrates except cellulose. The ability of silkworm to produce and secrete digestive enzymes is to a great extent influenced by the nutrient composition of the meal (Kellner et al., 1887). Silkworm requires specific essential sugars, amino acids, proteins and vitamins for its normal growth (Sengupta et al., 1972; Khedr, 2013). Silkworm midgut digestive enzymes have been studied in detail by various scientists (Abraham et al., 1992; Kanekatsu et al., 1978; Eguchi et al., 1976). Poor nutrition and low-nutrient diets have direct effects on primary biochemical and physiological systems, and thus may decrease the performance of insects by effecting changes in the detoxification system that can alter the susceptibility of the insect (Lindroth et al., 1991). In the present study activity of the enzyme amylase were high in those larvae reared with Jorhat mulberry variety followed TR10 mulberry variety which may be due to the sufficient amount of substrate resulting from high food intake. Digestion of leaf proteins is aided by the proteolytic enzymes, proteases. Late silkworms are generally eat coarse leafs, and are suppose to have a highly specific protease enzyme system that hydrolyzes the fibrous protein found in abundance in coarse mulberry leaves (Ito et al., 1966). The proteolytic activity of the alimentary canal in relation to feeding of proteins has been studied in many insects (Hamano et

al., 1970). In the present study, protease activity has been better on larvae fed with Jorhat mulberry plant variety and it is presumed that the leaf may activate the enzyme molecules to act on their substrates, or the enzyme molecules may be have sufficient amount of substrate. Approximate digestibility was found significantly high for TR10 mulberry plant variety followed by Jorhat mulberry plant variety reared larva, may be due to the presence of high amylase and protease in the larva reared on TR10 and Jorhat mulberry plant varieties.

Proteins are the building blocks of an organism. Hemolymph serves as a reservoir for nutrients and metabolites during metamorphosis. The cellular structures of silk gland also differentiate and repair themselves for synthesis of silk proteins by utilizing the free amino acids present in the hemolymph (Mathur et al., 1989; Mahmoud, 2013).The silk is secreted by the silk glands which is a reservoir for two important silk proteins such as fibroin and sericin (Zhang et al., 2006). In the present study, the silk gland registered more protein content when compared to hemolymph in majority of the experimental silkworm strain used except J112 during fifth instar larval stage where TR10 and Jorhat mulberry varieties fed larvae gives high hemolymph content whereas, silk gland protein was high in those larvae fed with BC2-59 variety followed by Jorhat variety. *B. mori* produces a delicate twin thread of silk fibroin, which is coated by a protective cover of sericin. Silk protein is a kind of protein like collagen, elastin, fibroin etc., is an essential constituent of cocoon filament (Komatsu, 1975). In our study, we have observed that fibroin percentage is significantly higher for most of the *B.mori* strain fed with TR10 and Jorhat mulberry plant variety may be due to the presence of high amino acids and carbohydrate. Presence of high protein implies, coordinated functioning of all the elements of the cell machinery devoted to fibroin assembling and maturation. The supply and metabolism of the amino acid used for fibroin synthesis and the mechanisms of amino acid activities prior to their

polymerization on polysomes. And also, hemolymph protein significantly high for TR10 mulberry plant variety reared larva followed by Jorhat mulberry plant variety reared larva, which is significantly increasing the fibroin percentage in silk gland because during intermoult stages, most of the amino acids resulting from digestion are transported directly to the silk gland via. hemolymph (Bricteux et al., 1965). Approximate digestibility was found significantly high for TR10 mulberry plant variety followed by Jorhat mulberry plant variety reared larva, may be due to the presence of high amylase and protease in the larva reared on TR10 and Jorhat plant variety.

The Deoxyribonucleic acids content in insect tissue is an index for expressing other biochemical contents like RNA and protein. 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. DNA and RNA are most important biomolecules of the cell as they controls overall metabolism of the cell or organism. Such biochemical growthrate indicators, such as RNA concentration or the RNA/DNA ratio, are routinely used for estimating growth rates and nutritional condition of larval fish in the field of marine ecology (Buckley, 1984). In our study, the DNA and RNA content was high in Local (Hmute) mulberry plant variety followed by TR10 and BC2-59 mulberry plant variety followed by Jorhat mulberry plant varieties, respectively. However, *B.mori* strain reared with Jorhat and TR10 mulberry plant varieties produced more silk than those larvae reared with other mulberry varieties. These results suggest that the silkworm strain reared with TR10 and Jorhat mulberry plant variety is producing moderate level of fibroin protein which result in increase silk productivity.

