Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2019; 8(6): 1749-1762
Received: 24-09-2019
Accepted: 28-10-2019

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Approaches of genetic divergence studies conducted in the silkworm *Bombyx mori* L.: A review

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Abstract
Genetic diversity is critical to success in any crop breeding and it provides information about the quantum of genetic divergence and serves a platform for specific breeding objectives. It is one of the three forms of biodiversity recognized by the World Conservation Union (IUCN) as deserving conservation. Genetic diversity is usually thought of as the amount of genetic variability among individuals of a variety or population of a species. It results from the many genetic differences between individuals and may be manifest in differences in DNA sequence, in biochemical characteristics (e.g., in protein structure or isoenzyme properties), in physiological properties (e.g., abiotic stress resistance or growth rate), or in morphological characters. Genetic diversity has been conventional, estimated on the basis of different biometrical techniques (Metroglyph, *D*2 divergence analysis, and principal component analysis) such as phenotypic diversity index (*H*), or coefficient of parentage utilizing morphological, economical, and biochemical data. In the present paper the study of genetic diversity is carried out in silkworm using divergent methods (quantitative traits, biochemical and molecular markers).

Keywords: Words genetic, metro glyph, biodiversity, breeding, biochemical

Introduction
Sericulture industry has been benefited tremendously through exploitation of heterosis. Silkworm hybrids are superior to pure breeds in growth, vigor and economic traits. In order to improve yield and quality of silk, it is pertinent to characterize the potential of available allelic resources in transferring desirable performance to hybrid progenies. Selection of parents on the basis of *per se* performance does not always lead to fruitful results. The selection should be based on a thorough knowledge of genetic diversity of parents with regard to traits that need to be improved. More diverse the parents, greater are chances of obtaining higher amount of heterotic expression and also more variability is expected to appear in segregating generations of such cross. A population with more diverse genotypes is of considerable importance- as success of any breeding programme relies on the genetic variability present in it, provides information about the quantum of genetic divergence and serves as a platform for specific breeding objectives [1]. Sericulture is a unique field of agriculture, because silkworms are reared on an extensive scale in rearing houses and their silk cocoons are utilized as fine material for clothing. Like agriculture, sericulture also requires a continuous flow of productive silkworm breeds and host plant varieties to meet the ever-lasting demand of people involved in the industry. To meet all these requirements, the breeders need very wide and inexhaustible genetic resources. Considering the great economic importance of *B. mori*, silk producing countries, such as China, Japan, India, Russia, Korea, Bulgaria and Iran have collected number of silkworm breeds suitable for a wide range of agro climatic conditions. More than 4000 strains are maintained in the germplasm of *B. mori* and 46 institutes are involving silkworm genetic resources maintenance, which includes Univoltine, Bivoltine and polyvoltine strains. These different genotypes display large differences in their qualitative and quantitative traits that ultimately control silk yield. A great diversity in silkworm *B. mori* L. has been reported to exist globally, considering the fact that many number of silkworm breeds are evolved by selection or cross breeding [2]. However, recent advent of different molecular techniques led breeders to estimate genetic diversity on the basis of data generated by different molecular markers which provide a mean of rapid analysis of germplasm and estimates of genetic diversity. This has enabled substantial improvement in quality, productivity as well as stability of silk production in India [3].

The genetic diversity of *B. mori* is derived from hybridization of different geographical races, mainly the Japanese, Chinese, European, and Indian strains with distinct traits.

Among these four geographical strains, silkworm of temperate origin produces a higher quantity of good, finer, stronger silk fiber, whereas the tropical strains are hardy, tolerant to pathogen load, and resistant to diseases. However, the tropical strains produce low amounts of silk, which is coarser and weaker. To help the breeders in the process to identify the parents that nick better, several methods of divergence analysis based on quantitative traits have been proposed to suit various objectives.

Genetic divergence using mahalanobis d^2 analysis

Mahalanobis D^2 statistics is an efficient tool in quantifying degree of divergence at genotypic level and has also been applied by various workers to study genetic divergence in silkworm [4]. Studied genetic divergence using Mahalanobis D^2 technique in forty-nine bivoltine silkworm genotypes. All forty-nine silkworm breeds were classified into three clusters with twenty-six genotypes in cluster-I, 13 genotypes in cluster-II and ten genotypes in cluster-III. The cluster pattern indicated a mixed trend. There was no relation between genetic diversity and geographical distribution of the breeds. Silkworm breeds of the same origin were included in different groups. The result indicated that the cocoon yield by number/10,000 larvae brushed, fecundity and total larval duration largely contributed to genetic divergence [5]. Studied genetic divergence among thirty two races of bivoltine silkworm, *B. mori* L., using D^2 technique. On the basis of five quantitative characters viz., larval duration, effective rate of rearing, cocoon weight, cocoon shell ratio and filament length, the silkworm genotypes were grouped into seven clusters. Geographical diversity did not contribute much to the genetic diversity. The character-wise contribution to divergence was: cocoon shell ratio (48.38%), single cocoon weight (18.55%), filament length (15.52%), larval duration (11.70%) and ERR (5.85%). The divergence ranged from 6.59 between cluster-V and cluster-VI to 113.88 between cluster-I and cluster-VII [6]. Further studied genetic divergence in fifteen multi-voltine stocks of *B. mori* L. using Mahalanobis D^2 technique. The fifteen genotypes were grouped into five clusters. The range of variation for D^2 values was from 0.70 (M_2 and CB_2) to 53.33 (Nistari and G). The genotypes from the same geographical region formed separate clusters [7]. Undertook a detailed analysis to test the efficacy of hierarchical agglomerative clustering (UPGMA method) in grouping the races and strains of mulberry silkworm, *B. mori* L. and to ascertain the importance of biochemical parameters in the clustering process. They studied 54 silkworm strains of different geographic origin and varying yield potentials. They realized five clusters (cluster 1- G92, NBJH (pc), NB_4D_2, G73 and NB_{18}; cluster 2- JZH (po), G74, French yellow and G102; cluster 3- Boropalu; cluster 4- NB_1, J122, C124, NBH (po), KA and AWC and cluster 5 – Nong 51, Pure Mysore, DA09, C' Nichi, 175, Moria, PCN(S), PCN, Mysore Princess, Nistari, PA1, Sarupat, MY1(S), G-Race, A4E, KollegalJawan, A_2, MW_1, Staple deep black, MU11, NK4, A4, Tamil nadu White, MHMP(w), AW8A, G176, HU204, Dong 34, MY1, NN6D, Goy Oval, Eq Plain, Rong Dazao, French plain, MHMP(y), Hosa Mysore, Kolar Gold and French Batik) on the basis of yield parameters [8]. Studied genetic divergence in twenty-six genotypes of tasar silkworm *Antheraea mylitta* D. applying Mahalanobis D^2 statistic. The genotypes got grouped into nine clusters. They recorded a wide range of variation in cluster means for fecundity, larval duration, cocoon weight, shell weight and absolute silk yield. The inter-cluster distance ranged from 1.55 (cluster-V and cluster IV) to 12.5 (cluster III and cluster VI). The analysis for estimating contribution of various characters towards the expression of genetic divergence indicated that fecundity (13.54%), cocoon weight (19.38%), shell weight (20.00%) and absolute silk yield (36.62%) contributed maximum to the genetic divergence. There was no relationship between geographical distribution and genetic divergence [9]. Carried out studies on the genetic divergence among nine bivoltine silkworm, *B. mori* L. breeds on the basis of seven important quantitative traits. Based on the divergence between the parents as measured by using Mahalanobis D^2 statistic, the parents were grouped into five distinct clusters. The clustering pattern revealed that there was no clear-cut relationship between genetic divergence and geographical origin of the races. The inter-cluster values ranged from 4.65-6.57 [10]. Studied the nature and magnitude of genetic divergence in eighteen diverse multivoltine strains of silkworm using Mahalanobis D^2 technique. Based on six important quantitative traits, which account for fitness and productivity, the genotypes were grouped into eight clusters. The inter-cluster distances ranged from 6.48-64.40. The intra-cluster distances ranged from 1.47-4.19. The study revealed that the breeds derived from same ancestry were included in different clusters and those of different genetic background occupied a single cluster indicating the uniformity in their selection pressure [11]. Studied genetic divergence in fifteen multivoltine and seventeen bivoltine silkworm breeds using D^2 statistic and the genotypes were grouped into five clusters. [12]. Carried out analysis of genetic divergence in fifty-six multivoltine silkworm races following hierarchical clustering method. The pooled data of five rearings on yield and yield parameters were analyzed which resulted in the formation of six clusters. C. Nichi and Pure Mysore formed separate clusters with single entity highlighting the genetic diversity between the two breeds and with that of rest of breeds. Exotic breeds were distributed among the various clusters and grouped with indigenously evolved races which highlight the importance of genetic diversity over the geographical origin of the breeds. Characters viz., larval weight, total larval duration and cocoon weight were mainly responsible for differentiating the breeds as these traits contributed maximum towards the total divergence [13]. Studied genetic diversity of twenty-five bivoltine silkworm (*B. mori* L.) genotypes, maintained at Division of Sericulture, Mirgund. The study revealed existence of sufficient genetic variability. The genotypes were grouped into 12 clusters on the basis of D^2 values for seven economic traits. Geographic diversity was found to have no association with genetic diversity. Highest genetic divergence was observed between cluster IV and X. Crosses between genotypes from divergent clusters could prove useful in hybridization programmes. The most important trait contributing to genetic divergence was shell weight followed by cocoon yield by number/10,000 larvae and cocoon weight [14]. Studied genetic divergence in twenty four genotypes of mulberry silkworm, *B. mori* L., using Mahalanobis D^2 statistic. Based on eight important quantitative traits, D^2 values were obtained and the genotypes were grouped into seven clusters using Tocher's method. Genotypes of temperate and tropical origin formed separate clusters. Silkworm breeds having common ancestry fell in a single cluster. The inter-cluster distances ranged from 16.01 to 70.84. Genetically the most divergent genotypes were included in cluster VI (C108) and cluster VII (KA) followed

