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"Genetic diversity and relationship among different varieties of eri silkworm *Philosamia ricini*"

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"GENETIV DIVERSITY AND RELATIONSHIP AMONG DIFFERENT VARIETIES OF ERI SILKWORM *PHILOSAMIA RICINI*"

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Sericulture is a technique of silk production and an important agro based labor intensive cottage industry with greater employment opportunities in rural areas. First time it was introduced in China by the Queen Hoshomin. Later, it was started practicing in India, Japan, Korea and some of European countries as well. Now, sericulture is practicing in more than 50 countries of the world under tropical and sub-tropical conditions. India is the second largest country in silk production stands next to China. Its cultivation has spread in the country since 140 BC (Rangaswamy *et al.,* 1976). In India, over three million people are employed in various fields of sericulture. It is a cottage industry and provides ample work for the womenfolk in the rural areas in rearing silkworms. Recently the enforcing of new ideas by research institutions both in mulberry cultivation and silkworm handling among sericulturists, the industry is now practiced as a main profession and as a major cash crop, of the country.

Although, India has unique distinction of being the only country producing all five commercially known silks viz. mulberry, tropical tasar, temperate or oak tasar, eri and muga. The climate of north-east India is suitable for growth of non-mulberry silkworms (Manoshi Goswami *et al.,* 2009).

Indian eri silkworm, *Philosamia ricini* (Lepidoptera: Saturniidae), a commercial silk producing insect, is believed to have originated in the Brahmaputra valley (Jolly *et al.,* 1979) and has restricted distribution in India, China and Japan for two centuries (Peigler 1993; Singh and Benchamin 2002). The primary food plant of this polyphagous insect is castor (*Ricinus communis*), but it also feeds on a wide range of food plants such as *Heteropanax fragrans* Seem, *Manihot utilissima* Phol, *Evodia flaxinifolia* Hook, *Ailenthus gradulosa* Roxb etc., (Suryanarayana *et al.* 2002). The wild eri silkworm completes three generations per year depending on geographical position and climatic conditions of the region, however, up to six generations occur in the domesticated cultures (Neupane *et al.,* 1990). Populations of eri silkworm that have been

commercially exploited and are present in different regions of north-east India showed wide variations in morphological and quantitative characters. Due to over exploitation of the silkworms for commercial uses coupled with deforestation, most of these natural populations are dwindling rapidly. In order to preserve the natural biodiversity present among these populations, attempts are being made to understand the genetic structure of each population. Preliminary studies based on some quantitative traits such as cocoon weight, shell weight, larval weight etc. were made to understand the genetic basis of this phenotypic variability (Siddiqui *et al.,* 2000). However, no systematic studies were made to generate substantial information on the genetic diversity of these populations so as to develop appropriate strategy for its conservation at the natural habitat. (Siddiqui *et al.,* 2000). In India, the North East region contributes 90% of the total eri silk production. Assam is the largest producer of eri silk was about 56.5% and remaining 33.5% was produced by Manipur (16.5%), Meghalaya (15%) and Nagaland (2%). Among other states, Bihar, West Bengal, Orissa, Karnataka and Rajastan together contribute the remaining 10% of eri silk production.

Systematic breeding methods evolved during the last six decades have enabled the breeders to synthesis desirable genotypes of known genetic constitutions with objectives to increasing the productivity of eri silk. Further, application of genetic principles in understanding their hereditary nature of the quantitative characters coupled with appropriate selection procedures have contributed a great deal in increasing the productivity of silkworms (Yucheng Wu *et al.*, 1994). Sericulturally advanced countries such as China and Japan have achieved a remarkable breakthrough in increasing the unit production of high grade silk by evolving highly productive mulberry silkworm races suitable to their local conditions and agronomical practices. In view of the existing demand for silk in India, it is high time to take necessary steps to evolve suitable silk yielding varieties for popularize vanya silk especially eri silk in different agro climatic regions of India. The improvement of race necessarily means an increase in productivity and viability traits. However, these two characters of high economic value are negatively correlated. In addition, the expression of most of the commercial characters of silkworm is under the control of composite action of polygene and the ambient environmental conditions to which the

silkworm larvae are exposed during growth and development. Moreover, the complex physiological associations, with unfavorable environmental conditions.

Understanding and preserving biodiversity is one of the most important global challenges that biologists are facing. Assessment of the genetic diversity present within a species is a prerequisite for developing a sustainable conservation program. The forested areas are the abode of many valuable flora and fauna including about ten species of wild silk moths (Chowdhury 1983; Thangavelu 1991).

Among insects, occurrence of genetic variants is a common phenomen in mulberry silkworm *Bombyx mori.* A large number of variants based on larval colour , shape, size, cocoons, and voltinism have been reported in mulberry, tasar, muga and eri silkworm (chowdhury 1965,. Jolly *et.al.,* 1974, Narsimhanna, 1964). However, suitable literature regarding systematic inheritance pattern of larval colour and larval marking variants in eri silkworm *Philosamia ricini* is scanty.

All living organisms are complex assemblages of chemical compounds at variable concentrations that react and perform all biological functions. Eri silkworm like any other organisms is characterized by possessing varied amount of biochemical constituents like proteins, carbohydrates, lipids, nucleic acids, amino acids, etc., functioning in different ways to maintain the life activities (Rockstein, 1978). Even though the biochemical profiles has been analyzed in eri silkworm by several authors (Singh and Singh, 1987; Poonia, 1985; Eid *et al.,* 1989). The literature pertaining to the concentrations of these bimolecules during late larval stage of eri silkworm was rather scanty. Hence, an attempt was made to study the changes in levels of protein and total reducing sugar in different tissues such as midgut, haemolymph and silkglad of fifth instar larval developmental stages of eri silkworm to know the variations in the quantity and utilization of these biomolecules.

The advent of molecular biological techniques clearly showed the advantages of molecular markers over morph biochemical markers to analyze population diversity. As the molecular markers are stable and environmentally independent, they are increasingly being

preferred to phenotypic traits to detect genetic variation not only among populations but also between individuals within a population. A number of DNA marker systems such as simple sequence repeats (SSR; Kimpton *et al.*, 1993; Estoup *et al.*, 1993; Reddy *et al.*, 1999a; Prasad *et al.*, 2005), Random Amplified Polymorphic DNA (RAPD; Williams *et al.*, 1990; Nagaraja and Nagaraju 1995; Chatterjee and Pradeep 2003), Inter-Simple Sequence Repeats (ISSR; Zietkiewicz *et al.*, 1994; Ehtesham *et al.*, 1995; Reddy *et al.*, 1999b; Chatterjee *et al.*, 2004; Kar *et al.*, 2005; Pradeep *et al.*, 2005), expressed sequence tag (EST; Vlachou *et al.*, 1997; Ciolfi *et al.*, 2005) and amplified fragment length polymorphism (AFLP; Vos *et al.*, 1995; Reineke *et al.*, 1998; Katiyar *et al.*, 2000) have been used to study the population genetics of different organisms including insects. Genetic diversity and differentiation among different populations of the wild silkworm *Antheraea mylitta* was examined using ISSR markers (Chatterjee *et al.*, 2004; Kar *et al.*, 2005).

In the present investigation an attempt has been made to understand the genetic diversity by using molecular marker (RAPD), biochemical, and breeding studies of six different varieties of eri silkworm *Philosamia ricini* viz., Yellow plain (YP), Blue Green Plain (BGP) larval colour and Yellow spotted (YS), Yellow Zebra (YZ), Blue Green Spotted (BGS) and Blue Green Zebra (BGZ) larval markings were reared and conducted experiments at the Department of Sericulture/Life Science, Bangalore University, Bangalore, Karnataka, India, during 2009-2012 based on the following objectives.

OBJECTIVES OF THE PROJECT

- Studies on the isolation and establishment of six pure lines of six varieties of eri silkworm *Philosamia ricini*
- Studies on the performance of quantitative characters of parental lines and their F1 hybrids of eri silkworm
- Biochemical profile of the eri silkworm during fifth instar development.
- Isolation of genomic DNA from different varieties of eri silk moth.
- Amplification of genomic DNA by PCR.
- Analysis genetic diversity of different varieties of eri silkworm by using RAPD Molecular marker.
- Analysis of data to construct the phylogenic dendrogram.

METHODOLOGY

Six morphologically distinct varieties of eri silkworm , *Philosamia ricini* viz., Yellow Plain (YP), Blue Green Plain(BGP), Yellow Spotted (YS), Yellow Zebra (YZ) Blue Green Spotted (BGS) and Blue Green Zebra (BGZ) were collected from Central Muga Eri Research & Training Institute, Central Silk Board, Ladoigarh, Assam, Central Sericultural Germplasm Resources Centre Ministry of Textiles, Hosur, Tamilnadu and Eri Silkworm seed production Centre, Central Silk Board, Peddapuram, Andra pradesh. Six eri silkworm varieties were reared with maximum care to avoid duplication at the rearing house of Department of Sericulture/Life Science, Bangalore University, Bangalore during 2009-2012.

Rearing techniques

Eri cocoons were collected from each population from above mentioned places; and kept until emergence of the adult moths. The male and female moths were allowed to copulate under standard laboratory condition to prepare eggs (Plate: 1). The above moths were depaired and female moths were tied by a piece of thread to kharika. Female moths laid 300-340 eggs in a cluster. Disease free eggs were prepared by conducting mother moth examination. The eggs were disinfected with 2% formalin solution and washed in a tap water under shade. The eggs were tied in a piece of cloth and hung under the roof for incubation at 250°c. The eggs were allowed to hatch on tenth day of oviposition. The cloth containing hatched eggs opened and placed on a tray. A few tender castor leaves were spread over the eri silkworms and transferred to the rearing room. Before brushing, the rearing room and rearing equipment were disinfected with 2% formalin solution.

Tray rearing method was adapted to rear young and late age eri silkworms under standard laboratory conditions followed by Sarkar (1986). The first and second instar eri silkworms were fed with whole tender castor leaves by providing four feedings per day. Late age worms were fed with entire matured castor leaves by providing four feedings per day.

Mounting and Harvesting

Towards late fifth instar stage, the larvae entered into spinning the body colour turned pale yellow and the silkworms crawled towards margin of rearing tray. The matured ripped worms produced hallow sound when rubbed gently between fingers. The matured spinning worms were picked and they were placed in clean spinning montage.

The eri larvae complete spinning on the third day during summer and on the fifth day in winter. The worms spins an open end cocoons, (Plate: 5) they were harvested on fifth day in summer and eight day in winter after spinning. The cocoons from each montage were stored in a clean white polyethylene sheet and kept in a ventilated room to avoid pest and predators.