CAT catalyzes the conversion of hydrogen peroxide to water and molecular oxygen (Dringen, 2000). In our study the increase in CAT activity in all the strains of *B. Mori* after

reared on different variety of mulberry could be expected in order to scavenge hydrogen peroxide. Hence, the Local (Hmute) and BC2-59 mulberry plant varieties was showing high amount of catalase activity than Jorhat and TR10 mulberry plant variety, it might be due to high production of hydrogen peroxide for Local (Hmute) and BC2-59 varieties. The decrease of CAT activity for Jorhat and TR10 mulberry plant varieties may be due to the fact that CAT is known to be inhibited by the accumulation of superoxide anion during destruction processes (Kono and Fridovich, 1982) because for Jorhat and TR10 mulberry plant variety high level of SOD activity was observed. MnSOD located in the mitochondrial matrix and CuZnSOD located in the mitochondrial intermembrane space, cytosol and extracellular space. These key enzymes catalyze the dismutation (disproportionation) of superoxide anion radical to hydrogen peroxide and molecular oxygen (Christianson, 1997). In doing so, they protect cells against oxidative damage and regulate the cellular concentration of O_2^- and its reactive progeny under both physiological and pathological conditions. Previous study reported that induction of SOD activity is the main response to organophosphate toxicity and other dietary pro-oxidant exposure in *B. mori* larva (Michael and Subramanyam, 2013). High level of SOD was found for larva reared on Jorhat and TR10 mulberry plant varieties because of low level of catalase activity. This endogenous mitochondrial antioxidant defense mechanism is essential in the *B. mori* mid-gut.

Protein gels were analyzed comparing the banding patterns; intensity and their molecular mass against protein molecular markers run parallely with samples using gel image analysis software-Syngen G-Box and ImageJ. In the present work, the molecular weight of the fibroin proteins we obtain from the seven silkworm hybrids viz., SK6 x SK7,SK7 x SK6, CSR2 x CSR4, CSR4 x CSR2, FC1 x FC2,FC2 x FC1 and J112 reared with Local, Jorhat, BC2-59 and TR10 mulberry plant varieties are quite similar with the results observed by molecular biologists

earlier (Sabina, 2012). Electrophoretic patterns of fibroin proteins at the fifth instar larva stage of different strains of *B. mori* reared with different host plant observed in silk gland tissues observed on SDS-PAGE differed in expression. Analysis of the transcript level of fibroin genes for the seven different strains of fifth instar *B. mori* larval silk gland reared with different host plant by semi-quantitative Reverse Transcriptase PCR analysis show significant differences for the level of fibroin gene expression. These results suggest that SK6 x SK7, SK7 x SK6, FC1 x FC2 is producing moderate level of fibroin protein followed by CSR2 x CSR4, CSR4 x CSR2, FC2 x FC1 when reared with Jorhat and TR10 mulberry plant variety, respectively. The accumulation of trehalose during the fifth instar is necessary for either growth or maintenance, or both of the silk glands. The stored trehalose may be used by the silk glands for the information of the cocoon or during the histolysis of the gland, or both. The pattern observed for carbohydrate affords additional evidence that released glucose is utilized for trehalose synthesis which may be used by the silk gland (Unni and Pant, 1985). This might be the cause for the high carbohydrate and amino acids contain in Jorhat and TR10 plant variety. The reserve of the four major amino acids – glycine, alanine, serine and tyrosine is used for fibroin synthesis. TR10 and Jorhat mulberry plant variety leaves contain high level of amino acids Zhou et al. (2000). Some of the glycine and serine is taken up directly from the hemolymph. The complement of these two amino acids and the major proportion of alanine are synthesized in the silk gland cells. Aspartic acids, glutamic acid and their amides are the source for the necessary carbon and nitrogen. And all the tyrosine is taken up from the hemolymph (Bricteux et al., 1965). Remarkably, Jorhat and TR10 mulberry plant variety leaves were showing high level of amino acids content and the larva reared on Jorhat and TR10 mulberry plant variety was also showing high amount of protein in hemolymph which was may be effecting the fibroin gene and protein expression in silk gland