by cluster II (Jam 2w, Jam 10, Jam 24 and NB7) cluster VII (KA). Single cocoon shell weight (31.159%), single cocoon weight (21.014%) and filament length (20.289%) contributed maximum towards the total genetic divergence [15]. Studied the genetic diversity of 47 silkworm genotypes of varied geographic origin. The data was subjected to Mahalanobis D^2 analysis and the genotypes were grouped into 12 clusters. The average inter-cluster distances ranged from 3.07-99.78. The genetic divergence was maximum (99.78) between cluster 10 (CSR_4) and cluster 12 (B_{36}) followed by (86.91) between cluster 7 (Gyandza, Belkokona, King Haung, KPG_4, KPG_5, BL_1, Sannish-8, Sannish-21, SS, Sannish-18P and Chinese farmer) and cluster 12 (B_{36}) and (77.41) between cluster 6 (SH_6) and cluster 12 (B_{36}). The minimum inter-cluster distance (3.07) was found between cluster 2 (SKUA-R-6) and cluster 3 (SK_{13}). The contribution of shell weight (20.25%), renditta (13.97%) and single cocoon weight (12.86%) towards genetic diversity was maximum. The clustering pattern showed a mixed trend. Silkworm genotypes having different geographic origin fell in the same cluster while genotypes having same geographic origin fell in different clusters. [16] carried out cluster analysis of sixteen silkworm strains according to [17] distance based on eight traits (silk gland weight, larval body weight, larval body length, cocoon length, cocoon weight, cocoon width, shell weight and raw silk percentage). The grouping from UPGMA (unweighted pair group method average) revealed a clear division into three groups. However, the strains of the same origin did not group together, demonstrating they can have different biological developmental performance.

Genetic divergence using isozyme analysis

Among the available reliable techniques to quantify the variability, isozyme techniques and protein profiles are important ones. Isozymes offer many advantages for the investigation of micro-evolutionary process, because they have a simple genetic basis and their phenotypes can be objectively and accurately quantified. Difference in allozyme functions is shown to have different physiological manifestations. In silkworm the genetic divergence has also been studied using isozyme markers [18]. Reported that to investigate the isozyme pattern the electrophoretic method should be accepted as a tool for detecting proteins with enzymatic activity due to their specificity and activity. Further the electrophoretic technique of isozyme analysis is more useful at the low level of family relation (species and race) especially in case of insects. It is by this technique that the analysis of racial differentiation can be made to make clear and even the origin of *B. mori* L [19]. Reported that by the development of separation technique of proteins and enzymes a lot of variants showing different mobility on gel electrophoresis have been demonstrated in some enzymes and proteins of silkworm. These variants are useful for genetic mapping by determining crossover frequencies in appropriate hybrids of genetic markers and the localization of gene loci in some enzymes and proteins in the silkworm [20]. Assessed genetical distances between different groups of silkworm races in tropical and temperate regions by using biochemical markers like blood albumen, acid phosphatase, lipoprotein complex, esterase and amylase. The tropical races of South East Asia were shown to have a higher number of gene substitutions than the Chinese, European and Japanese races [21]. Reported the gene frequencies in polymorphic loci by using the gel electrophoresis and made the phylogenetic differentiation of silkworm strains. He also reported the effects of these polymorphic variants on the qualitative characters of the silkworm and also on the gene loci coding silk proteins, fibroin and sericin [22]. Studied the activities of acid and alkaline phosphatase during the embryonic development in tropical tasar silkworm, *Antheraea mylitta* D. They reported a sharp increase in the acid and alkaline phosphatase activity on the second day of embryogenesis which may be due to the initial requirement of energy for the rapid division of cells. Moreover, alkaline phosphatase activity shows a gradual decrease throughout embryogenesis which may be due to the gradual increase in the activity inside the cells with simultaneous decrease in the alkalinity [23]. carried out biochemical studies of amylases in the silkworm, *B. mori* L. and reported that different amylase enzymes were identified by analysis of digestive fluid and haemolymph in diapausing and non-diapausing strains. The diapausing strain showed negligible digestive amylase activity, while the non-diapausing strain registered strikingly higher amylase activity. Developmental specific expression of haemolymph amylase activity was seen in both the strains. In the non-diapausing strain the digestive amylase activity was at its peak during inter-moult and depressed during moult. PAGE analysis revealed the occurrence of only anodal digestive and haemolymph amylases in the diapausing strain, whereas both cathodal and anodal enzymes were seen in the digestive fluid and haemolymph of the non-diapausing strain [7]. Ascertained the importance of biochemical parameters in the clustering process. They realized two broad groups (Group 1 containing 24 races and Group 2 containing thirty races on the basis of biochemical parameters, one having all the breeds with high cocoon and shell weight while other having all low yielding silkworm strains. These two groups were further classified on the basis of amylase, invertase and alkaline phosphatase activity [24]. Have highlighted the prospects of using amylase as a marker for breeding, in view of its divergence in terms of activity and isozyme polymorphism, in addition to its role in better digestibility and close association with survival [25]. In a study on prospects of isozyme marker based selection in silkworm breeding adopted integrating digestive amylase as a marker for improving the survival potential of productive breeds. Three productive bivoltine breeds viz., NB_{18}, CSR_2 and CSR_5 with 'null' type of amylase were used as recurrent parents (RPs) and they were introgressed with '4 band/5 band' isozymes from indigenous multivoltine donor parents (DPs) viz., Pure Mysore and Nistari by backcross breeding with the RPs followed by selection of progeny showing the isozyme pattern of the DPs in each generation. The performance estimates of the three near isogenic lines (NILs) evolved (NNB, NC_2 and NN1) with respect to yield, cocoon weight, shell weight and shell ratio in comparison with those of their respective RPs have shown promising results [26]. Studied tyrosine hydroxylase isozyme patterns in the developmental stages of silkworm, *B. mori* L. and found that the pattern varied as three distinct bands with different mobilities. The slow-moving bands were more or less identical in the developmental stages whereas the single fast moving band was prominent and broad in the larva and adult while it was faint in the pupal stage [27]. While reviewing the role of genetic and molecular biology in strengthening silkworm breeding advocated the use of linked molecular markers (isozyme/DNA) for improvement of desirable traits and further reported that identification of suitable biochemical markers holds the key to successful implementation of marker assisted selection.

Esterase and phosphatase profiles were studied in eleven bivoltine and eight multivoltine silkworm races chosen on the basis of geographical origin and morphological diversity. The results placed bivoltine and multivoltine races into separate groups whereas the races within the bivoltine and multivoltine groups were placed in 5 and 4 groups respectively [28].

Chattopadhyay *et al.* (2001) studied esterase isozyme polymorphism in the digestive juice and haemolymph of the tropical multivoltine silkworm, *Bombyx mori* L., breed (CB5) and its syngenic lines (CB5Lm-1 and CB5LM-2 and CB5LM-5) and reported that polymorphism existed in the isozyme pattern of α-esterase with two or three bands in digestive juice and three to five bands in haemolymph. No polymorphism was observed in β-esterase isozyme pattern having four bands in digestive juice and two bands in haemolymph.

Mahesha and Honnaiah (2002) studied amylase and succinate dehydrogenase activity levels in F_1 progeny raised from ethylmethane-sulphonate treated silkworm, *B. mori* L. The results of this study indicated that the silkworm batches obtained from the EMS treated larvae exhibited almost similar pattern with altered levels of enzyme activity when compared to their respective control sets.

Miao (2002) in a study on the activity of alkaline phosphatase in the midgut of infected silkworm, *B. mori* L. reported that the activity varied greatly among tested strains and that there was positive correlation between alkaline phosphatase activity and cocoon quality. Ram and Lal (2002) categorized 20 silkworm genotypes into five groups on the basis of α-esterase activity. Group 1- Jam 11, Jam 23, Jam 27p; Group 2- Jam 21M, Jam 25, Jam 18M, Jam 19; Group 3- Jam 27 M, Jam 22, Jam 2w, Jam 2y, Jam 24, Jam 18p, J122; Group 4- Jam 10, J12 x CN, J112 and Group 5- Changnaung, Haulak and Yakwei.

Farooq *et al.* (2003) assessed genetic diversity in silkworm using PCR anchored ISSR wherein fourteen silkworm genotypes were separated into two major clusters. One cluster consisting of three non-diapausing and the other consisting of eleven diapausing genotypes. The eleven diapausing genotypes were further classified into sub-clusters and the genetic distances among these genotypes were ascertained.

Stoykova *et al.* (2003) carried out electrophoretic analysis of non-specific haemolymph esterases during silkworm ontogenesis and identified five loci controlling non-specific esterases. Four of them were polymorphic, two showing diallelic and two other showing triallelic polymorphism. The degree of polymorphism showed race specificity and a stage specific gene expression of esterase loci was observed.

Etebari *et al.* (2005) studied intra-specific biodiversity of eight groups of silkworm (*B. mori* L.) by biochemical markers and reported that the activity level of total protein, cholesterol, glucose, alanine aminotransferase and aspartate aminotransferase in haemolymph of 5th instar larvae was significantly different in all the groups.

Stoykova (2008) while studying genetically determined polymorphism of non-specific esterases and phosphoglucomutase in eight introduced breeds of the silkworm, *B. mori* raised in Bulgaria ascertained a polylocus control of non-specific esterases. Biallel and triallel polymorphism in three of the esterase loci was determined. The allelic frequencies of the polymorphic loci in each breed were analyzed. Inter-breed differences were found in different allelic frequencies, different heterozygosity and polymorphism.