Bio-chemical studies:

Larvae were separated from the general rearing after second moult based on their physical appearance such as body colour and larval markings and reared separately. Immediately after fourth moult, the fifth instar larvae (5-6 worms) were collected from each experimental rearing at an interval of 24 hours up to spinning stage and t3his material was used for the estimation of soluble protein and reducing sugar.

Collection and preparation of tissue sample:

Haemolymph

The haemolymph was collected in a clean tube containing a pinch of thiourea (to prevent oxidation) from the eri silkworm larvae by cutting the caudal horn. The haemolymph was diluted 10 times with ice cold distilled water and centrifuged at 3000 rpm for 10 minutes. The supernatant was collected and used for the estimation of soluble protein and total sugar.

Midgut

After collecting the haemolymph, the mid gut was excised from the same silkworm larvae and cleaned with ice cold distilled water to remove the midgut contents. The midgut tissue was blotted dry on blotting paper. A 10% homogenate (W/V) of the tissue was prepared in ice cold distilled water using a tissue homogenizer fitted with a Teflon pestle. The homogenate was

centrifuged at 3000 rpm for 10 minutes. The supernatant was collected and used for the estimation of soluble protein and total sugar.

Silk gland

The silkgland was also excised from the same larva. It was cleaned with ice cold distilled water and blotted dry on a filter paper. A 10% homogenate (W/V) of the tissue was prepared in 10% TCA using tissue homogenizer. The homogenate was centrifguged at 3000 rpm for 10min. The supernatant was taken for the estimation of total sugar. The residue was dissolved in 5 ml of 1N NaOH again centriguged and the supernatant was used for the estimation of protein.

Biochemical analysis:

Estimation of soluble protein

The modified method of Lowry *et al.*,(1951) was followed for the estimation of proteins.

Estimation of Protein

Heamolymph, Midgut and Silkgland of fifth instar eri silkworm were used for the estimation of protein, 1ml of the given sample was taken in a test tube to that 5ml of protein reagent was added and incubated for 10 minutes at room temperature. After incubation 0.5ml of Folin's reagent was added and mixed thoroughly. Allowed to stand for 30 minutes at 25o C. The absorbacy was measured at 660 nm. The concentration of protein was determined using Bovine Serum Albunin (BSA) as standard.

Estimation of total sugar

Heamolymph, Midgut and silkgland of fifth instar eri silkworm were used for the estimation of reducing sugar, 1ml of the given sample was taken in a test tube, to that 1ml of distilled water was added followed by 0.5ml of DNS reagent. This mixture was boiled over a boiling water bath for 10 minutes. The test tubes with this mixture were allowed to cool at room temperature. To this 2.5ml of distilled water was added and optical density of each tube was measured at 540nm used by spectrophotometer.

A blank was prepared by taking 1ml of distilled water in place of sample. The amount of reducing sugar present in the sample was calculated using the standard curve prepared by taking different concentrations of standard glucose solution.

Collection and preservation of six varieties of Eri silkworm *Philosamia ricini* for RAPD molecular marker studies:

Six morphologically distinct varieties of eri silkworm *Philosamia ricini* were collected from different regions of India, used for RAPD molecular marker study. From each population, 10 to 15 cocoons were collected and kept until emergence of the adult moth. Genomic DNA from 10 individual moths of each variety was extracted separately followed the phenol: chloroform extraction method (Suzuki *et al.,* 1972). Eri silkworms were frozen at -80^0 C or preserved in 70% ethanol until further use for DNA isolation and subsequently to DNA finger printing.

Genomic DNA isolation

Genomic DNA was extracted from six morphologically different varieties of eri silkworm moths according to the procedure of (Suzuki *et al.*, 1972; Thananantа *et al.*, 1997; Nagaraja, 2002; Nagaraja and Nagaraju, 1995). Eri Silk moth were first frozen with liquid nitrogen and then grounded in cooled mortar with pestle. After adding 5ml of the extraction buffer (100mM Tris-HCl, pH 8.0, 50mM EDTA and 1% SDS) and proteinase -K (100mg/ml), it was incubated at 37 °C for 2 h with occasional swirling. The DNA was extracted twice with phenol-chloroform. The supernatant DNA was subjected to ethanol-precipitated in presence of 3M sodium acetate (pH 5.2) re-suspended in TE (10 mM Tris-HCl, 1 mM EDTA, pH 8.0) buffer. The RNAse treatment was done by adding RNase- A (100mg/ml) and incubating at 37 °C for 1 h. DNA was re-extracted with phenol-chloroform and ethanoprecipiated as described earlier. The genomic DNA was quantified on 0.8% agarose gels and diluted to uniform concentrations (10mg/ml) for the RAPD study.

RAPD Amplification

RAPD amplification was performed in a volume of 25 µl that contained: 50 ng of template DNA, 2.5 mM each of dNTPs (Chromus Biotech Pvt. Ltd), 5 pM of primer (Sigma-Aldrich), 10x *Taq* assay buffer (10mM Tris-HCl, pH -8.8, 500 mM KCl, 15 mM $MgCl_2$, 0.1 % gelatin, 0.05 % Tween 20 & 0.05 % Nonidet P 40), 1 unit of *Taq* DNA polymerase (Chromus Biotech Pvt. Ltd.,) and sterilized

water. Amplification was performed in a thermal cycler (MJ Research Inc.). Initial denaturation was performed at 94 °C for 3 min before beginning the cycling protocol followed by 1 min at 92 °C, 2 min at 36 °C and 2 min at 72 °C. A total of 40 cycles were performed. The cycling was terminated with a final extension at 72 °C for 6 min. The amplification products were analyzed on 1.2 % agarose gels with low range molecular weight marker. The gel was stained with ethidium bromide, visualized under ultraviolet light and documented using HEROLAB gel documentation system.

RAPD data analysis

The presence of amplified bands with different intensities and locations were detected using Gel-Compare II software. All calculations were done using computer based software program. A pair wise similarity matrices and dendrogram was generated by using Jaccard's similarity coefficient and unweighted pair group method with arithmetic mean (UPGMA) method respectively using Gel-Compar II software.

RESULTS AND DISSCUSSION

The results of the UGC Major Research Project are being presented under the following headings.

Studies on isolation and establishment of six pure lines of eri silkworm *Philosamia ricini*

Six morphologically distinct varieties of eri silkworm , *Philosamia ricini* viz., Yellow Plain (YP), Blue Green Plain(BGP), Yellow Spotted (YS), Yellow Zebra (YZ) Blue Green Spotted (BGS) and Blue Green Zebra (BGZ) were collected from different geographical region in India.(figure 3,Table 1)

The present work was undertaken to probe into genetic architecture of the quantitative characters such as duration of all instars, duration of the fifth instar, effective rate of rearing, fifth instar larval weight, cocoon yield, cocoon weight, shell weight, shell ratio, larva - pupa rate, weight of pupae, rate of moth emergence, fecundity and hatching percentage of the six parental lines. It was found to that, the Yellow plain was superior with Blue Green plain larval colours and Blue Green Zebra was superior among larval markings(Table 2). The improvement of a race necessarily means an increase in productivity

and viability traits. However, these two characters of high economic value are negatively correlated. In addition, the expression of most of the commercial characters of silkworm is under the control of composite action of polygenes and the ambient environmental conditions.

It was found that the **Yellow plain** was superior with regard to duration of fifth instar (162 h), effective rate of rearing (92 %), cocoon yield (90.00), cocoon shell rate (12.85 %), larva-pupa rate (89.22%), weight of pupae (21.591 g), rate of moth emergence (89 %), fecundity (339 eggs) and hatching percentage (89.45 %) when compared to **Blue Green plain (BGP)** These findings are in agreement with the results of Nagaraja *et al.*, (1996) on *P. ricini.*

On the other hand. **Blue Green Zebra** larval marking parental line was superior with regard to duration of all instars (535 h), duration of the fifth instar (159 h), effective rate of rearing (94 %), fifth instar larval weight (72.645 g), cocoon yield (93.26), cocoon shell rate (13.22 %) larva-pupa rate (92.22 %), weight of pupae (23.102 g), rate of moth emergence (92 %), fecundity (356 eggs) and hatching percentage (93 %) when compared to Yellow spotted, Yellow Zebra, Blue Green Spotted and Blue Green zebra (BGZ) larval markings. In general, all the larval markings are superior with regard to cocoon shell rate and larva - pupa rate when compared to larval colours(Table 2).

Studies on the performance of quantitative characters of parental lines and their selected F1 hybrids.

Utilizing the known genotypes, choice of mating system and judicious method of selection is a vital importance in the conventional breeding methods such as line breeding and cross breeding. Among them cross breeding was more dependable than line breeding for the evolution of silkworm races and to obtain high productive hybrids for commercial utilization (Kovalov, 1970). Generally cross breeding programme involve mating of two or more varieties which are good in some characters but poor in others followed by inbreeding and selection over generation to isolate races with a harmonious blend of desired economic characters. In view of the above in the present study the six eri silkworm varieties which are known for their distinct phenotypic expression with respect to commercial traits. They were involved in positive and negative combinations in order to extract the most promising F1 genotypes(Table 3).

The phenotypic expression of varieties or species are determined by the interaction between genetic and environmental factors in the mulberry silkworm *Bombyx mori* as reported by Mather and Harrison (1949), Robertson (1955), Levins (1968), Sengupta (1969), Barlow (1981), Rajanna (1989) and Revanasiddaiah (1994). Similarly the expression of quantitative characters in the eri silkworm *Philosamia ricini* were largely dependent on the complex interaction between polygenes governing them and the dynamic environmental conditions to which they are exposed. Therefore, suitability of any race is dependent on its genotypic expression to produce consistently high phenotypic expression under a set of environmental conditions. However, the performance of the variety itself is the best indicator of the suitability of genotype to a known environment, (Rajanna 1989, Revanasiddaiah 1994, Yucheng *WuetaL,* 1994).

It was well known that crossing of two races with different genetic background produce heterozygosity in F1 hybrids and results in heterozygous population to choose the desirable alleles. However, Frankham *et al.,* (1968), James *et.al.,* (1968), James (1972) and Harris (1976) suggested such desired alleles are present at very low frequency in F1 generation. Therefore, large population of F1 hybrids were raised in the present study to ensure a greater scope for the expression of such desired alleles.

The results on the **duration of all larval instars** in hybrids of positive and negative crosses were found to be significantly different from that of parents. However, interactions between varieties and seasons were observed to be no significant. The comparison of this character with regard to F1 hybrids of negative crosses Blue Green Zebra x Yellow plain, by recorded a shortest larval duration of 519.15 h indicating superiority of a negative cross(Table 4). Whereas, longest larval duration of 575.25 h is found to be in the F1 hybrid of the negitive cross Blue Green plain x Yellow plain(Table 4). The present results are not in agreement with the findings of Nagaraj *et al.,* (1996).