and cocoon for the strain reared on Jorhat and TR10 mulberry plant variety. Although BC2-59 mulberry plant variety reared strain also has moderate level of fibroin gene and protein expression, the silk fiber filament length and denier were not satisfactory when compared to TR10 and Jorhat mulberry plant variety reared larva. This may be because a disulfide linkage between the heavy (H) chain and light (L) chain of fibroin protein is not formed because of partial deletion of the L-chain gene, which is essential for the intercellular transport and secretion of fibroin (Inoue et al., 2005). In this condition, the posterior silk glands are not sufficiently developed and the liquid fibroin is scarcely secreted, but there is no such disorder in the middle silk glands. These might be for the not getting proper nutrition from the host plant or wrong selection of the host variety (Yamamoto et al., 2002). Based on view point of economic parameters, BC2-59 is not a good host plant for the selected strains for this region due to the bad quality of silk fiber filament. In case of Jorhat and TR10 mulberry plant varieties, the silkworm strains produced high quality of silk fiber filament, moderate amount of fibroin in cocoon and moderate level of fibroin expression in silk gland. So, Jorhat and TR10 mulberry plant varieties are the economically best varieties of host plants for the selected insect strains under these environmental conditions. In the present study, we also analysed the genetic variation between the seven different strains of *B. mori* using COX1, ND1, Cytb and ITS1 but no variation was observed between the strains. So the *B. mori* strains are showing relative host plant specificity not due to their genetic diversity.

Sericulture hold the tremendous scope for development of rural economy of the Mizoram as it has the advantages of close association with the tradition and culture of local populace, eco-friendly production process and skilled household in rearing, reeling and weaving. It is one of the most promising income resources to this region without spending much for its cultivation and

better utilization is the call of the hour. This is the first scientific research done on host plant relationship on different *B. Mori* strain reared with different mulberry variety in Mizoram. These findings can form a platform for further research on sericulture especially under the agro-climatic conditions of Mizoram. From the results, we have observed that, *B.mori strain* CSR4 x CSR2, FC1 x FC2 and FC2 x FC1 and mulberry varieties Jorhat and TR10 turns out to be superior in silkworm rearing tests compared to other silkworm strains and mulberry varieties examined respectively under same agro climatic conditions. Such mulberry variety and silkworm strains can be recommended for more trials at field level by farmers and could be exploited for commercial purpose in Aizawl, Mizoram state for sustainable growth and development of sericulture industry.

6. SUMMARY OF THE STUDY

1. In the present study, the effect of the host plants was analyzed by rearing seven different bivoltine strains (SK6 x SK7, SK7 x SK6, CSR2 x CSR4, CSR4 x CSR2, FC1 x FC2, FC2 x FC1 and J112) on four different mulberry plant varieties (Local- Hmute, Jorhat, BC2-59 and TR10).
2. TR10 showed significant optimum amount of protein, lipid, carbohydrate, reducing sugar, magnesium, phosphorus and high amount of amino acid, calcium, potassium, water contents in the leaves.
3. Significant higher concentration ($p<0.05$) of total phenol, flavonoid was observed in methanolic extract than aqueous extract. Total phenol and total flavonoid contents were high on Local (Hmute) and BC2-59 mulberry variety, whereas; DPPH radical scavenging activity was highest on Jorhat mulberry plant variety followed by TR10 mulberry plant variety.
4. SK6 x SK7 strain showed high consumption rate, consumption index, approximate digestibility, shell weight, shell percentage, denier, sericin percentage and protease for TR10 mulberry plant variety whereas; growth rate, ECI and ECD were high for Local (Hmute) mulberry plant variety. Cocoon weight, filament length, filament weight, shell weight, fibroin percentage, hemolymph and silk gland protein, amylase and protease were high for Jorhat mulberry plant variety.
5. SK7 x SK6 strain shows high consumption rate, consumption index, approximate digestibility, shell weight, shell percentage, fibroin percentage, hemolymph protein and

amylase for TR10 mulberry plant variety whereas, growth rate, filament length, filament weight, denier, sericin percentage, silk gland protein and protease were high for Jorhat mulberry plant variety. ECI, ECD and cocoon weight were high for Local (Hmute) mulberry plant variety.