In a study on molecular and biochemical diagnosis of three scarab beetles (Coleoptera: Scarabaeidae) which commonly infect sugarcane plantation in upper Egypt, Ibrahim *et al* (2009) employed esterase, protein banding pattern and RAPDs to discriminate between three scarab beetles and found two esterase bands which distinguished the beetles..

Association and path between yield attributing traits and isozyme markers

A knowledge of nature and magnitude of mutual relationships among components of economic worth helps to improve the efficiency of selection by use of favourable combination of characters and minimise the retarding effect of characters that are negatively correlated. Further, it is necessary to partition the total correlation between yield and its attributing traits into direct and indirect effects to determine the relative contribution of various metric traits towards the effect by path coefficient analysis. In silkworm, the association and path between yield and yield attributing characters has been worked out by several researchers. Intensive studies on the correlation between yield and biochemical parameters as well as isozyme markers have been carried out for both crop plants and domestic animals (Tanksley and Rick. 1980). These studies have opened new areas of research in animal and plant breeding. Attempts have also been made to analyse correlations between multiple biochemical parameters and yield in the silkworm.

Sen *et al.* (1976) carried out path analysis in *Antheraea mylitta* and reported positive direct effect of cocoon weight and shell ratio on shell weight. These characters also had indirect effects on shell ratio via egg number.

Jeong *et al.* (1986) observed direct effect of 5th age larval duration, total larval duration, cocoon shell weight and shell ratio on filament length.

Ozdzenska and Kremky (1987) found high correlations between cocoon weight and shell weight (0.95) as well as between shell weight and silk richness (0.92). Negative correlation was recorded between hatchability and number of eggs/gram (-0.89).

Studies of Siddiqui *et al.* (1989) revealed positive direct effect of larval weight, fecundity and ERR on silk yield both at phenotypic and genotypic levels. However, cocoon weight and shell weight exhibited indirect effects on silk yield via larval weight and fecundity.

Jayaswal *et al.* (1990) reported in silkworm that fecundity has negative correlation with shell weight and shell ratio. There exists a positive correlation between larval weight and cocoon weight. Cocoon yield has been found positively correlated with survival rate. The cocoon yield was also positively correlated with pupation rate and cocoon weight. Cocoon weight has been found significantly and positively correlated with pupal weight. However, pupal weight has positive correlation with fecundity. High significant positive correlations have been reported between cocoon weight, shell weight and filament length. Rajanna and Reddy (1990) reported positive direct effect of 5th age larval duration on cocoon weight. In a study on correlation between female pupal weight and fecundity in bivoltine silkworm, *B. mori* L., Kamili *et al.* (1992 b) reported positive and highly significant correlation between female pupal weight and fecundity.

Krishna *et al.* (1992) reported that cocoon weight was directly influenced by pupal weight, cocoon yield, filament length and ERR, whereas, shell weight was influenced by larval duration, cocoon yield, fecundity and filament length. Likewise shell weight was observed to be dependent on cocoon yield, 5th age larval duration and filament length. Liw (1992) observed that

cocoon yield revealed positive and significant correlation with cocoon weight and pupation rate. The author also carried out studies on genetic path network among quantitative characters in *B. mori* and revealed that larval duration had direct positive contribution towards cocoon weight and indirect contribution towards cocoon yield/10,000 larvae brushed, shell weight and shell ratio via cocoon weight.

Rangaiah *et al.* (1992) observed maximum direct effect of cocoon shell weight on cocoon weight, whereas, larval weight, fecundity and shell ratio had direct negative effect on cocoon weight both at genotypic and phenotypic levels. Fecundity exerted positive indirect effect on cocoon weight via shell weight and shell weight in turn exerted indirect positive effect on shell percentage.

Siddiqui *et al.* (1992) [8]. observed the contribution of nine quantitative characters to yield in *Antheraea mylitta*. Cocoon weights, Shell ratio, ERR and larval duration contributed positively and directly towards silk yield. However, the direct effect of fecundity, hatching percentage, larval weight and shell weight on silk yield was found to be negative. It was also noticed by the authors that the cocoon weight which registered the maximum direct effect, influenced the silk yield via silk ratio and ERR. Likewise shell ratio influenced the silk yield indirectly through cocoon weight and ERR.

Chatterjee *et al.* (1993 a) reported positive and significant correlation of fecundity with larval weight, cocoon weight, cocoon shell weight and shell ratio. However, ERR exhibited negative significant correlation with larval weight and larval duration. Chatterjee *et al.* (1993 b) proved that there is a significant correlation between some biochemical parameters of the haemolymph and the mid-gut fluid (amylase, invertase, trehalose, protease and alkaline phosphatase) of the silkworm larvae with biological and economical characteristics (weight of matured larvae, single cocoon weight, single shell weight and ERR) of this insect of which the most important compounds are amylase, invertase and alkaline phosphatase. Kamili (1994) reported that genotypic correlation coefficients were higher than their corresponding phenotypic correlation coefficients for all the quantitative traits that were studied. Cocoon yield was found to exhibit significant positive association with ERR, pupation rate, cocoon weight, shell weight, shell ratio, hatching rate, filament length, larval weight and larval duration. Girdhar *et al.* (1995) reported that high positive genotypic (0.75) and phenotypic (0.95) correlations were found between cocoon yield and cocoon weight. The same was also true between single shell weight and filament length (0.66; 0.70) and single shell weight and denier (0.78; 0.85).

In another study on nature and degree of association between various economic attributes of bivoltine silkworm, *B. mori* L., Kamili *et al.* (1996) reported significant and positive association between fecundity, number of cocoons/kg and pupation rate and between hatching and number of cocoons/kg. Pupation rate showed positive correlation of low magnitude with cocoon weight and cocoon yield. Kamili *et al.* (1999) carried experiment on genetic analysis and character association for cocoon yield and its components in silkworm (*B. mori* L.) and reported that phenotypic correlation coefficients were lesser in magnitude than the corresponding genotypic correlation coefficients for fourteen metric traits in heterozygous populations of *B. mori* L. Jayaswal *et al.* (2000) reported that larval weight, cocoon weight, shell ratio and filament length had highly positive significant correlation with single shell weight. Positive and significant correlation was also found between fecundity and cocoon yield by number, fecundity and larval weight, fecundity and single cocoon weight. Kumar *et al.* (2000) identified RFLP markers closely linked to cocoon shell ratio, which primarily determines the silk yield. The results showed the prospects of using RFLP markers as molecular tags for directional breeding in silkworm for improving cocoon shell character to maximize the silk yield. Kumaresan *et al.* (2000) [2]. reported that the correlation between single cocoon weight and single shell weight as well as single shell weight and shell ratio were highly significant. The filament length showed highly significant and positive correlation with four economic characters.

Miao (2002) in a study on the activity of alkaline phosphatase in the mid-gut of infected silkworm, *B. mori* L. reported that the activity varied greatly among tested strains and that there was positive correlation between alkaline phosphatase activity and cocoon weight, shell weight and shell ratio. Ram and Lal (2002) in a study on α-esterase isozyme activity and its relation to yield performance in silkworm, *B. mori* L. revealed a close parallelism between morphological traits and biochemical parameters. He studied a positive and highly significant correlation between the enzyme activity and yield contributing characters such as larval weight, cocoon weight, shell weight, fecundity and cocoon yield. Chatterjee and Mohandas (2003) identified eight ISSR markers generated by six primers and concluded that certain markers have a significant association for a number of parameters, whereas, others were selected for only one yield variable. Farooq *et al.* (2003) studied the association of ISSR markers with yield parameters (yield by weight, yield by number, cocoon weight, egg number, larval weight and shell weight) in thirteen diverse strains of silkworm. Gaviria *et al.* (2006) studied 11 Chinese and 12 Japanese silkworm strains for association between AFLP markers and productivity characters. The analysis permitted the identification of molecular markers associated with cocoon weight or shell weight separately. Some markers were associated with both characters. Ahsan and Rahman (2008) studied genotypic variability and correlation coefficients in thirty five hybrid populations of mulberry silkworm, *Bombyx mori* L. for five egg characters (hatching percentage, total number of eggs laid, blue egg percentage, unfertilized egg percentage and dead egg percentage). Hatching percentage was found to have negative correlation with unfertilized egg percentage and blue egg percentage while, blue egg percentage and unfertilized egg percentage were positively correlated to each other.

Genetic diversity in silkworm using molecular markers

Williams *et al.* (1990) reported that RAPD markers are well suited to genetic mapping, for plant and animal breeding applications, and for DNA fingerprinting studies. Nagaraja and Nagaraju (1995) used random amplified polymorphic DNA (RAPD) technique to study DNA profiling of thirteen silkworm genotypes of tropical and temperate regions including six diapausing (HU_{204}, KA, NB_1, NB_7, NB_{18}, NB_4D_2) and seven non-diapausing genotypes (C.nichi, Gungnong, Moria, Nistari, Pure Mysore, Diazo and Surapat). Genotype-specific amplification products were obtained. The hierarchical clustering technique placed the silkworm genotypes into two distinct groups consisting of six diapausing and seven non-diapausing genotypes. The genotype, Pure Mysore was found to be the most divergent genotype among the genotypes studied. The results of this study suggest that the RAPD technique could be used as a

powerful tool to generate genetic markers that are linked to traits of interest in the silkworm.