In the present investigation, **fifth instar larval duration** was the lowest (147 h) in the F1 hybrids of the negative cross Blue Green Zebra x Yellow plain(Table 4), but all other F1 hybrids, showed moderate values. This was an agreement with the findings of Revanasiddaiah (1994) who has reported the lowest larval duration of the F1 hybrids of the negative cross of Chinese sex-limited normal marking female with Japanese sex - limited plain male of *Bombyx mori.*

The **effective rate of rearing** was found to be the highest (97%) in F1 hybrids of the negative cross Blue Green Zebra x Yellow plain, but other F1 hybrids of the negative crosses revealed moderate effective rate of rearing (96%)(Table 4).

Analysis of the result with regard to **fifth instar larval weight** is found to be maximum 75.542 g in the F1 hybrid of the negative cross Blue Green Zebra x Yellow plain (Table 4)and minimum of 58.425g is recorded in Blue Green plain x Yellow spotted in F1 hybrids of the positive cross. It was clearly indicates that, the above negative cross is superior to positive crosses(Table 5). This is not in agreement with the findings of Govindan *et al*, (1987) and Tayade (1987).

The comparison of **cocoon yield** was the highest i.e., 96.00 in the negative cross Blue Green Zebra x Yellow plain (Table 4) and the lowest (85.00 g) in the positive cross Yellow plain x Yellow zebra(Table 5) . This observation does not support the views of Banuprakash(1990).

The analysis of the result reveals the maximum **cocoon weight** of 28.275g in the negative cross Blue Green Zebra x Yellow spotted(Table 6), whereas moderate cocoon weight of 25.755g in Blue Green Zebra x Yellow plain(Table 4) , F1 hybrid of the positive cross and minimum cocoon weight of 22.465 g is observed in the negative cross Blue Green Spotted x Yellow plain(Table 4). This indicates that negative crosses are better than that of positive crosses with regard to this trait.

The comparison of **cocoon shell weight** is found to be the highest (3.690g) in F1 hybrid of negative cross Blue Green Zebra x Yellow plain(Table 4), while the lowest value of 2.590g is recorded in the negative cross Yellow zebra x Yellow spotted(Table 6). It was showed that, the performance of negative cross was better than positive cross. This observation does not agree with Tayade (1987), Benchamin *et al.,* (1988) and Govindan *et al.,* (1987) in *Bombyx mori.*

The analysis of the F1 hybrids of different crosses for **cocoon shell rate** reveals that the negative crosses of Blue Green Zebra x Yellow plain and Yellow Zebra x Yellow plain with 14.327 % occupy first position(Table 5). Blue Green plain x Blue Green Zebra with 13.99% occupy second position(Table 5) and the lowest of 10.665% is observed in the negative cross Yellow zebra x Yellow spotted(Table 6).

Analysis of **larva - pupa rate** among F1 hybrids of all positive and negative crosses shows that the negative cross Blue Green Zebra x Yellow plain(Table 4) has the maximum larva - pupa

rate (96 %), followed by the positive crosses Blue Green Zebra x Yellow zebra (95.92 %)(Table 7). Yellow plain x Blue Green plain (85.25 %) (Table 4)and other crosses showed moderate values. This is in agreement with Satenahalli (1986) in *Bombyx mori* but is not in agreement with the findings of Nagaraja *et al';* (1996) who has reported high pupation rate in parents when compared to their F1 hybrids.

Analysis of the F1 hybrid of different crosses with regard to **weight of pupae** registered significant differences. The F1 hybrid of the negative cross Blue Green Zebra x Yellow plain(Table 4) showed the highest pupal weight 26.486g than that of other hybrids.

The comparison of the rate of **moth emergence** registered highest of 96% in the F1 hybrid of the negative crosses Blue Green Zebra x Yellow plain(Table 4), Blue Green Zebra x Yellow spotted(Table 6) and the positive cross Blue Green Zebra x Yellow zebra(Table 7) , followed by 95% in the positive cross Yellow spotted x Blue Green Spotted(Table 5) , Yellow spotted x Blue Green zebra(Table 6) , whereas the lowest percentage was noticed (86%) in the positive cross Yellow plain x Blue Green plain(Table 4).

Analysis of the **fecundity** among the different positive and negative crosses reveals highest mean fecundity rate of 399 eggs in the F1 hybrids of the negative cross Blue Green Zebra x Yellow plain(Table 4), while the lowest fecundity rate of 320 eggs is observed in the positive cross Yellow plain x Blue Green Zebra(Table 4). Further, the F1 hybrids of all the crosses registered moderate values.

The comparison of the **hatching percentage** among positive and negative crosses reveals the highest (97%) in the F1 hybrids of the negative cross Blue Green Zebra x Yellow plain (Table 4)and the lowest (89%) in Yellow plain x Blue Green plain(Table 4) , F1 hybrids of the positive crosses showed moderate values.

Comparatively higher effective rate of rearing, fifth instar larval weight, cocoon yield, cocoon weight, cocoon shell weight, cocoon shell rate, larva-pupa rate in the F1 hybrids of negative cross between Blue Green Zebra x Yellow plain can be attributed to the inheritance of additive gene effect as described by Govindan *et al* (1997) in diallelic crosses *of Philosamia ricini*. Marginal increase in hatching percentage observed in F1hybrids may be due to physiological status of both male and female moths (parents) and also the environmental factors like

temperature, humidity and light during incubation of eggs (Rajanna 1989 and Revanasiddaiah, 1994). Comparatively shorter larval duration observed in F1 hybrid was attributed to the higher rate of metabolism and speed of development due to heterozyosity. The positive crosses show reduced variation with regard to the expression of characters studied, since heterozygous individuals are bound to carry a greater diversity of alleles offering greater versatility in growth and development.

From the rearing results it was showed that, all the quantitative characters are superior in the F1 hybrids of the negative cross Yellow plain x Blue Green Zebra. When compared to other crosses. Because of this achievement of the parent Blue Green Zebra larval marking and Yellow plain larval colour, they can be used as superior basic breeding material for tropical and subtropical agroclimatic conditions. The performance of the present check variety Yellow plain (Table 2) used in India for commercial exploitation is compared with the rearing results of other parents. It was found that, the larval marking variety female Blue Green Zebra can be utilized with Yellow plain larval colour male variety to produce F1 hybrids for better quality and higher quantity of eri silk.

An evident from the data on the breeding experiments aimed at analyzing the newly evolved larval colour and larval marking varieties to obtain promising F1 hybrids from different combinations for tropical and subtropical climates, the major characters namely, genetic diversity, productivity and viability were found to be complexly related, being influenced by intrinsic and extrinsic factors. It is paradoxical to point out that almost nothing is known about the exact genetic basis of quantitative characters except for measuring the phenotypic and environmental components for variance (Baton, 1986). Therefore, it can be safely concluded that the stability in the performance of larval colour and larval marking varieties exhibiting higher cocoon productivity and moderate viability could be due to the probable influence of large gene effects, linkage, gene interaction or a combination of all of them.

Biochemical profile of the six different varieties of eri silkworm during fifth instar Development:

Biochemical studies were carried out to analyze the changes in the levels of proteins and total sugars in different tissues such as midgut, haemolymph and silkgland of six different

varieties of eri silkworm during larval development and presented in the form of tables and figers.

Changes in the level of soluble protein in the midgut

Among six different varieties of eri silkworm, Blue Green Spotted showed the lowest (17.23 mg/g) protein content followed by Yellow Spotted (18.23 mg/g), Yellow Zebra(18.24 mg/g), Blue Geen Plain (19.23mg/g), Yellow Plain (20.31mg/g) and Blue Green Zebra (22.72 mg/g). It was found to be the highest in the Blue Green Zebra (66.42 mg/g) and lowest in Blue Green spotted (50.12 mg/g) on the third day of fifth instar (Table 8, Chart 1).

Protein plays an important physiological role in growth and development of the body during fifth instar larval development of eri silkworm. In the midgut, the protein content shows an increasing trend form first day to third day. This shows that, these occur an accumulation of protein in the midgut during the early part of the fifth instar. This might be due to the result of high digestive activity for digestion of leaf proteins, their absorption and assimilation during this period. From third day onwards the protein content in the midgut decreases due to decrease in the digestive activity. This supports the earlier findings of Seo *et al.,* (1985) and Horie (1959).

Changes in the level of protein in the Haemolymph

It is well known that, the haemolymph the only extracellular fluid in insect, was having diverse functions (Pawar et al., 1977). Haemolymph as circulatory tissue has important role in the transport and distribution of nutrients to different tissues. It was the reservoir for the products that are required for and produced by every physiological activity of the insect. Thus, the changes in the comparision of haemolymph reflects the morphogenic and biochemical changes taking place in insect tissues. The haemolymph of insect perform several physiological functions such as immunity, transport and storage reserve (Mullins, 1985) in content of the haemolymph increased constantly throughout the fifth instar larval development reaching the peak level on the last day. This might be due to the active synthesis and secretion of proteins by the fat body into the haemolymph which carries them to different tissues to support the active growth, development and silk synthesis during fifth instar development which is in conformity with the earlier findings of Yashitake and Nagata (1079).

The protein content in the haemolymph showed a constant increase throughout the fifth instar larval development reaching the peak on the last day. It showed the same in all the six varieties of eri silkworm. Further, the Yellow plain showed the maximum protein content (51.97 mg/ml) followed by Yellow Zebra (50.64mg/g), Blue Green Plain (49.56 mg/g),Blue Green spotted (48.76 mg/g),Yellow spotted (45.46 mg/g) and Blue Green Zebra (23.69 mg/g) during the last day of the fifth instar. The minimum value (13.42 mg/g) was recorded in White plain on the first day of fifth intstr development (Table 9, Chart 2).

Changes in concentration of soluble protein in the Silkgland

The concentration of protein in the silkgland showed an increasing trend in all the six varieties of eri silkworms. This was the synthesis of silk protein occurs slowly at the beginning and was accelerated during the later part of fifth instar larval development. This supports the earlier findings of Shimura (1978). The similar trend was also observed in the haemolymph protein this might be mobilization of haemolymph protein for silk synthesis in silkgland.

The Blue Green Zebra larva showed the highest protein content in sillkgland was (70.21 mg/g) followed by White Plain (64.01 mg/g),Blue Green spotted (63.76 mg/g),Yellow spotted (62.64 mg/g),Blue Green plain (61.95mg/g) and Yellow zebra (61.22 mg/g) on the last day of fifth instar. The lowest value was recorded in Blue Green Plain (19.96 mg/g) and highest value recorded in Yellow Plain and Blue Green Zebra (30.98 mg/g) on the first day of fifth instar (Table 10, Chart 3).