6. CSR2 x CSR4 strain showed high consumption rate, consumption index, denier, hemolymph protein for TR10 mulberry plant variety whereas; approximate digestibility, shell weight, shell percentage, filament length, filament weight, fibroin percentage, amylase and protease were high for Jorhat mulberry plant variety. Growth rate, ECI, ECD and sericin percentage were high for Local (Hmute) mulberry plant variety. Cocoon weight, silk gland protein was high in BC2-59 mulberry plant variety.
7. CSR4 x CSR2 strain shows high consumption rate, consumption index, denier and hemolymph protein for TR10 mulberry plant variety whereas; approximate digestibility and cocoon weight, shell weight, shell percentage, filament length, filament weight and fibroin percentage were high for Jorhat mulberry plant variety. Growth rate, ECI, ECD, sericin percentage and silk gland protein were high for BC2-59 mulberry plant variety. Amylase and protease were high for Local (Hmute) mulberry plant variety.
8. FC1 x FC2 strain showed high consumption rate, consumption index, approximate digestibility, ECI, ECD, shell percentage, fibroin percentage and amylase for TR10 mulberry plant variety whereas; growth rate, cocoon weight, shell weight, filament length, filament weight, denier and hemolymph protein were high for Jorhat mulberry plant variety. Sericin percentage and silk gland protein were high for BC2-59 mulberry plant variety and protease was high in Local (Hmute) mulberry plant variety.

9. FC2 x FC1 strain shows high Consumption rate, approximate digestibility, shell percentage, denier, hemolymph protein and sericin (Hmute) for TR10 mulberry plant variety whereas, consumption index, growth rate, cocoon weight, shell weight, filament length, filament weight, amylase and protease were high for Jorhat mulberry plant variety. ECI and ECD were high for Local (Hmute) mulberry plant variety. Fibroin percentage and silk gland protein were high for BC2-59 mulberry plant variety.

10. J112 strain shows high consumption rate, consumption index, approximate digestibility, shell weight, shell percentage, Fibroin percentage, hemolymph and silk gland protein, amylase and protease for TR10 mulberry plant variety whereas, growth rate, ECI, ECD and filament length were high for Jorhat mulberry plant variety. Cocoon weight, filament weight and denier were high for Local mulberry plant variety. Sericin percentage was high for BC2-59 mulberry plant variety.

11. Catalase activity was observed relatively high activity in the CSR4 x CSR2 strain, followed by SK7 x SK6, respectively. BC2-59 variety showing highest activity relatively followed by Local (Hmute) variety.

12. SOD activity was observed relatively high activity in the FC1 x FC2 strain, followed by FC2 x FC1, respectively. Over all Jorhat variety showing highest activity followed by TR10 variety.

13. Catalase expression was observed to be highest *B. mori* larvae reared with BC2-59 mulberry plant variety followed by Jorhat, TR10 mulberry plant. Among the *B. mori* strains used SK7 X SK6 shows high expression followed by CSR2 x CSR4, CSR4 x CSR2 and FC1 x FC2.

14. SOD expression was observed to be highest in Jorhat mulberry plant variety for all the *B. mori* strain used followed by TR10 mulberry plant variety where expression was also high in all the *B. mori* strain used except for the strain SK6 x SK7. BC2-59 mulberry plant variety reared *B.mori* strain also showed high expression except for the *B. mori* strain SK6 x SK7, SK7 x SK6 and J112.

15. FC2 x FC1 strain has highest expression for fibroin protein and gene for all the four different host plants. *B. mori* strain SK6 x SK7, SK7 x SK6, FC1 xFC2 and FC2 x FC1 showed highest protein and gene expression level for Jorhat mulberry plant variety followed by CSR2 x CSR4, CSR4 x CSR2, FC2 x FC1 for TR10 mulberry plant variety and CSR2 x CSR4, CSR4 x CSR2, FC1 x FC2 and FC2 x FC1 for BC2-59 mulberry plant variety showing moderate level of fibroin protein and gene expression.