Nagaraju *et al.* (1995) used banded krait minor satellite DNA Bkm-2(8) as a probe to reveal polymorphism in different silkworm ecotypes including six diapausing and seven non-diapausing genotypes and reported that, although polymorphism level was relatively lower than the SSR-anchored PCR, yet Bkm probe revealed very clear fingerprint profiles as such is reliable to estimate genetic diversity of silkworm germplasm based on DNA fingerprint information. Nagaraju and Singh (1997) carried out DNA fingerprinting assays on silkworms of diverse origin based on random amplified polymorphic DNA (RAPD) and banded krait minor satellite DNA (Bkm) 2(8) multilocus probes and successfully characterized the diverse silkworm genotypes at their DNA level. They reported that RAPD assay was reliable and gave consistent results in grouping the genotypes. They further highlighted that DNA fingerprinting should be considered a worthwhile approach for studying genetic diversity in silkworm genotypes. Using RAPD-PCR technique, Thananata (1997) studied differentiation and identification in five morphologically distinguished silkworm *B. mori* strains including three indigenous Thai multivoltine types namely Nanglueng, Nanglai indigenous, S_5 and two improved bivoltine strains, namely KUl05 and KUl06 of Chinese and Japanese origin respectively. The author reported that RAPD-PCR technique is an efficient tool in silkworm differentiation and identification studies which could separate five morphologically different races into diapausing and non-diapausing varieties.

While estimating the genetic relatedness within and among different silkworm verities including six diapausing (Hu_{204},KA, NB_1, NB_7, NB_4D2 and NB_{18}) and seven nondiapausing (C. nichi, Gungnong, Moria, Nistari, Pure Mysore, Diazo and Sarupat) strains using Bkm-derived probe, Sharma *et al.* (1999) observed a high degree of genetic similarity within each variety studied. The results of this study demonstrated that variability of DNA fingerprints within and among silkworms which would help in producing potentially heterotic hybrids. Based on microsatellite markers, Reddy *et al.* (1999*a*) carried out genetic diversity studies in 13 silkworm strains of diverse origin reported that microsatellite-based analysis reflected the geographical pedigree and morphological relations of strains used in this study. While analyzing the fingerprints of diverse silkworm stocks including six diapausing (HU204, KA, NB1, NB18, and NB4D2) and seven non-diapausing (C. nichi, Moria, Nistari, Pure Mysore, Diazo, Gungnong and Sarupat) strains using SSR-anchored PCR Reddy *et al.* (1999*b*) established that ISSR-anchored PCR method is potentially useful for genetic fingerprinting of silkworm genotypes and as a mapping tool in the silkworm.

Nagaraju *et al.* (2001) while comparing the multi-locus RFLP's and PCR-based marker systems for genetic analysis of the thirteen diverse silkworm *B. mori* L. stocks reported that RAPD, ISSR-PCR and RFLP assay clearly separated the diapausing and non-diapausing silkworm varieties. Balvasi (2003) used inter simple sequence repeat (ISSR) markers to characterize eleven populations of silkworm *Bombyx mori* L. from the silkworm germplasm bank of Iran and reported that ISSR technique is efficient in studying the genetic divergence among silkworm stocks. Chatterjee and Tanushree (2004) carried out molecular profiling of Silkworm biodiversity in India and assessed molecular diversity among *Anthereaemylitta*, *A. assama*, *A. pernyi*, *A. proylei*, *A. roylei* and *Philosamiacynthia* with 11 ISSR and 8 non-random primers on agarose gel and established closest relationship between *A. pernyi* and *A. proylei*. Chatterjee *et al.* (2004) carried out ISSR Profiling of genetic variability in the ecotypes of tropical Tasar silkworm, *Antheraea mylitta* Drury. The analysis identified nine markers, which can be utilized to characterize specific population and will be of help to follow the ongoing genetic changes triggered by various ecological factors and human influences on the Railey ecotype. The study, further, substantiated the ability of ISSR finger printing to differentiate closely related individuals with reasonably higher fidelity as the 12 selected ISSR primers have generated very high degree of polymorphism among the different ecotypes and also among populations of the Railey ecotype. Srivastava *et al.* (2005) carried out genetic analysis of twenty stocks of silkworm (*B. mori* L.) through RAPD markers and reported that certain silkworm stocks like Kalimpong-A, C.nichi, Nistari and Mysore Princess were having higher genetic distance from others and thus could be used for heterosis breeding and also in breeding programmes aimed at introgressing hardy genes into the bivoltine high yielding stocks through marker assisted selection.

Li *et al.* (2005) estimated the genetic relationship among thirty-one diverse silkworm (*B. mori* L.) strains with SSR markers and reported that SSR markers are an efficient tool for fingerprinting cultivars and conducting genetic diversity in silkworm. Prasad *et al.* (2005) carried out survey and analysis of the silkworm, *B. mori* based on microsatellite markers. The microsatellite map generated using these polymorphic markers resulted in 8 linkage groups. *B. mori* microsatellites were the most conserved in its immediate ancestor, *Bombyx mandarina* followed by the wild Saturniid silk moth, *Anthereae assama*. The results of this study provide an opportunity to use SSR markers for investigating the wide range of genetic diversity that exists in the wild species outside the gene pool of domesticated silkworm, *B. mori*. The investigation of Li *et al.* (2006) revealed considerable genetic diversity among silkworm stocks preserved in germplasm by SSR markers and reported that SSR markers are appropriate in undertaking diversity studies and in characterizing large number of silkworm stocks with unique morphological characters for further breeding programmes. Mahendran *et al.* (2006) studied genetic variability and phylogenetic relationship among the different ecoraces of tropical tasar silkworm, *Antherea mylitta.* D (Andhra Local, Bhandara Local, Daba, Modal, Nalia, Raily, Sarihan, Sukinda, Tera) using RFLF technique and reported that phylogenetic relationships of different ecoraces obtained on the basis of RFLP pattern support the phenotypic and geographical relations. Hou *et al.* (2006) used Thirty-five SSRmarkers to conduct fingerprint analysis in introduced germplasm resources. Their finding revealed that all the SSRmarkers were polymorphic and unambiguously separated silkworm strains from each other. A total of 467 alleles were detected with a mean value of 13.34 alleles/locus (range 3-28). The mean polymorphism index content (PIC) was 0.71 (range 0.299-0.919). UPGMA cluster analysis of Nei's genetic distance grouped silkworm strains on the basis of their origin. The results indicated that SSRmarkers are efficient tools for fingerprinting cultivars and conducting genetic diversity studies in the silkworm.

Vijayan *et al.* (2006) used inter simple sequence repeat (ISSR) system to assess genetic diversity and differentiation among six commercially exploited eri silkworm *Samia Cynthia ricini* populations (Nongpoh, Borduar, Titabar,

Dhanubhanga, Khanapara and Mendipathar). Twenty ISSR primers produced 87% of inter population variability among the six populations. Regression analysis showed positive correlation between genetic distance and geographic distance among the populations studied. In a study, Huang *et al.* (2006) obtained 11 cleaved amplified polymorphic sequence (CAPS) markers to assess the genetic diversity among 29 strains of *Bombyx mori* from China, Japan and Europe. Cluster analysis, based on the genetic similarities calculated from CAPS data, grouped these strains roughly according to their geographical origin. One group contained silkworm strains from Europe and some of the Japanese strains were interspersed into the Chinese groups, whereas other Japanese strains clustered together. Li *et al.* (2007) while analyzing genetic relationship among different ecotypes of silkworm strains using ISSR markers observed that univoltine, bivoltine and multivoltine strains clustered separately based on their origin. The results of this study revealed that ISSR amplification is a valuable method for determining genetic variability among silkworm varieties and is an efficient technique for characterizing the large number of silkworm strains held in national and international germplasm centers.

Hou *et al.* (2007) while conducting analysis of SSR fingerprinting in introduced silkworm germplasm found that RAPD based dendogram resulted in a clear separation of two groups, one comprising of diapausing and other comprising of non-diapausing genotypes. Among the diapausing genotypes, all the "Chinese type" genotypes which spin oval cocoons grouped separately, while the "Japanese type" genotypes which spin peanut shaped cocoons were found in another group. Further genotypes, which share the same geographical origin, were grouped in the same cluster. Dalirsefat *et al.* (2007) estimated the genetic diversity in three Iranian native silkworm *B. mori* strains (Khorasan Lemon, Khorasan Orange and Khorasan Pink) and three commercial Japanese lines (P31, P103 and P107) using AFLP markers. The study revealed two distinct groups as Khorasan native (Iran) and Japanese lines and also high genetic diversity (75.4%) between the strains. Mirhosseini *et al.* (2007) studied the genetic relationship within and among Iranian native silkworm strains determined by DNA fingerprinting by using AFLP markers. The study revealed that the variability of DNA fingerprints within and among silkworm strains could provide an essential basis for breeders in planning cross breeding strategies to produce potentially heterotic hybrids in addition to contributing in conservation programmes. Salhei *et al.* (2008) assessed genetic diversity among three Iranian Silkworm lines using the AFLP markers. Considerable genetic diversity was reported with maximum and minimum polymorphism observed in Lines 103 and 31, respectively. The study revealed that AFLP markers established genetic diversity efficiently among the silkworm lines hence these markers can be used in assessing genetic diversity and phylogenetic relatedness in vast germplasm resources. In a study carried out by Velu *et al.* (2008), mutant silkworm stocks were evaluated to determine their genetic relationship using ISSR Primers. The dendogram produced using the Unweight Pair Group Method with Arithmetic Mean (UPGMA) and Cluster Analysis resulted in the formation of one major cluster and six sub-clusters and separated 20 mutant silkworm strains belonging to the same origin and similar voltinism. The findings of this study revealed considerable polymorphism among strains and indicate that ISSR markers can be efficiently utilized to analyze phylogenetic relationships and heterozygosity in silkworm.