Changes in concentration of total sugar in the midgut:

Among the six varieties of eri silkworm the Blue Green zebra (53.69mg/g) showed the highest and the lowest value exhibited in Yellow spotted (45.46 mg/g) on fifth day of fifth instar. The lowest value was recorded in Yellow plain (13.42 mg/g) and highest value recorded in Blue Green Zebra (18.58 mg/g) on first day of fifth instar (Table 11, Chart 4).

In the midgut total sugar concentration was found to be the highest on the first day and decreased gradually on the last day of the fifth instar development. This shows that the digestion of sugars occur very early during the fifth instar which are observed by the midgut and utilized for the production of energy required for the growth and development throughout the fifth instar period. This result supports the earlier findings of pant (1969), Morris (1972) and Poonia (1985).

Among the varieties there was no significant difference in the concentration of total sugar. This shows that, the efficiency of digestion and accumulation of sugars in the midgut do not vary significantly in different varieties.

Changes in concentration of total sugar in the Haemolymph:

The concentration of total sugar was found to be low initially which increased significantly thereafter during fifth instar. This might be due to release of sugar form the other tissues (midgut and fat bodies) into haemolymph to transport to other tissues to supply their energy requirement to support active growth and metabolism. This confirms that, haemolymph being a circulatory tissue transport the body material to other tissues during fifth instar larval development (Mullins, 1985).

There was no significance difference observed among six varieties of eri silkworm with respect to total sugar in the haemolylmph at the lowest level on first day as Yellow plain (0.41 mg/g) and highest in Yellow zebra (0.51 mg/g). The Blue Green Zebra (6.53 mg/g) showed hiest value and Yellow zebra (6.22mg/g)showed lowest value in fifth day of fifth instar (Table 12, Chart 5).

Changes in concentration of total sugar in the Silkgland:

The total sugar content of the silkgland was found to be lower initially and then increased constantly towards the end of the fifth instar in order to provide the required energy for the increased sysnthesis of silk proteins in the silkgland as sugars were the reserve energy for the insect (Buck, 1953).

The minimum total sugar content was found to be in Yellow spotted (0.73mg/g) and highest value recorded in Yellow zebra (1.02 mg/g) in first day of the fifth instar. Similarly, the minimum total sugar content was found to be in Blue Green Plain (3.62 mg/g) and higest value recorded in Yellow Zebra (5.81 mg/g) in fifth day of fifth instar (Table 13, Chart 6).

The study with regard to changes in the concentration of soluble protein, total sugar with different tissues among six varieties of eri silkworm gave a clear indication that, the increase and decrease in the level of these biomolecules are co related to the degree of digestion and absorption in the midgut, transport by the haemolymph and utilization by the silkgland during

fifth instar development. Blue Green Zebra and Yellow plain are better as far as silk production concerned when compared to the other varieties of eri silkworms.

Analysis of genetic diversity of different varieties of eri silkworm by using RAPD Molecular marker.

RAPD analysis promises to become a valuable tool for analysis of genetic variation, estimating genetic distance among populations and generating molecular markers for economic traits of the eri silkworm. The varietal- specific amplification of distinct bands permits their use in genetic fingerprinting of eri silkworm and their eco types.

Genomic DNA was extracted from the eri silkworm moth according the procedure of Suzuki *et al.*, 1972; Thananantan *et al.*, 1997; Nagaraja, 2002; Nagaraja and Nagaraju, 1995. A total of 20 RAPD primers were screened, out of which 18 primers generated good amplification and hence they were chosen for fingerprinting of six morphologically different eri silkworm varieties for accessions. Poorly stained, unique, and very low-frequency bands from the data set were discarded for further analysis. Among the 20 primers tested, 18 selected primers generated 533 bands, of which 64 bands were polymorphic. RAPD profile generated with primers ES-6, ES-8, ES-9 and ES-15 are depicted in figure 5. The level of polymorphism revealed by this study was low. But the selected accessions were dissimilar at morphological level. The total number of DNA fragments amplified and the number of polymorphic bands from each accessions with individual primer was shown in Table 14. Cluster analysis (UPGMA) was generated by computing polymorphic as well as monomorphic markers to construct the dendogram(Table 16) Jaccard's similarity coefficients ranged from 0.1 to 0.66 (Table 15) and the maximum similarity was noticed between the Asc-05 and Asc-06, the minimum similarity was observed between Asc-01 and Asc-02.

Associations among six eri silkworm population accessions revealed by UPGMA cluster analysis based on RAPD profile are presented (Table 16). All the accessions could be split into two major clusters, First cluster at 85% and Second cluster at 75% similarity level. In the first cluster consists of four accessions viz., Asc-03, Asc-04, Asc-05 and Asc-06. Among the four accessions, the 65% similarity levels have exhibited in Asc-03 and Asc-04 form the cluster "a". Similarly the

35% similarity level has exhibited in Asc-05 and Asc-06 form the cluster "b". The second cluster form Asc-01 and Asc-02.

Since RAPDs are random selection of DNA sequence, it was apparent in the study that RAPD technique was sensitive enough to detect differences between accessions of eri silkworm in which differentiation is not always possible morphologically. In the present study, 12.00% polymorphism demonstrated the potentiality of the method in evaluating genetic diversity within the eri silkworm populations. The range of similarity 0.1 to 0.66 indicated low genetic diversity in eri silkworm germplasm.

Eri silk moths are poor flyers with a short life span, which is likely to reduce chances of genetic mixing among geographically close populations. This could account for the genetic differentiation among the geographically close eri populations of Assam. It is known fact that, high gene flow between populations precludes local adaption that results from fixation of alleles favored by local climatic conditions, and will prevent speciation(Barton and Hewitt 1985). At the same time new gene combinations in populations upon which selection can act. Environment has got profound influence on biology, reproduction, behaviour and physiology of the silkworms. Climatic conditions and food can have influence on their evolution, hence they might be sharing more nucleotides in genome level, creating variation within the populations of eri silkworms. RAPD analysis of parthenogentic and bi parental species of *Aporrectodea* species observed genetic similarity of 85% and the changes in intra specific diversity of *A. caliginosa* populations resulted in a genetically different population due to high competition (Dyer *et al.*, 1998).

The increased rate of inbreeding and genetic drift can be considered as possible causes of erosion in genetic diversity in small eri silkworm populations (Keyghobadi *et al.*, 2005; Ouma *et al.*, 2005). The decrease in disequilibrium with the increase in altitude impels low genetic diversity in high altitude populations. A significant decrease in gene diversity coinciding with the increase in altitude in eri populations places an evidence with influence of the heterogeneous topographical conditions of northeast India on population build-up, as reported for Drosophila melanogaster (Hoffmann *et al.*, 2001; Anderson *et al.*, 2005) and other animals (Pariset *et al.*, 2009), under varying geographical conditions. Both enlargement in effective size of eri silkworm

populations and their habitat protection are essential for maintaining genetic variability and increasing gene flow .

India being a country with diverse environmental conditions, the local races are rich reservoirs of many resistant genes, and molecular markers are inevitable tools to study inheritance of such complex traits. The amplification products resulting from the RAPD assay vary between populations. Hence, it can be used as genetic markers and as well as to construct linkage maps. Considering the genetic diversity of the genotypes belonging to different groups will constitute promising parents for hybridization in silk improvement program. Thus, the above study exhibit the range of genetic diversity and distance was low between the eri silkworms populations were due to restricted environmental acclimatization majority in Assam. To increase the genetic diversity of eri silkworm population, it is required to introduce them under wide environmental condition which for further study of microevolution, breeding and several fitness benefits on eri silkworm populations for commercial exploitation.

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SIGNATURE OF
THE PRINCIPAL INVESTIGATOR

Eri Male moth

Eri Female moth

Eri moths on Kharika

Coupulated Male and Female eri moths

Eri eggs

Figure 1: Egg production of eri silkworm *Philosamia ricini*

Castor Plantation

Chawki Rearing

Late age Rearing

Figure 2: Rearing of eri slikworm *Philosamia ricini*

Scr-01 Yellow Plain (YP)

Scr-02 Blue Green Plain (BGP)

Scr-03 Yellow Spotted (YS)

Scr-04 Blue Green Spotted (BGS)

Scr-05 Yellow Zebra (YZ)

Scr-06 Blue Green Zebra (BGZ)

Figure 3: Morphologically different six varities of eri silkworm *Philosamia ricini*