16. These results suggest that the strains SK6 x SK7, SK7 x SK6, FC1 x FC2 and FC2 x FC1 are producing moderate levels of fibroin protein followed by CSR2 x CSR4, CSR4 x CSR2, FC2 x FC1 when reared with Jorhat and TR10 mulberry plant varieties, respectively.

17. Jorhat and TR10 mulberry varieties reared silkworm strains showed better rearing performance as well as produced good quality of silk fibre filament, moderate amount of fibroin content in cocoon and moderate level of fibroin protein and gene expression in silk gland.

18. Jorhat and TR10 mulberry plant varieties are the economically best varieties of host plants for the selected *B. mori* strains under these agro-climatic conditions and hence, could meet the immediate requirement of the improved mulberry varieties for Mizoram, India and other states of North-Eastern region.

19. Recommendation to be given here for Mizoram Central Silk board and farmers: According to the economic parameter Jorhat and TR10 variety are equally good as host plant for the CSR2 x CSR4, FC1 x FC2 and FC2 x FC1 *B. mori* strain, respectively.

20. Phylogenetic analysis using CO1, ND1 and ITS1 genes for the seven different strains of *B. mori* results in a monophyletic tree with different tree building method (ML and ME).

21. Genetically, there is no variation between the experimental *B. mori* strains. So, the *B. mori* strains are showing relative host plant relationship due to their genetically homogeneity.

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8. Research publications

Lalfelpuii R, Choudhury BN, Gurusubramanian G, Kumar NS (2014) Effect of different mulberry plant varieties on growth and economic parameters of the silkworm *Bombyx mori* in Mizoram. Science Vision 14(1): 34-38.

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Lalfelpuii R, Souvik Ghatak, Kumar N S and Gurusubramanian G (2016) Effect of different mulberry plant varieties on the expression of Fibroin gene and protein in commercially available strains of silkworm *Bombyx mori.* Insect Physiology and Biochemistry (Manuscript Communicated).

Lalfelpuii R, Souvik Ghatak, Kumar N S and Gurusubramanian G (2016) Comparative studies on the influence of host plant chemical stimulants on the feeding and biochemical parameters in commercially available strains of silkworm *Bombyx mori.* Journal of Insect Physiology (Manuscript Communicated).

9. Conference/ Seminars/ Workshop Attended

National Seminar on "Environment, biodiversity, veda and traditional systems" (10^{th}-12^{th} April, 2012) jointly organized by Department of Zoology, MZU, MANU - International Council for Man and Nature, Asia Chapter, and Action for Sustainable, Efficatious Development and Awareness (ASEA), Rishikesh, Uttarakhand.

One Day State Level Seminar on "Sustainable energy for all" (24^{th} August 2012) organized by MIPOGRASS and Mizoram Council of Science, Technology and Environment, Directorate of Science and Technology, Govt. of Mizoram.

Workshop on "Sensitization workshop for development of location specific R&D and demonstration project for SC/ST" (28^{th} August 2012) organized by Mizoram Council of Science, Technology & Environment, Govt. of Mizoram.

Workshop on "1^{st} Summer school cum workshop" (23^{rd} – 27^{th} May, 2011) organized by Institutional Level Biotech Hub, Department of Pharmacy, Regional Institute of Paramedical & Nursing Sciences, RIPANS, Aizawl".

National Seminar on "Recent trends in research & development in muga culture- Ideas to action" (3^{rd}-4^{th} May, 2012) organized by Central Muga-Eri Research & Training Institute, Lahdoigarh; Muga Silkworm Seed Organization (MSSO) and Regional Office, Guwahati, Central Silk Board, Govt. of India.

5th Interactive Meeting of North East Bioinformatics Centers (NEBInet) (11-12 october, 2012) organized by Mizoram University, Aizawl.

Lecture series on "Seri biotechnology" (10th January, 2014) organized by Biotech Hub, Centre for the Environment, Indian Institute of Technology, Guwahati.

Workshop on " Techniques in molecular biology and bioinformatics" (26th- 31st January, 2014) organized by DBT- State Biotech Hub and Bioinformatics Infrastructure Facility sponsored by Department of Biotechnology (DBT), Ministry of Science and Technology, Government of India, New Delhi.

Workshop on State Level Hands-on Workshop in "Biostatistics using sigma plot" (27th June, 2014) sponsored by Department of Biotechnology (DBT), New Delhi .