Awasthi *et al.* (2008) evaluated bivoltine, polyvoltine and mutant silkworm (*Bombyx mori* L.) with RAPD, ISSR and RFLP-STS markers and reported that on clustering with UPGMA and PCA, RAPD and ISSR markers clearly discriminated the bivoltine and multivoltine and a multivoltine Tamil Nadu white occupied the position among the bivoltine, since it has bivoltine parentage. Boropolu, an original land race from North-East, India and Feng Shang, a Chinese silkworm strain also showed a close genetic relationship. Arunkumar *et al.* (2009) developed microsatellite markers for the Indian golden silkmoth, *Anthereae assama* and reported thatthese microsatellite markers will be useful resources for population genetic studies of *A. assama* and other closely related species of saturniids. An investigation was carried out by Furdui*et al.* (2009) on RAPD profiles of six Romanian silkworm races (AB, IBV, B1, RG_{90}, AC_{29}-T and AC-T) and two hybrids viz; AC-T x S_8XAC $_{29}$-T; AC-T xAC_{29}-T. The amplification products resulted by RAPD markers pointed out significant differences between strains and hybrids at genotype level. The study proved that RAPD technique can be successfully applied for characterization of *B. mori* genotypes. Malik *et al.* (2009) reported that molecular markers, namely, RAPD, RFLP, ISSR, and SSR, have been effectively utilized in analyzing the genetic diversity and phylogenetic relatedness in the domesticated silkworm *B. mori*. Reddy *et al.* (2009) while exploring the genetic variability in multivoltine and bivoltine silkwormswith molecular markers reported that there was distinct grouping between the multivoltine and bivoltine races when grouped with marker generated by ISSR-PCR and the 2D diagram of the Principle Component Analysis (PCA) of the marker generated by the different ISSR primers helped to visualize the two major clusters which included the multivoltine and bivoltine separately.

Using Inter-Simple Sequence Repeat (ISSR) markers, Srivastava *et al.* (2009) assessed genetic diversity in different populations of eco-races of Indian Tasar silkworm, *Anthereae mylitta* Drury. All the primers exhibited polymorphism which is an indicative of the genetic variation in individual Raily silkworm. Among the populations, total polymorphism recorded was 76%. Nei's gene diversity (h) ranged from 0.194 to 0.337 exhibiting high heterozygosity. Relevance of this study is of high significance in formulating conservation strategies and sustainable utilization of the economically important Railyecorace of *Antherea mylitta*. Erogolu and Alica (2009) analyzed three Turkish silkworm breeds viz; (Bursa Beyazı, Alaca and HataySarısı) by RAPD-PCR method. Out of 23 arbitrarily primers five primers revealed 34 polymorphic loci. One of the primers OPA-02 (800bp) was observed in HataySarısı which is a local nondiapausing genotype of Turkey which reflects that these markers can easily discriminate between diapausing and non diapausing silkworm varieties. The results revealed that such DNA markers can be potentially utilized in improvement programmes of silkworm strains in future besides applying such molecular techniques could reveal more genetic information for characterization of silkworms. Vijayan *et al.* (2010) assessed genetic diversity among 13 tropical non-diapausing strains of silkworm, *B. mori* stocks collected from different tropical sericultural zones in West Bengal, Assam, Karnataka, Bangladesh and China. Using SSR and mtDNA-SSCP markers, the study revealed considerable genetic diversity among 13 silkworm genotypes that were developed in, India, China and Bangladesh. Based on the phenotypic and genetic analysis, BL_2, KollegalJawan and Mysore princess

were identified to have high potential to be used as parents for developing high yielding summer specific strains for commercial exploitation.

Akir *et al.* (2010) carried out molecular analysis of Turkish silkworm breeds (Alaca, Bursa Beyazı and HataySarisi) by RAPD-PCR method and reported that diapausing and non-diapausing local breeds were separated successfully and the percentage of Polymorphic loci were determined higher in non-diapausing breeds and lower in diapausing breeds. It was further reported that the results obtained from the study can be used for the improvement programs of Turkish silkworm breeds in future. A comparative assessment of genetic diversity and genetic structure based on RAPD markers for three silkworm species viz; *B. mori* L., *Anthereae pernyi* and *Samia Cynthia ricini was* done by Liu *et al.* (2010). The study showed highest levels of genetic diversity between *Anthereae pernyi* and *B. mori* at the species level. However, at the strain level, *Anthereae pernyi* had relatively highest genetic diversity and *B. mori* had lowest genetic diversity.

In an investigation, Kim *et al.* (2010) typed 54 silkworm strains for nine polymorphic microsatellite loci. A total of 17 strain-specific isomorphic alleles, which discriminated 14 among 54 silkworm strains, were obtained from eight loci. The study suggested that these strain-specific alleles can be casually utilized to discriminate between applicable strains without any further typing of other loci. Furthermore, a substantial number of homozygote strains, represented by 24 among 67 alleles in nine loci, were also detected. These results collectively implicate silkworm microsatellite DNA as useful and potentially important molecular markers for the eventual discrimination of silkworm strains preserved in silkworm germplasm centers.

To gain better understanding on genotyping in nutritionally efficient silkworm breeds/hybrids, Ramesha *et al.* (2010) carried out nutri-genomic analysis of mulberry silkworm (*B. mori* L.) strains using PCR-SSR technique and reported that a single yet varying size amplified band in all four parental silkworm strains (RMG_4,RMW_2,RBD_1 and RBO_2) and two clear bands in the hybrids (RMG_1 x RBD_1 and RMW_2 x RBO_2) with different molecular weight from three PCR-SSR primer loci *viz*; F_{11139}, F_{10429} and F_{110705}. The PCR-SSR results demonstrated homozygosity in newly evaluated nutritionally efficient parental silkworm strains and heterozygosity in hybrid and confirm their being nutritionally efficient with superior nutrigenomic traits. Jain *et al.* (2010) reviewed RAPD marker system in insects and revealed that RAPD markers are the most common yardsticks for measuring genetic differences between individuals, within and between related species or population. These markers are well suited for genetic mapping, plant and animal breeding application and DNA fingerprinting, with particular utility for studies on population genetics and provide an efficient assay for polymorphism which allows rapid identification and isolation of chromosome specific DNA fragments. Neog *et al.* (2010), studied analysis of genetic diversity of Muga silkworm (*Anthereae assamensis*, Helfer; Lepidoptera; Saturniidae) using RAPD-based molecular markers and reported that although there lies little morphological differences among the collected Muga silkworm populations, the populations are highly polymorphic which might have enabled the silkworm to survive under restricted geographical location i.e. North-East region of India only but under adverse climatic conditions for a longer period. Reddy *et al.* (2010) carried out an investigation to analyze the diversity in different silkworm races using Inter Simple Sequence Repeat (ISSR) molecular marker for the selection of diverse parents. All the primers exhibited 100 per cent polymorphism across 30 silkworm races analyzed. The similarity coefficients ranged from 0.33 to 1.00. Of the pair wise combinations, NB7 and Kollegal Jawan showed the lowest similarity index (0.33), whereas the highest similarity index was recorded between *C. nichi* and P_4D_3 (1.00), followed by CSR_3 and CSR_2xCSR_4 (0.98). The PCA analysis of the markers generated by the different ISSR primers helped to visualize the two major clusters which included the multivoltine and bivoltines separately. The grouping of bivoltines in the PCA analysis clearly showed higher similarity among bivoltines as compared to the multivoltine and the combining ability of Pure Mysore was reported excellent than bivoltine races for hybridization and owing to its high amylase activity, it was selected as donor parent for the F_1 cross while as CSR_2 was selected as the other parent, owing to its better qualitative and quantitative parameters.

The study of Murugesh *et al.* (2010) revealed 100 percent polymorphism across 30 silkworm races analyzed with ISSR markers. The similarity coefficients ranged from 0.33 to 1.00. Of the pair wise combinations, NB7 and Kollegal Jawan showed the lowest similarity index (0.33), whereas the highest similarity index was recorded between C.nichi and P4D3 (1.00), followed by CSR_3 and CSR_2xCSR_4 (0.98). The mean similarity index was 0.68. There was distinct grouping between the multivoltine and bivoltine races when grouped with marker generated by ISSR PCR. The 2D diagram of the PCA analysis of the markers generated by the different ISSR primers helped to visualize the two major clusters which included the multivoltines and bivoltines separately. The grouping of bivoltines in the PCA analysis clearly showed higher similarity among bivoltines as compared to the multivoltines. Superiority over the parents (Pure Mysore and CSR_2) with regard to biological parameters was found with F_1. Talebi *et al.* (2011) studied RAPD markers for understanding the genetic variability among the four silkworm races namely $C_{108,}$ NB_4D_2, Pure Mysore, Nistari and their hybrids and reported that the lowest percentage of polymorphism (0.0%) was observed when amplified by OPA-01 and OPA-02. The cluster analysis based on UPGMA (unweighted pair group method with arithmetic mean) method revealed that dissimilarity index values has generated the dendogram separating the bivoltine and multivoltine races into two groups and has revealed that Pure Mysore was further away from the group. Renuka and Kamble (2011) carried out RFLP analysis among ten bivoltine silkworm genotypes. The restriction fragment length polymorphism analysis revealed polymorphic patterns among these races. These polymorphic bands were further analyzed through Wards method which placed high silk yielding genotypes together in one cluster whereas the low silk yielding genotypes formed a unique cluster. The results of the study revealed considerable genetic polymorphism among the silkworm stockscharacterized and restriction pattern grouped silkworm stocks based on silk yield.

Singh *et al.* (2011) investigated the RAPD profile of isolated DNA from eleven morphologically distinct strains of muga silkworm *Antherea assama* and reported that RAPD technique is sensitive enough to detect differences and high degree of polymorphism between strains of muga silkworms in which differentiation is not always possible morphologically. Their study further revealed clear domestication between the wild and domesticated muga silkworm strains based on the dendogram generated.

Srivastava *et al.* (2011) used Inter simple sequence repeats (ISSR) and random amplified DNA polymorphism (RAPD) markers to estimate the genetic diversity among 14 tropical silkworm races (*B. mori* L.) for identifying potential parents to be used for hybrid preparation for commercial exploitation. High polymorphism (70.91 and 74.70%) was revealed by ISSR and RAPD markers. The dendrogram generated, using unweighted pair group method using arithmetic average from these markers, grouped the 14 silkworm races into two major groups, which corroborates the differences in cocoon characteristics.