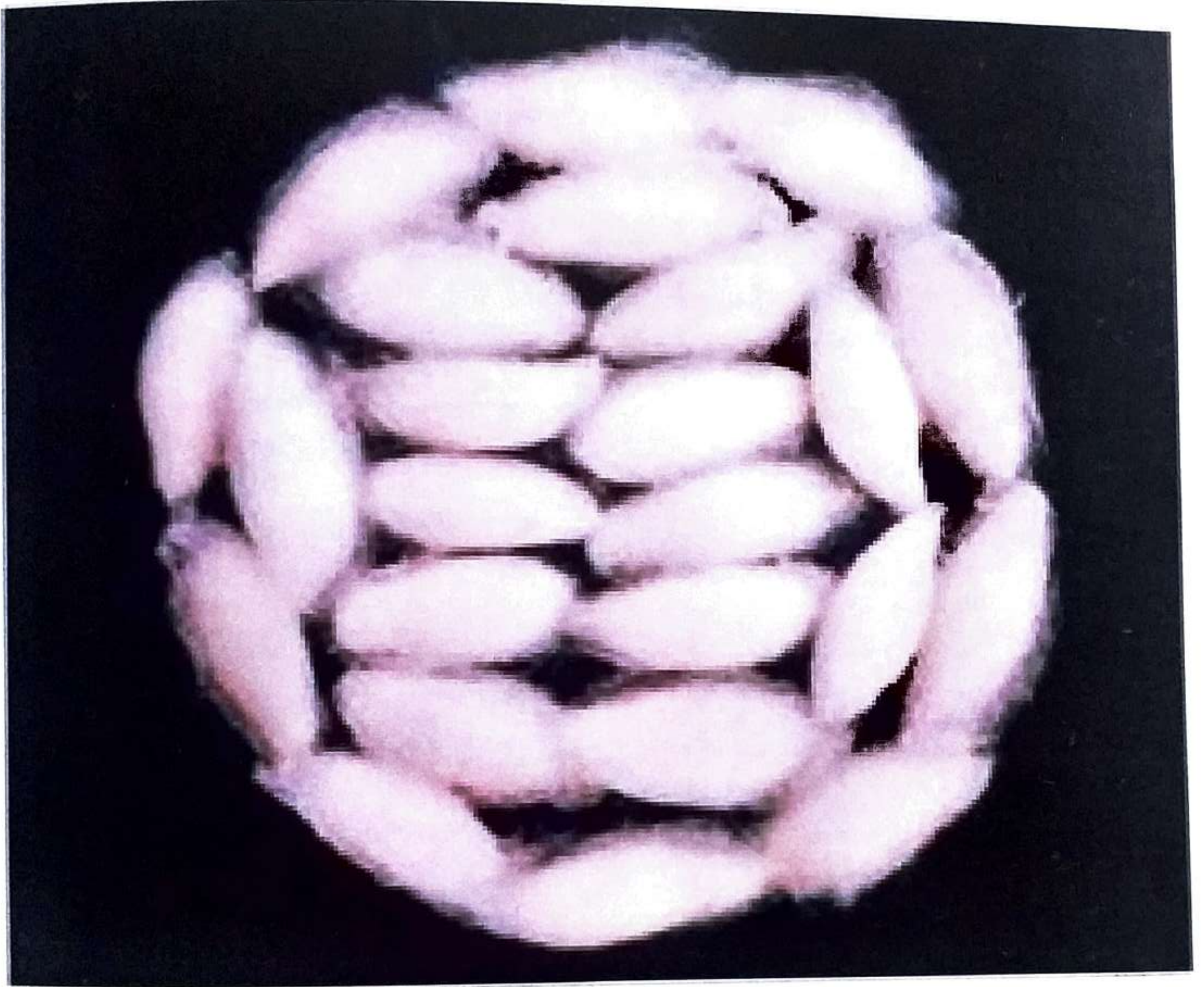


Figure 4: Eri cocoons

Accession number	Eri silkworm varieties	Place of collection	Larval colour	Cocoon colour
Scr-01	Yellow plain	Assam	Yellow	White
Scr-02	Yellow spotted	Assam	Yellow	White
Scr-03	Yellow zebra	Andrapradesh	Yellow	White
Scr-04	Blue green plan	Andrapradesh	Blue green	White
Scr-05	Blue green spotted	Tamilnadu	Blue green	White
Scr-06	Blue green zebra	Tamilnadu	Blue green	White

Table 1: Collection of six morphologically different eri silkworm accessions

Sl No	Metric Traits	Yellow Plain (YP)	Blue Green Plain (BGP)	Yellow Spotted (YS)	Blue Green Spotted (BGS)	Blue Green Zebra (BGZ)	Yellow Zebra (YZ)
1	Duration of all instanrs (h)	575.24	580.00	550.30	548.25	535.00	551.30
2	Duration of the fifth instar (h)	162.00	168.00	161.00	160.00	159.00	162.00
3	Effective rate of rearing (ERR) (%)	92.00	88.00	93.00	91.00	94.00	93.00
4	Fifth instar larval weight (10 worms) (g)	69.202	60.204	70.730	68.625	72.645	69.846
5	Cocoon yield by number per 100 larvae (No)	90.00	87.00	91.00	91.00	93.00	92.00
6	Weight (10 cocoons) (E)	22.278	25.921	22.457	25.942	25.150	22.457
7	Cocoon shell weight (10 shells) (g)	2.864	3.221	2.946	3.361	3.120	2.946
8	Shell rate (%)	12.85	12.42	13.11	12.95	13.22	13.11
9	Larva-pupa rate (%)	89.22	86.00	90.14	90.00	92.22	91.24
10	Weight of 10 pupae (g)	21.591	20.162	21.786	21.686	23.012	22.124
11	Rate of moth emergence (%)	89.00	86.00	90.00	90.00	92.00	91.00
12	Fecundity (No)	339.00	329.00	341.00	339.00	356.00	331.00
13	Hatching (%)	89.45	88.95	90.00	91.00	93.00	92.00

Table 2: Mean values of the quantitative characters of six parent varieties of eri silkworm *Philosamia ricini*

POSITIVE CROSS	NEGATIVE CROSS
Yellow plain (YP) x Blue Green Plain (BGP)	Blue Green Plain (BGP) x Yellow plain (YP)
Yellow plain (YP) x Yellow Spotted(YS)	yellow Spotted (YS) x Yellow plain (YP)
Yellow plain (YP) x Blue Green Spotted (BGS)	Blue Green Spotted (BGS) x Yellow plain (YP)
Yellow plain (YP) x Blue Green zebra (BGZ)	Blue Green zebra (BGZ) x Yellow plain (YP)
Yellow plain (YP) x Yellow zebra (YZ)	Yellow zebra (YZ) x Yellow plain (YP)
Blue Green Plain (BGP) x Yellow Spotted (YS)	Yellow Spotted (YS) x Blue Green Plain (BGP)
Blue Green Plain (BGP) x Blue Green Spotted (BGS)	Blue Green Spotted (BGS) x Blue Green Plain (BGP)
Blue Green Plain (BGP) x Blue Green zebra (BGZ)	Blue Green zebra (BGZ) x Blue Green Plain (BGP)
Blue Green Plain (BGP) x Yellow zebra (YZ)	Yellow zebra (YZ) x Blue Green Plain (BGP)
Yellow Spotted (YS) x Blue Green Spotted (BGS)	Blue Green Spotted (BGS) x Yellow Spotted (YS)
Yellow Spotted (YS) x Blue Green zebra (BGZ)	Blue Green zebra (BGZ) x Yellow Spotted (YS)
Yellow Spotted (YS) x Yellow zebra (YZ)	Yellow zebra (YZ) x Yellow Spotted (YS)
Blue Green Spotted (BGS) x Blue Green zebra (BGZ)	Blue Green zebra (BGZ) x Blue Green Spotted (BGS)
Blue Green zebra (BGZ) x Yellow zebra (YZ)	Yellow zebra (YZ) x Blue Green zebra (BGZ)
Yellow zebra (YZ)x Blue Green Spotted (BGS)	Blue Green Spotted (BGS) x Yellow zebra (YZ)

Table 3: Positive and negative crosses by using six varieties of eri silkworm *Philosamia ricini*

		YPxBGP	BGPxYP	YPxYS	YSxYP	YPxBGS	BGSxYP	YPxBGZ	BGZxYP
	Metric Traits	F1 hybrid	F1 hybrid	F1 hybrid	F1 hybrid	F1 hybrid	F1 hybrid	F1 hybrid	F1 hybrid
1	Duration of all instars (h)	545.00	575.25	574.24	574.00	546.00	545.00	525.00	519.15
2	Duration of the fifth instar (h)	157.25	163.00	162.00	159.00	161.00	160.00	158.00	147.00
3	Effective rate of rearing (ERR)	91.00	91.64	93.00	94.00	92.00	90.00	92.48	97.00
4	Fifth instar larval weight (10 worms) (g)	69.650	65.725	68.00	70.640	68.325	73.464	73.632	75.542
5	Cocoon yield by number per 100	94.00	90.00	90.00	91.00	90.00	91.00	92.00	96.00
6	Cocoon Weight (10 cocoons) (g)	25.675	23.450	24.928	24.265	24.692	22.465	24.256	25.755
7	Shell weight (10 shells) (g)	3.240	3.169	3.286	3.375	3.129	3.102	3.374	3.690
8	shell rate (%)	12.619	13.51	13.18	13.91	12.67	13.80	13.91	14.327
9	Larva-pupa rate (%)	85.25	90.00	86.54	90.00	89.49	89.23	93.36	96.00
10	Weight of 10 pupae	23.759	21.206	21.567	21.675	21.665	22.762	23.690	26.486
11	Rate of moth emergence (%)	94.00	88.00	91.00	93.00	91.00	92.00	93.00	96.00
12	Fecundity (No)	385.00	322.00	350.00	350.00	330.00	340.00	355.00	399.00
13	Hatching (%)	89.25	90.00	90.00	91.00	90.00	92.00	92.00	97.00

Table 4: Mean value of 13 quantitative characters of F1 hybrids YPxBGP, BGPxYP, YPxYS, YSxYP, YPxBGS, BGSxYP, YPxBGZ, BGZxYP (positive and negative cross)

		YPxYZ	YZxYP	BGPxYS	YSxBGP	BGPxBGS	BGSxBGP	BGPxBGZ	BGZxBGP
Sl No	Metric Traits	F1 hybrid	F1 hybrid	F1 hybrid	F1 hybrid	F1 hybrid	F1 hybrid	F1 hybrid	F1 hybrid
1	Duration of all instars (h)	574.00	550.45	555.40	565.35	558.25	568.40	558.25	538.54
2	Duration of the fifth instar (h)	163.00	158.00	162.00	161.50	166.00	165.00	166.00	158.00
3	Effective rate of rearing (ERR)	91.58	92.00	92.00	93.00	90.00	92.00	90.00	96.00
4	Fifth instar larval weight (10 worms) (g)	70.645	74.250	58.425	60.245	60.525	65.245	60.525	72.824
5	Cocoon yield by number per 100 larvae	85.00	93.00	88.00	90.00	90.00	91.00	90.00	94.00
6	Cocoon Weight (10 cocoons) (g)	26.220	25.864	24.649	25.894	25.648	24.249	25.648	24.649
7	Shell weight (10 shells) (g)	3.468	3.450	3.142	3.558	3.292	3.186	3.588	3.142
8	shell rate (%)	13.22	14.327	12.74	13.856	12.835	13.13	13.99	12.74
9	Larva-pupa rate (%)	89.49	90.50	89.00	91.15	90.00	92.00	92.50	93.22
10	Weight of 10 pupae	21.678	22.356	21.646	21.794	21.646	22.875	21.646	23.625
11	Rate of moth emergence (%)	90.00	92.00	91.00	92.00	92.00	91.00	92.00	92.00
12	Fecundity (No)	345.00	345.00	340.00	342.00	343.00	344.00	343.00	358.00
13	Hatching (%)	91.25	93.00	91.00	92.00	92.00	92.00	92.00	94.00

Table 5: Mean value of 13 quantitative characters of F1 hybrids YPxYZ, YZxYP, BGPxYS, YSxBGP, BGPxBGS, BGSxBGP, BGPxBGZ, BGZxBGP (positive and negative cross)