Pradeep *et al.* (2011) carried out genetic analysis of scattered populations of Indian Eri silkworm, *Samia Cynthia ricini by* SSR markers and found 98% inter-population and 23-58% intra-population polymorphism. It was inferred from the study that the genetic distances among the eri populations increased with the increase in altitude and geographical distances. They further reported that deforestation and exploitation has led to the fragmentation of habitats and scattering of populations of the economically important eri silkworms in North East India. Further, Patchy habitats prompted low genetic variability, high linkage disequilibrium and colonization by new sub-populations. In an investigation to analyze genetic diversity and phylogenetic relationship among pure line and hybrids of Romanian silkworm *B. mori* stocks, Furdui *et al.* (2011) amplified the DNA profiles of one pure line (S_8) and 8 hybrids (S_8 x AC_{29}-T, AC_{29}-T x S_8, AC x B_1, B_1 x AC, $Hesa_1$ x $Svila_2$, B_1 x $Svila_2$, B_1 x $Hesa_2$, $Vratza_{35}$ x $Svila_2$) with 35 highly polymorphic RAPD primers. The results of the studies revealed that out of 35 markers 21 markers generated polymorphic bands that were used to analyze genetic phylogeny and diversity. Further, a total of 921 polymorphic bands were detected and UPGMA cluster analysis grouped silkworm strains on the basis of their origin, obtaining a dendogram reflecting their genetic relationship. Jinghade *et al.* (2011) while reviewing the implications of heterozygosity and inbreeding on germplasm biodiversity and its conservation in the silkworm, *B. mori L.* reported that application of molecular markers help to estimate genetic diversity much more accurately than that of morphological traits, since a minimal amount of heterozygosity in each silkworm strain is essential for better conservation by avoiding inbreeding depression. Furthermore,the use of DNA markers to relate genetic homozygosity and genetic distance of the parental strains to the manifestation of hybrid vigor, and understanding the genetic basis of heterosis, will be quite useful to select suitable parental lines for hybridization programs.

Singh *et al.* (2011*a*) characterized four strains of eri silkworm *Samia Cynthia ricini* (Yellow, Yellow spotted, Green and Green spotted) based on their DNA by RAPD technique. Eight random primers and one universal primer used for RAPD analysis generated a total of 79 bands, of which 49 were polymorphic. The RAPD analysis grouped Yellow spotted and Green in cluster-I and Green spotted and Yellow in cluster-II. The range of genetic diversity observed among the strains affirms the potentiality of RAPD technique for identification and selection of distant parents for silkworm hybridization for high silk yield. Singh *et al.* (2011*b*) while studying the RAPD profile of eleven morphologically distinct strains of muga silkworm *A. assama* (Mangaldoi, $Tura_1$, Jorhat, $Tura_2$, $Tura_3$, $Tura_4$, $Tura_5$, Lahing, $Mokokchung_1$, $Mokokchung_2$ and Baghmara) reported that among the eleven muga silkworm strains, three high yielding strains were genetically different from the remaining strains. Utilizing ISSR molecular markers, Radjabi *et al.* (2012) studied the interspecific biodiversity of Iranian local races of silkworm *B. mori* (Kh-lemon, Ha-lemon, Ha-white, Ha-yellow, Kh-pink, Baghdadi, Gi-orange and Kh-orange). Results of their study revealed that 5 Primers yielded 81 scorable bands with fragment size between 250-300 base pairs. Maximum of the amplified bands showed in UBC807 primer which has maximum percentage of polymorphism among primers. The outcome of the study highlighted that ISSR markers have high reproducibility in screening many polymorphisms in a single assay hence; this technique is suitable for large scale screening of silkworm germplasm.

Estimating the genetic variability with microsatellite markers among six populations of Indian golden Saturniid silk moth (*Anthereae assama*), Arunkumar *et al.* (2012) observed highly significant diversity in one of the populations and reported that *Anthereae assama* inhibiting the WWS-region was genetically divergent from the five populations studied. He also reported that this study laid base for further investigation to identify and study such populations from muga silkworms as well as other habitat to generate information useful in conservation of dwindling muga culture in North East India. Devi *et al.* (2012) subjected six populations of *Anthereae proyeli* and *Anthereae frithi* more to Inter Simple Sequence Repeat (ISSR) marker analysis and found very high polymorphism among the breeds and reported that ISSR markers are suitable to study intra and inter specific variation in this group of insects. They concluded that the findings made in this study are of much importance for germplasm conservation as well as breeding of these economically important insects since, the study revealed phylogenetic relationship among *Anthereae proyeli* and its breeds.

The genetic diversity and phylogenetic relationships of 150 individual eri silkworm *Philosamia ricini* ecoraces were studied by Pragyan and Rao (2012) using RAPD analysis. The polymorphisms within and between ecoraces were assayed using 60 random primers and182 loci were amplified ranging from 350bp to 2500bp. The percentage of polymorphic loci was found to be 35.22, 17.3 and 16.2%, for Borduar, Mendipathar and Titabarecoraces respectively. Genetically, the Borduarecorace was more closely related to Mendipatharecorace than to the Titabarecorace. The authors reported that genetically distinct genotypes identified in the present study could be potential sources of germplasm for eri silkworm improvement. To point out the genetic similarity and phylogenetic relationships among the strains of Romanian silkworm genotypes Vlaic *et al.* (2012) analyzed four local breeds (AB, B75, S8, B1) and four local hybrids (AC_{29}-T x S_8, AC x B_1, S_8 x AC_{29}-T, B_1 x AC) through RAPD-PCR technique and observed a high genetic diversity among the studied silkworms groups. Genetic distances estimated by Nei and Li method varied from 0.28889 to 0.92437 with an average of 0.58497. The amplification products resulted from the RAPD assay varied between strains. The outcome of this study suggested that RAPD markers can be used as genetic markers to construct linkage maps. Singh *et al.* (2012) used molecular markers based on PCR of Inter-simple sequence repeats (ISSR) and simple sequence repeats (SSR) with four populations of wild insects and eleven populations of cultivated insects. They reported that wild populations had high genetic diversity estimates (Hi = 0.25; HS = 0.28; HE = 0.42) and at least one population contained private alleles. The results of this investigation indicated that genetic variability within populations examined was significantly high.

Shivashankar *et al.* (2013) while conducting genetic diversity analysis of six populations of eri silkworm*Samia Cynthia ricini* (Yellow plain, Yellow spotted, Yellow Zebra, Blue Green Plain, Blue Green spotted and Blue Green Zebra) by RAPD markers observed low genetic distance between the eri silkworm populations and reported that the range of genetic diversity and low genetic distance between the Eri silkworm populations is attributed to restricted environmental acclimatization. He further reported that RAPD technique is sensitive enough to detect differences between accessions of Eri silkworms were differentiation is not always possible morphologically. Jagadeesh Kumar (2013) studied molecular dynamics of genomic DNA in bivoltine silkworms namely CSR2, CSR4, KA, NB4D2 and JROP and reported low level of genetic distance between the breeds on the basis of gene frequency evidenced by the boot strap values in the constructed dendrogram with the help of molecular ISSR markers. RAPD marker-based diversity analysis was undertaken by Moorthy *et al.* (2013) to evaluate genetic relationships among 20 silkworm breeds collected from different sericulture research Institutes of India. Selected 40 primers generated 402 bands, out of which 302 were found to be polymorphic with an average of 10.05 fragments per primer. UPGMA cluster analysis grouped the genotypes into two distinct clusters, one with polyvoltine and another with bivoltine breeds with an average genetic distance of 0.237. Further, bivoltines were sub grouped into two, those with high silk content were in one group and those with moderate silk contents were in other group. The overall grouping pattern of clustering corresponds well with principal component analysis confirming patterns of genetic diversity observed among the genotypes. The result of this study provided valid information on differentiation of silkworm breeds based on voltinism and silk content. This result may help to identify suitable parents for their use in the yield improvement programme and effective and efficient measures for germplasm conservation.

Pereira *et al.* (2013) utilized RAPD technique to verify genetic polymorphism and divergence as well as susceptibility to *B. mori* Nucleopolyhedrosis virus (BmNPV) and reported that there is marked difference in diverse characteristics including geographical origin, body weight, larval span, cocoon weight and other biological traits. Bakappa and Subramanaya (2014) used ISSR markers to understand the genetic polymorphism and phylogenetic relationships among the eighteen silkworm races/breeds comprising six each of bivoltines (C_{108}, KA, NB_4D_2, CSR_2, P_{31} and NB_{18}, multivoltines (Pure Mysore, Nistari, C.nichi, MU_1, *npnd*and Hosa Mysore) and autosomal mutants (zebra, ursa, knobbed, pere, lemon and pre). They reported that marker profiles clearly discriminated the bivoltines whereas multivoltines and an autosomal mutant namely *pre* and a bivoltine race namely C_{108} were genetically inter-linked together in cluster-I and II respectively. The results indicated high efficacy of ISSR markers in genetic diversity studies. Genetic relationships among ten silkworm strains comprising of five each of bivoltine (CSR2, CSR50, CSR51, BHR3 and SK4C) and polyvoltine (Pure Mysore, ND7, Nistari, Cambodge and L14) were investigated by Chandrakanth *et al.* (2014). 15 SSR markers generated 54 alleles with an average of 3.6 alleles per locus. The silkworm strain CSR_{51} had 3 specific alleles, SK4C had 2 specific alleles and Pure Mysore, Cambodge, L14, CSR_2, CSR_{50} and BHR3 each had one specific allele. The bands produced by FI0619 and LFL0944 markers were specific for voltinism character. The dendrogram generated by UPGMA based on 15 microsatellite loci differentiated silkworm strains into two major groups. Group I consisted of bivoltines and group II contained polyvoltines. Further, the bivoltine group was subdivided based on cocoon shape and polyvoltine group was subdivided based on the cocoon colour spun by the silkworm strains. The authors reported that this study was also able to identify divergent parent which can be used in further breeding programme.