Sl No	Metric Traits	BGPxYZ F1 hybrid	YZxBGP F1 hybrid	YSxBGS F1 hybrid	BGSxYS F1 hybrid	YSxBGZ F1 hybrid	BGZxYS F1 hybrid	YSxYZ F1 hybrid	YZxYS F1 hybrid
1	Duration of all instars (h)	561.50	540.24	546.00	545.00	527.00	524.00	534.00	525.30
2	Duration of the fifth instar (h)	160.00	159.00	158.45	157.25	156.00	154.00	160.00	159.25
3	Effective rate of rearing (ERR)	90.00	91.00	90.00	91.00	95.00	94.00	92.00	92.00
4	Fifth instar larval weight (10 worms) (g)	65.430	70.246	69.740	69.650	70.645	67.240	68.240	69.450
5	Cocoon yield by number per 100 larvae	91.00	90.00	92.00	94.00	93.00	95.00	93.00	92.00
6	Cocoon Weight (10 cocoons) (g)	26.240	24.246	26.246	25.675	26.655	28.275	25.285	24.285
7	Shell weight (10 shells) (g)	2.998	3.107	3.452	3.240	2.985	3.250	3.480	2.590
8	shell rate (%)	11.42	12.81	13.152	12.619	11.194	11.494	13.763	10.665
9	Larva-pupa rate (%)	92.00	92.24	93.50	93.75	95.25	94.50	93.85	93.45
10	Weight of 10 pupae	22.565	22.545	23.458	23.759	24.662	25.256	23.230	24.281
11	Rate of moth emergence (%)	91.00	92.00	95.00	94.00	95.00	96.00	92.00	94.00
12	Fecundity (No)	345.00	347.00	365.00	385.00	375.00	398.00	386.00	394.00
13	Hatching (%)	92.00	92.25	95.00	95.00	93.00	96.00	93.65	94.00

Table 6: Mean value of 13 quantitative characters of F1 hybrids BGPxYZ, YZxBGP, YSxBGS, BGSxYS, YSxBGZ, BGZxYS, YSxYZ, YZxYS (positive and negative cross)

		BGSxBGZ	BGZxBGS	BGZxYZ	YZxBGZ	YZxBGS	BGSxYZ
Sl No	Metric Traits	F1 hybrid	F1 hybrid	F1 hybrid	F1 hybrid	F1 hybrid	F1 hybrid
1	Metric Traits	525.40	520.50	524.50	550.25	535.00	534.00
2	Duration of all instars (h)	159.00	148.00	158.50	160.00	158.00	156.00
3	Duration of the fifth instar (h)	92.00	94.00	93.00	96.00	94.00	91.00
4	Effective rate of rearing (ERR)	68.524	70.524	74.561	73.481	72.854	73.584
5	Fifth instar larval weight (10 worms) (g)	93.00	94.00	94.00	94.00	92.00	93.00
6	Cocoon yield by number per 100	25.450	24.450	27.375	25.210	26.678	25.678
7	Weight (10 cocoons) (g)	3.261	3.362	2.927	3.246	3.469	3.358
8	Shell weight (10 shells) (g)	12.813	13.75	10.692	13.45	13.003	13.07
9	Cocoon shell rate (%)	91.00	94.00	95.92	93.82	91.56	92.00
10	Larva-pupa rate (%)	22.264	23.264	25.271	24.246	24.526	24.826
11	Weight of 10 pupae (g)	91.00	93.00	96.00	93.00	93.00	92.00
12	Fecundity (No)	345.00	350.00	369.00	362.00	351.00	340.00
13	Hatching (%)	93.00	94.00	95.00	94.00	92.00	91.00

Table 7: Mean value of 13 quantitative characters of F1 hybrids BGSxBGZ, BGZxBGS, BGZxYZ, YZxBGZ, YZxBGS, BGSxYZ (positive and negative cross)

Varieties	Days				
	1	2	3	4	5
YP	45.45	53.69	62.64	39.23	20.31
BGP	41.25	48.68	59.25	28.21	19.23
YS	42.46	50.72	60.12	30.23	18.23
YZ	50.59	53.69	55.41	37.17	18.24
BGS	42.64	48.23	50.12	30.24	17.23
BGZ	40.96	49.60	66.42	38.20	22.72

Table 8: Changes in concentration of soluble protein (mg/g) in the midgut of eri silkworm during fifth instar larval developement

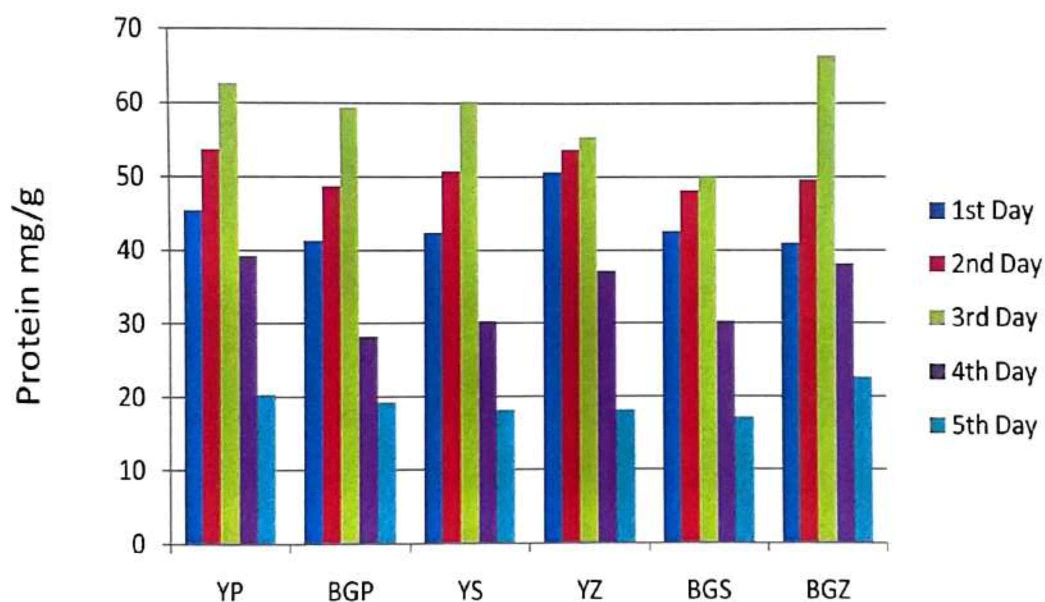


Chart 1: Changes in concentration of soluble protein (mg/g) in the midgut of eri silkworm during fifth instar larval developement

Varieties	Days				
	1	2	3	4	5
YP	13.42	16.52	25.12	39.23	51.97
BGP	16.86	20.31	24.78	33.23	49.56
YS	15.64	19.24	23.70	28.30	45.46
YZ	14.52	17.52	20.13	38.20	50.64
BGS	17.64	21.24	23.64	34.38	48.76
BGZ	18.58	24.78	26.84	39.58	23.69

Table 9: Changes in concentration of soluble protein (mg/g) in the haemolymph of eri silkworm during fifth instar larval development.

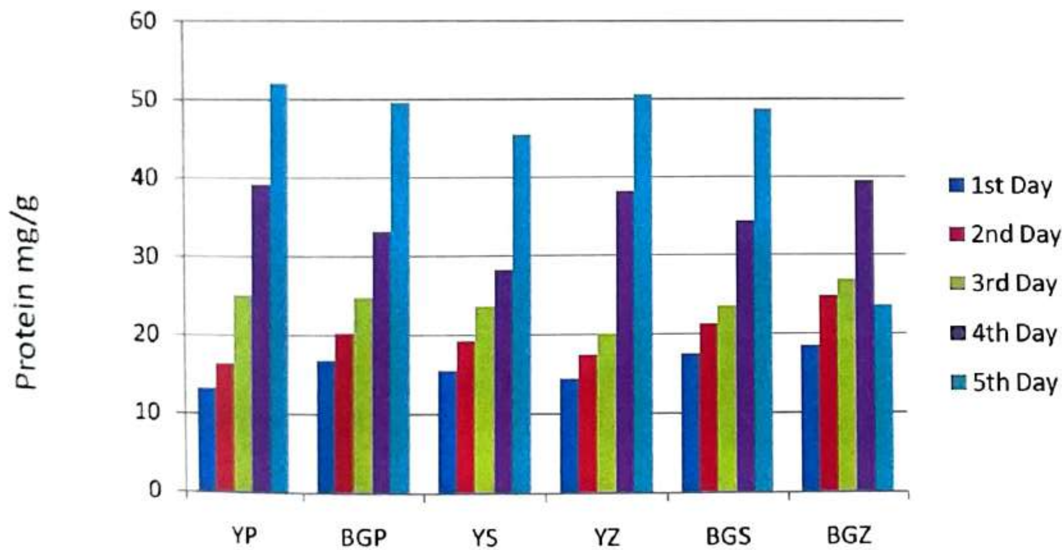


Chart 2: Changes in concentration of soluble protein (mg/g) in the haemolymph of eri silkworm during fifth instar larval development.

Varieties	Days				
	1	2	3	4	5
YP	30.98	35.11	36.83	40.27	64.01
BGP	19.96	29.25	35.11	47.50	61.95
YS	20.90	28.45	36.12	48.64	62.64
YZ	22.41	30.26	38.42	50.76	61.22
BGS	28.43	32.73	39.34	53.70	63.76
BGZ	30.98	37.17	39.92	44.05	70.21

Table 10: Changes in concentration of soluble protein (mg/g) in the silkgland of eri silkworm during fifth instar larval development.

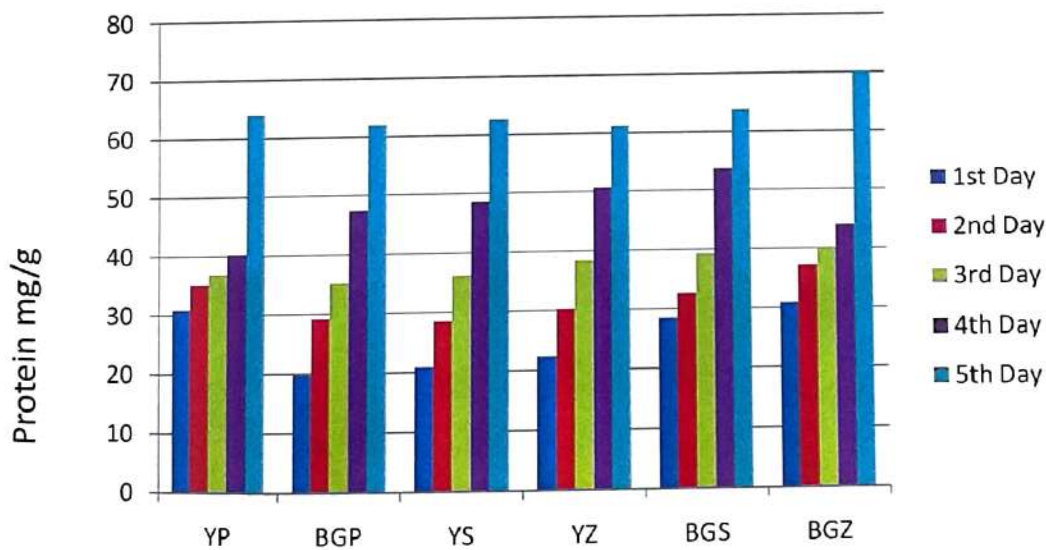


Chart 3: Changes in concentration of soluble protein (mg/g) in the silkgland of eri silkworm during fifth instar larval development.