Paramanik and Chikkaswamy (2015) analyzed seventeen diverse strains of silkworm (*B. mori*) using RAPD markers. Differential polymorphism was noted among ten silkworm varieties showing variation in percentage of polymorphic bands from 20 to 80%. Some of the DNA fragments were strain specific and some could differentiate the multivoltine from the bivoltine strains. The observed high proportion of polymorphic loci revealed profound variation among the silkworm varieties. The results demonstrate that DNA content and RAPD markers provide an effective tool for detection and evaluation of genetic variation existing among silkworm varieties.

Correlation studies among different quantitative traits of silkworm, *bombyx mori* l.

The silkworm, *B. mori* L., most important source of natural silk has played an important role in the life of man ever since its discovery by the Chinese some 4000 years ago. It is also traditionally associated with the socio-economic life of many countries. Even today it enjoys the supremacy over all other fabrics and it is appropriately called “Queen of Textiles” for its unparallel lustre, delicacy, elasticity, crispness and artistic appearance. According to Krishnaswami *et al*. (1973), the mulberry silkworm, *B. mori* L., is the greatest source of natural silk. Generally we have two type of silkworm, viz. mulberry and non-mulberry silkworm. The silkworm larva passes trough five different stages called instars. The final or 5th instars spin to form cocoon within which the larva undergoes pupation. The cocoon shell is the source of the natural silk, which is the driest form of that secretion. Basically there are two proteins which go to form the silk fibre viz., “fibroin” which constitutes the core of the fibre and “sericin” a waxy substance which encases the fibroin. The origin of sericulture, silk production and weaving is ancient and clouded with legend. They are closely interlinked with the emergence of China as one of the great civilizations. Chinese mythology ascribes the invention of this art to the mythical Empress Shih Xiling, the consort of the no less mythical “Yellow Emperor”, Shi Huang Ti, one of the fathers of Chinese civilization. Ancient Chinese annals give an exact date for Empress Xiling’s discovery of silkworm rearing, namely 2640 B.C. It is said that one day 14-year-old queen was enjoying a tea ceremony in her palace garden with her friends and maids under a mulberry tree, suddenly a golden coloured cocoon dropped in her teacup. When she tried to remove the cocoon from the teacup, an end of the filament comes out of the cocoon and it was a continuous one. Then she collected some more cocoons, carried them to her palace, preserved them till emergence and reared in the next generation and later invented the method of reeling (Sarkar, 1958). In reality, sericulture evolved through a gradual process and by the middle of the third millennium B.C. silk was in human use. China mastered this art well before any other civilization, and for nearly 5000 years China was believed to have been the sole possessor of the techniques of silk production. For many centuries the Chinese zealously guarded the source and methods of production of silk for

about 3000 years. According to legend, in about 140 B.C., sericulture as well as silk spread overland form China to India (Krishnaswami *et al*., 1973). Some sources believed that the first country after China to learn the secret of silk production was Korea where Chinese immigrant started sericulture in about 1200 B.C. The industry later spread to Japan and other countries. The transfer of silkworm and silk is also flooded fables where many stories have taught from mouth to mouth. The silk industry flourished in our sub-continent in the beginning of the 17thcentury. At that time Bengal was known as the store house of silk (Sarkar, 1958). A large quantity of silk and silk fabrics was exported from Bengal to various parts of Mughal Empires, to the neighbouring countries and to Europe.

On the other hand, others thought that the Hindus discovered it in the sub-Himalayan regions. The history of which was older than the Chinese (Sarkar, 1958). According to them B. mori was first domesticated at the foothills of Himalayas. Latter on silkworm rearing began to spread and established on the bank to the rivers Brahmaputra and the Ganges (Krishnaswami, *et al*., 1973). As the demand of silk expanded the industry set on spreading. The silk industry flourished in our territory in the beginning of the seventeenth century. At that time Bengal was known as the storehouse of silk (Sarkar, 1958). A large quantity of silk and silk fabrics was exported from Bengal to various parts of the Mughal Empire, to the neighbouring countries and to Europe. The lending silk producing countries are China, Japan, India, South Korea, U.S.S.R and Brazil and that is to say 97% of raw silk of the world is produced in these countries. During the middle of the eighteenth century as many as six varieties of raw silk and silk clothes were exported to Europe and to the markets of the West Asia including Basra, Mecca, Jedda, Pegu and Malacca. Silkworm is reared in Bangladesh particularly in the district of Rajshahi and Nawabganj where silkworm is cultivated extensively as a major source of earning for the rural people. Sericulture is well known as a highly employment and a low capital intensive activity ideally suited for the transfer of wealth from richer sections of the society to poorer sections since silk is consumed mostly by the affluent people and the money so spent on purchase of silk is distributed among the Seri culturists, reelers, twisters, weavers and traders. Persons from the age of 8 to 80 may engage themselves in the activity of silk production and earn their livelihood.

Mulberry silkworm is variously classified according to the difference in their physiological and ecological characteristic. According to the voltinism they are classified into univoltine, bivoltine and multivotine or polyvotine, while on the basis of moulting, they are classified into trimoulter, teramoulter, pentamoulter and rarely bio-moulter as well as hexamoulter. Furthermore, they are grouped into Japanese race, Chinese race, European race and Tropical race on the basis of place of origin and geographical distribution. Collection of varietal materials of *B. mori* L. and their evaluation under different environmental condition is a prerequisite for the improvement of the commercial characters. All of the Seri culturally advanced countries maintain different varieties of silkworm to serve the above objective. The different varieties of *B. mori* L. reared by the farmers in Bangladesh are mainly indigenous in origin. Therefore, these varieties are highly resistance but shows very poor silk yielding performance with respect to both the qualitative and quantitative traits. To overcome these difficulties and to boost up silk production, the sericulture authority of Bangladesh introduced exotic and developed high yielding multivoltine varieties of silkworm for commercial rearing (Rahman *et al*., 1978; Islam, 1980; Salam *et al*., 1980; Rahman, 1983; Rahman *et al*., 1984). Rearing of different varieties of silkworm is dependent on the environmental conditions like temperature, humidity, quality of mulberry leaves and population density in the rearing bed. Most of the varieties including indigenous, developed and exotic showed varied response to different environmental conditions (Sengupta, 1969; Sidhu *et al*., 1969; Petkov and Yolov, 1979; Biswas and Rahman, 1987; Rahman, 1995). Correlation studies were conducted under the influence of different environment by Hayes (1922), Immer and Ausemus (1931) and Kelly *et al*. (1932). In breeding work, knowledge of correlation between different characters is of paramount importance. Hazel (1943) first demonstrated the estimates of genetic correlation. The genetic correlation might be due to three different causes (i) Pleiotrophy (ii) Linkage and (iii) Heterozygosity (Reeve 1953; Tallis, 1959; Mode and Robinson, 1959; Abe, 1969; Grossman 1970; Hammond and Nicholas 1972).

A series of studies on correlations between various traits were available in many insect groups. Phenotypic and genotypic correlations of egg production and longevity in wild out-bred stocks of D.melanogaster and D. simulaus had been worked out by Tantawy and EL-Helw (1966), Tantawy and Rakha (1964), Rose and Worth (1981), Giesel *et al*. (1982), Rose (1984), Roff and Mousseau (1987). Collins *et al*. (1984) worked on the correlation for several characters in the honey bee. In non-mulberry silkworms, significant positive correlation between pupal weight and fecundity were shown by Miller *et al*. (1982) inA. polyphemus by Singh and Prasad (1987) and in P. ricini by Kotikal *et al*. (1989). The correlation regression between pupal weight and fecundity in muga silkworm were studied by Barah and Sengupta (1991). In *A. mylitta*, Sen *et al*. (1976) reported highly positive and significant correlation between cocoon weight and shell weight (r=0.86), Siddiqui *et al*. (1985) reported positive and significant correlation between shell weight and cocoon weight; shell weight and larval weight; shell weight and moth weight, both at phenotypic and genotypic level. Siddiqui *et al*. (1989) reported positive and significant correlation between fecundity and cocoon weight, fecundity and silk yield, cocoon weight and shell weight and cocoon weight at phenotypic level and genotypic level in a half diallel populations of *A. mylitta*. References are also available on correlation studies between various quantitative characters in *B. mori* L. Legay (1961) reported correlation between various quantitative characters. Nataraj and Ganesh (1969) worked on the inter relationship between cocoon weight and shell weight in different breeds. Samachari and Krishnaswami (1980) studied the correlation between cocoon weight and pupal weight, shell weight and egg weight. Petkov (1981a) observed negative phenotypic correlation between cocoon weight and silk richness. Petkov (1981b) reported high and positive correlation between cocoon and shell weight (r=0.679 and 0.681). Oshiki and Sato (1986) showed the relationship between egg size and manifestation of quantitative characters, while Long and Petkov (1987) studied the correlations among different quantitative characters. On the other hand, Ozdzenska and Kremky (1987) observed positive and significant genetic correlation between cocoon weight and silk richness. Rajanna and Sreeama (1990) studied the cocoon productivity traits such as cocoon weight, pupal weight shell weight and shell percentage on the basis of 5th instar 5th day larval weight in tropical multivoltine and bivoltine races. Jayaswal *et al*. (1991) and Shaheen*et al*. (1992) worked on the

correlation between female pupal weight and fecundity of *B. mori*. Giridhar *et al*. (1995) inferred from the study of correlation that cocoon weight, shell weight, filament length and denier could be considered as selection criteria for improvement of cocoon yield. It is evident from the above review that there is very meagre information available on correlation among different quantitative traits in respect of silkworm, B. mori varieties of Bangladesh. Therefore, an attempt was made to study the relationship exists among different cocoon and pupal characters of four verities of *B. mori* L. Nagaraja and Nagaraju (1995) studied the genome fingerprinting of diapausing and non-diapausing silkworm genotypes using random arbitrary primers and reported that RAPD technique could be used as a powerful tool to generate genetic markers that are linked to traits of interest in the silkworm.