Varieties	Days				
	1	2	3	4	5
YP	13.42	16.52	25.12	39.23	51.97
BGP	16.68	20.31	24.78	33.38	49.56
YS	15.64	19.24	23.70	28.30	45.46
YZ	14.52	17.52	20.13	38.20	50.64
BGS	17.64	21.24	23.64	34.38	48.76
BGZ	18.58	24.78	26.84	39.58	53.69

Table 11: Changes in concentration of total sugar (mg/g) in the midgut of eri silkworm during fifth instar larval development.

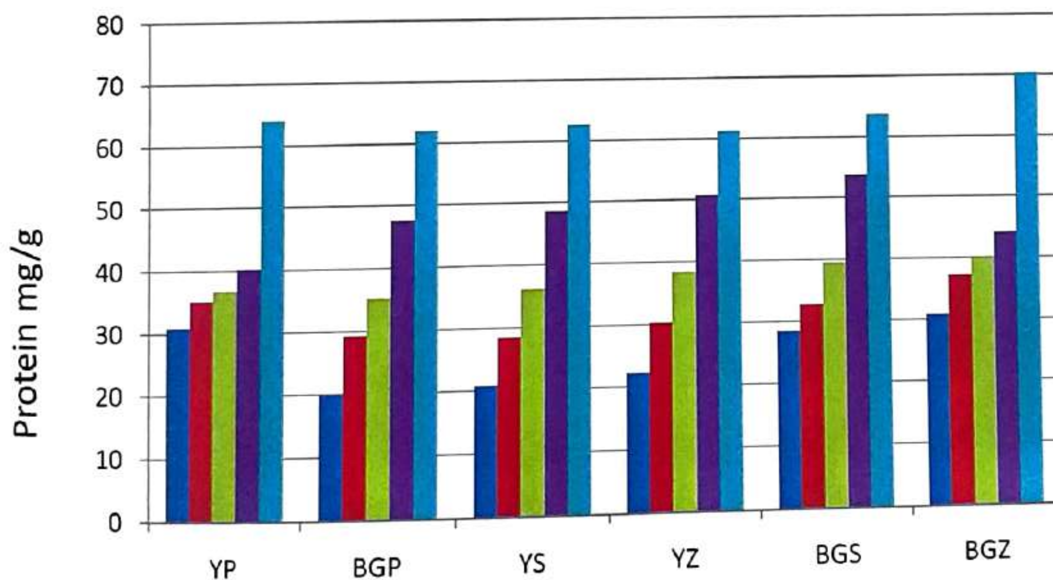


Chart 4: Changes in concentration of total sugar (mg/g) in the midgut of eri silkworm during fifth instar larval development.

Varieties	Days				
	1	2	3	4	5
YP	0.41	3.26	5.15	5.61	6.43
BGP	0.45	3.50	4.60	5.49	6.34
YS	0.43	3.48	4.56	5.42	6.29
YZ	0.51	3.57	4.64	5.52	6.22
BGS	0.44	3.40	4.59	4.50	6.36
BGZ	0.46	3.67	4.79	5.46	6.53

Table 12: Changes in concentration of total sugar (mg/g) in the haemolymph of eri silkworm during fifth instar larval development.

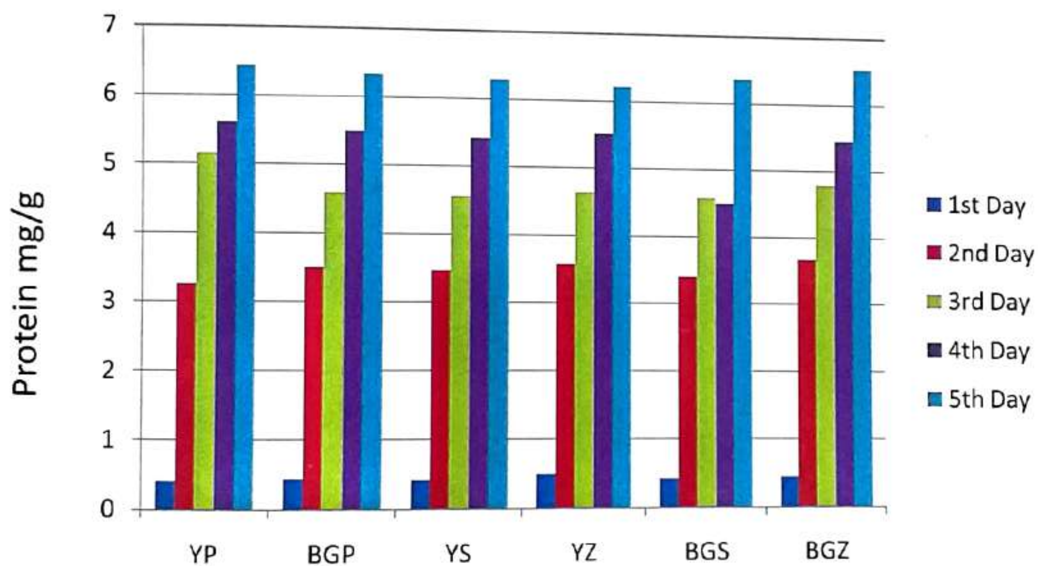


Chart 5: Changes in concentration of total sugar (mg/g) in the haemolymph of eri silkworm during fifth instar larval development.

Varieties	Days				
	1	2	3	4	5
YP	0.81	0.94	2.02	3.89	3.98
BGP	0.76	0.97	1.78	2.14	3.62
YS	0.73	1.10	2.20	3.69	4.22
YZ	1.02	1.22	3.06	4.79	5.81
BGS	0.86	1.16	2.39	3.56	4.03
BGZ	0.92	1.22	3.37	4.89	5.51

Table 13: Changes in concentration of total sugar (mg/g) in the silkgland of eri silkworm during fifth instar larval development.

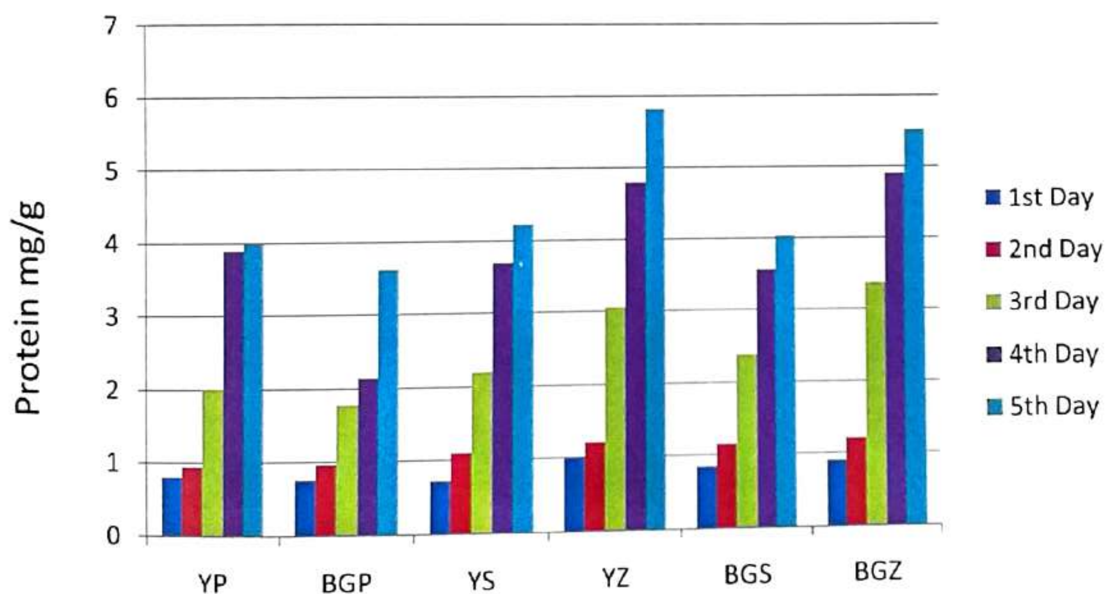
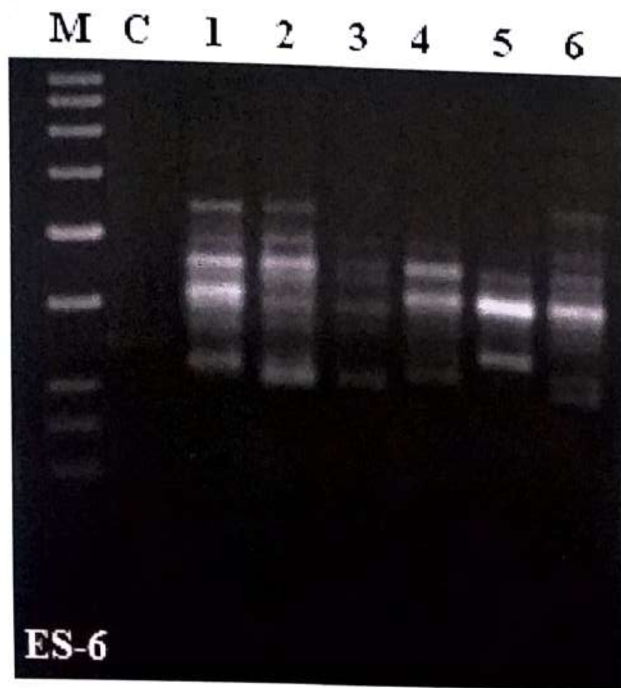
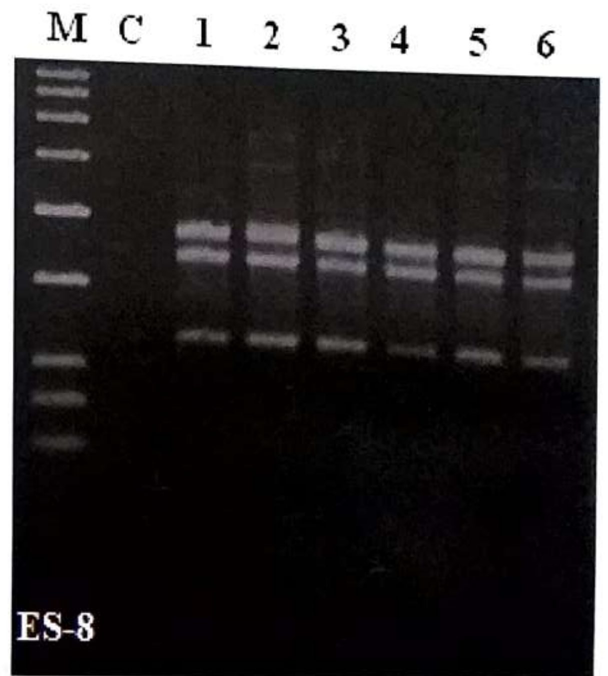


Chart 6: Changes in concentration of total sugar (mg/g) in the silkgland of eri silkworm during fifth instar larval development.

Serial number	Primers	Nucleotide sequence (5'→3')	Total bands	Polymorphic bands	PPB*
1	ES 1	TGCCGAGCTG	45	3	6.66
2	ES 2	TGTCATCCCC	24	2	8.33
3	ES 3	TGGGCGTCAA	78	2	2.25
4	ES 4	AGCCTGAGCC	44	11	25.00
5	ES 5	GACCGACCCA	44	3	6.81
6	ES 6	AAGCGACCTG	39	10	25.64
7	ES 7	ACCCCGCCAA	49	2	4.08
8	ES 8	GTCCGGAGTG	40	15	37.50
9	ES 9	AAGGCTCACC	45	1	2.22
10	ES 10	GTGGAGTCAG	12	2	8.33
11	ES 13	CATTCGAGCC	14	3	21.42
12	ES 14	CAGGGAAGAG	11	1	9.09
13	ES 15	CTGTTGCTAC	16	1	6.25
14	ES 16	ACTGAACGCC	11	1	9.09
15	ES 17	CTCAGTGTCC	16	2	12.50
16	ES 18	ACGCCAGAGG	20	2	10.00
17	ES 19	AGGCAGAGCA	13	2	15.38
18	ES 20	GACGTGGTGA	12	1	8.33
Total			**533**	**64**	
Average			30		12.00

Table 14: Banding profile produced by selected RAPD primers
*PPB = Proportion of polymorphic bands.





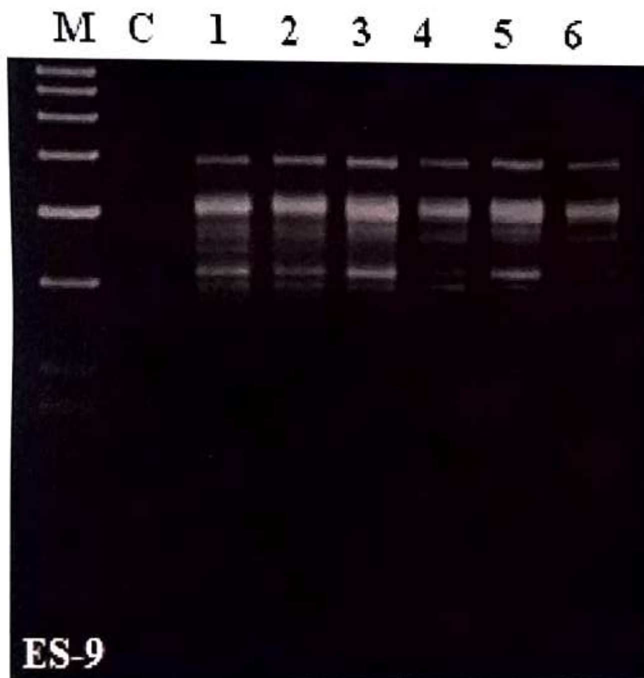


Figure 5: PCR fingerprinting of six varieties of eri silkworm *Philosamia ricini* with RAPD primers (ES-6, ES-8, ES-9, ES-15).

Yellow Zebra	1.0					
Blue Green Plain	0.33	1.0				
Blue Green Spotted	0.00	0.60	1.0			
Blue Green Zebra	0.00	0.00	0.66	1.0		
Yellow plain	0.00	0.00	0.00	0.10	1.0	
Yellow Spotted	0.28	0.00	0.00	0.00	0.25	1.0

Table 15: Genetic distance among the six varieties of eri silkworm *Philosamia ricini*

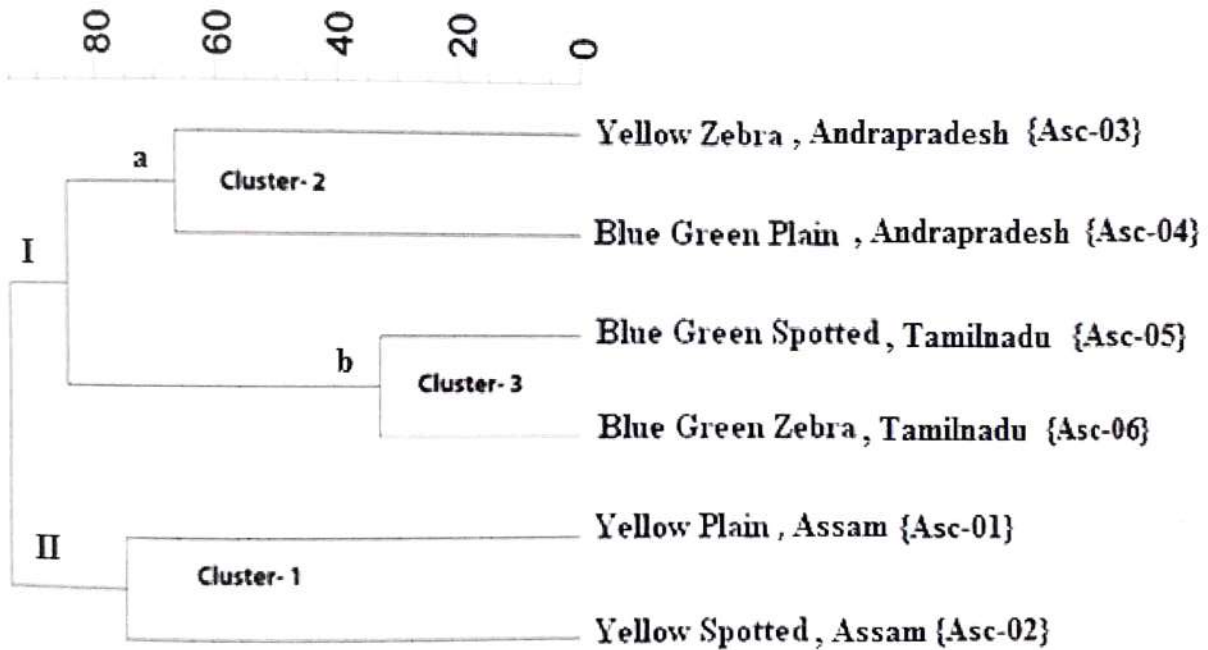


Table 16: Dendrogram of illustrating genetic relationships among the six varieties of eri silkworm accessions.

Journal of Biosciences

Genetic Diversity Analysis of Eri Silkworm by RAPD

--Manuscript Draft--

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Abstract:

Non-mulberry eri silkworm, Samia cynthia ricini is an commercial exploitation of Vanya silk. To enhance the productivity and quality of silk fibers many attempts have been made through genetic manipulation. Since the information on the genetic basis, phenotypic variability and genetic diversity within populations of eri silkworm is scanty, an attempt had been made to understand the genetic diversity among six populations viz., Yellow plain (YP), Yellow spotted (YS), Yellow Zebra (YZ), Blue Green Plain (BGP), Blue Green Spotted (BGS) and Blue Green Zebra (BGZ) by using Randomly Amplified Polymorphic DNA (RAPD) marker. Out of 20 RAPD primer screened, 18 primers generated good amplification of which 64 were polymorphic. RAPD profile generated for selected accessions with morphological dissimilarity showed 12% of polymorphism with primers ES-6, ES-8, ES-9 and ES-15. Cluster analysis (UPGMA) was generated to construct the dendogram. Jaccard's similarity coefficients range (0.1 to 0.66) with maximum & minimum similarity noticed between Asc-05 /Asc-06 and Asc-01/Asc-02. The range of similarity 0.1 to 0.66 indicated low genetic diversity in eri silkworm germplasm which would be due to restricted environmental acclimatization.

“DNA Fingerprinting of *Samia cynthia* using Arbitrary Primers”

Dissertation submitted to the Bangalore University

in partial fulfillment for the degree of

Master of Science in Life Science

By

PRAMODINI

Reg. No. [illegible]

Guide

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2010

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RAPD AND ISSR MOLECULAR MARKER STUDIES IN SILKWORM VARIETIES

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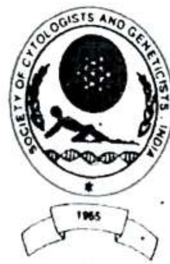
Twenty diverse strains of Silkworm (Bombyx mori) were analysed using polymeric chain reaction for RAPD and ISSR molecular markers. The genomic DNA twenty silkworms were amplified with different RAPD and ISSR pimes revealed 150 RAPD and 130 ISSR bands out of which 95% is polymorphism and 5% is monomorphic, silkworm is polygenic. Some of the DNA fragments were strain specific and some could differentiate the multivoltine from the bivoltine strains or vice versa.

Silkworm genetic resources that are being maintained in Hosur, Tamil Nadu, India, are yet to be adequately topped to develop elite varieties that are subjected to different agro-eco-climatic condition of the country like India. Molecular markers are known to provide unambiguous estimated of genetic variability of silkworm populations. Since they are independent confounding effects of environment, the genetic similarity among the twenty silkworm varieties/strains and their genetic diversity and relationships were discussed.using Nice, Dice and Jaccord method and resolved the problem using population genetic equation especially by Hardy -Weinberg equations

ABSTRACTS



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29. Analysis of genetic diversity among six varieties of cashew (*Anacardium occidentale L.)* using Random Amplified Polymorphic DNA

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Cashew is a perennial commercial crop of family *Anacardiaceae*. It is said to be a native of Brazil. It is also a plantation crop of export value with considerable foreign exchange earning attributed in India. In order to encash the global opportunity, the cashew producing countries lay greater emphasis on the strengthening their research programme. RAPD molecular markers are of good choice for assessing the genetic diversity and relationship in cashew species, Therefore, molecular marker studies of different cashew species were studied for their genetic diversity and relationship. Different universal primers were used and the target gene for introgression of the characters were cloned. The study included six differint cashew plants viz., Damodar, Dhanashree, Priyanka, Ullal-1, UN-50 and VRI-1 and eight 10-mer primers were used. A total of 74 distinct DNA fragments ranging from 0.2 to 3.0 Kb were amplified by using 8 selected random 10-mer primers. Genetic similarity analysis was conducted on the presence or absence of bands in the RAPD profile. Cluster analysis constructed between six genotypes formed clusters at 4.1 linkage distance indicating their difference for 4.1% and similarity for 95.9%, at this linkage distance the varieties are grouped into 2 major clusters with UN-50, Priyanka, VRI-1 forming one cluster and Damodar Dhanashree and Ullal-1 forming another cluster. In cluster 1 Priyanka and VRI-1 shared close linkage distance of 2.6 and UN-50 joining this cluster. In another cluster Dhanashree, Ullal-1 formed one cluster and Damodar joining it. Based on similarity indices, Analysis of genetic relationships in cashew using RAPD banding data is useful for genetic characterization, identification diversity in cashew.