Nagaraju and Singh (1997) assessed the genetic diversity of diapausing and non-diapausing silkworm (*B. mori* L.) by DNA profiling through RAPD markers and reported that RAPD analysis showed potential to become a viable tool for analysis of genetic variation among populations and for identifying molecular markers for economic trait of silkworm. Reddy *et al.* (1999*a*) analyzed 28 microsatellite (SSR) loci and identified a marker, SAT-211, with specific alleles for strains with and without diapause. A very polymorphic marker, SAT-2763, was also identified with a total of 17 alleles and very high heterozygosity (90%). Kumar *et al.* (2000) identified RFLP markers closely linked to cocoon shell ratio which primarily determines the silk yield. The results showed the prospects of using RFLP markers as molecular tags for directional breeding in silkworm for improving cocoon shell character to maximize the silk yield. Restriction Fragment Length Polymorphism (RFLP) profiles of thirteen silkworm *B. mori* L. stocks subjected to correlation and multiple regression analysis resulted in the identification of nine specific markers associated with the expression of eleven yield components. The study indicated that RFLP markers are highly useful in molecular mapping, genotype characterization and marker assisted selection thus; create an opportunity of using germplasm stocks directly for isolating specific RFLP bands for their use in marker assisted selection (Sethuraman *et al.*, 2002). Farooq *et al.* (2003) have reported significant association of ISSR markers with yield parameters (yield by weight and number, cocoon weight, egg number, larval weight and shell weight) in thirteen diverse strains of silkworm. RAPD profiles generated with seven UBC primers for fourteen silkworm stocks, originated from China, Japan, India, and Russia, subjected to correlation and multiple regression analysis established significant association of 45 markers with larval span, growth indices and four cocoon yield parameters relevant for silk production and *t*-test attested the significance of the association of markers viz; 89.5_{1500bp} and 54.13_{300bp}, with longer larval duration and high cocoon weight respectively. The two indices of yield/growth were also tested with DFA, which helped in identifying a few markers and thereby opened scope of using such marker (e.g., 91.11_{900bp}) for incorporating molecular markers in the breeding program for crop improvement in silkworm (Chatterjee and Pradeep, 2003).

Polymorphic profiles of 147 markers generated with 12 ISSR primers across the genomic DNA of 20 silkworm stocks of diverse yield status subjected to correlation and multiple regression analysis led to the identification of 8 ISSR markers significantly associated with yield traits in silkworm (Chatterjee and Mohandas, 2003). The authors reported that DNA markers projecting a significant association with specific yield components need to be tested for their use in marker assisted breeding programmes for modifying the yield potential of silkworms. Pradeep *et al.* (2005) reported association of three polymorphic microsatellite simple sequence repeat (SSR) and inter simple sequence repeat (ISSR) viz; SAT-106, UBC-873_{1074}bp and UBC-873_{823}bp with larval duration in the longer larval duration silkworm (LLD) and could separate larval populations of Nistari strain based on the shortest larval duration (SLD) and the longest larval duration (LLD). RAPD and ISSR primers generated polymorphic profiles in LLD and SLD lines. Distinct markers specific to LLD individuals were observed from the 3rd generation and indicated selection induced differentiation of allelic variants for longer larval duration. This finding implies that selection combined with inbreeding could result in lines with different genetic properties following separation from the original parental populations. Li *et al.* (2006) carried out linkage and mapping analysis to the densonucleosis non-susceptible gene nsd-Z in the silkworm *Bombyx mori* using SSR markers. Their investigation revealed considerable genetic diversity among silkworm germplasm and reported seven SSR markers linked nsd-Z. Gaviria *et al.* (2006) studied 11 Chinese and 12 Japanese silkworm strains for association between AFLP markers and productivity characters. The correlation and multiple regression analysis projected the significant association of molecular markers with cocoon weight and shell weight separately. Some markers were found to be associated with both characters. However, Correlation analysis between the bands and productive characters showed both positive and negative association. The outcome of the study suggests that such markers might help in programs of marker assisted selection. Gaviria *et al.* (2006) and Srivastava *et al.* (2007) have reported that molecular markers having strong associations with cocoon traits are highly desirable. Once the molecular markers linked to desirable traits are identified, they could be introgressed into silkworms for higher productivity through molecular assisted breeding programmes.

Genomic DNA samples from fifteen multivoltine silkworm races were analyzed by Srivastava *et al.* (2007) for their thermo tolerance behavior using ISSR PCR technique. Stepwise multiple regression analysis (MRA) revealed a total of five bands (807_{1300}, 808_{3000}, 808_{4000}, 834_{4000}, and 834_{3000}) showing correlation with pupation rate after thermal treatment. Out of them, marker 808_{3000} showed maximum and highly significant correlation ($r = 0.757$, $p < 0.001$, $t = 4.182$) with pupation rate among the silkworm races. The identified putative markers are being used to develop DNA marker to be used in evolving thermo tolerant silkworm breeds using marker assisted selection programme. Pradeep *et al.* (2007) studied molecular markers for biomass traits, association, and genetic divergence in silkworm *Bombyx mori.* Correlation analysis (R = 0.9) revealed significant interrelation among biomass traits *viz*., larval duration (TLD), larval weight (LWT), cocoon weight (CWT), shell weight (SWT), shell ratio (SR) and floss content. PCR using inter simple sequence repeat (ISSR) primers revealed 92% polymorphism among 14 tropical and temperate strains of *B. mori*. Stepwise multiple regression analysis (MRA) selected 35 ISSR markers positively or negatively correlated with different biomass traits, illustrated polygenic control. ISSR marker 830.81_{bp} was significantly associated with LWT, CWT, SWT, SR and floss content, indicated its pleiotropic role. Two ISSR markers, 835.51_{bp} and 825.97_{bp} showed significant association with

floss content and TLD. The authors reported that identification of several potential markers that continue to develop genetic characteristics of silkworm population and reveal genetic divergence within and low yielding strains could have potential practical utility in prospective silkworm breeding programme.

Simple sequence repeats (SSR) and inters SSR (ISSR) marker systems were used to reveal genetic changes induced by artificial selection for short/long larval duration in the tropical strain Nistari of the silkworm *B. mori*. Significant associations of the SSR and ISSR loci with long larval duration were established in the F_2 generation. The inheritance of the polymorphic SSR and ISSR loci was tested in the F_2 population developed from a cross of long larval duration (LLD) female and (short larval duration SLD) male parents. The significant difference in larval duration between short and long duration F_2 individuals as well as the inheritance of the trait (long larval duration) along with the loci SAT-106 and UBC-873_{1074bp} implies a genetic contribution of these loci to this trait (Pradeep *et al.*, 2008).

Mirhosseini *et al.* (2009) constructed linkage map for silkworm based on AFLP markers and reported that AFLP amplification is highly reproducible, the development of an AFLP linkage map provides an invaluable tool for studying silkworm genetics, such as identification of strain specific markers for tracking allele frequency changes and QTL analysis for economically important traits. Ashwath *et al.* (2010) while conducting studies on Identification of RAPD markers linked to digestive amylase genes using Near Isogenic Lines of the silkworm, *B. mori* reported that characterization of PCR products closely associated with the amylase gene can lead to identification of DNA sequences that may be responsible for better digestibility and higher survivability in *B. mori* which could be used for the development of improved and robust *B. mori* breeds for commercial exploitation through the strategy of DNA marker assisted selection. Zhao *et al.* (2010) used SSR markers to identify and map thermo tolerant gene (KN) in two silkworm strains viz; Dong34, a thermo tolerant strain and OU_{17}, a highly susceptible strain reported that five SSR markers were linked to thermo tolerance in silkworm and is present in 8th linkage group.

Lie *et al.* (2010) while mapping major quantitative trait loci in silkworms, identified nine putative major QTLs for cocoon traits, five QTLs for whole cocoon weight, four QTLs for cocoon shell weight, six QTLs for pupal weight and four QTLs for cocoon shell rate, scattered across nine linkage groups in silkworm *B. mori*. They further reported that there were eleven effective QTLs whose contribution rate was over 10%. Among them two QTLs accounted for over 20% of the contribution rate controlling the whole cocoon weight (cw-33), cocoon shell weight (sw-33) and these two QTLs could be very helpful in improving cocoon traits. Mirhosseini *et al.* (2010) studied identification of AFLP markers linked with cocoon weight genes in silkworm (*B. mori* L.) and reported that content genomes (amount and combination) in each F_2 generation are different. Renuka and Kamble (2011) used Restriction fragment length polymorphism (PCR-RFLP) of storage protein gene and yolk protein gene to identify trait specific markers among ten bivoltine silkworm stocks. The restriction fragment length polymorphism revealed polymorphic patterns among these races. These polymorphic bands were further analyzed through Wards method and two major groups in the cluster were obtained. The silkworm races possessing high shell ratio (19.88-23.97%) were grouped in one cluster and the races with low shell ratio (15.91-20.94%) grouped in another cluster. The result indicated both storage as well as yolk protein gene restriction patterns have revealed a similar pattern grouping of high and low shell cluster separately indicating that these vital genes have strong association with quantitative traits in silkworm.

Sreekumar *et al.* (2011) worked out Single Nucleotide Polymorphism (SNP) DNA markers linked to cocoon traits in mulberry silkworm. Out of 240 Primer Pairs used, 48 Primers revealed distinct Polymorphism between the parents which was confirmed by the co-dominant expression of PCR products in F_1 generation. However, only one base pair 04124 was found to show clear cut differences in the amplified products. Srivastava *et al.* (2011) observed high polymorphism (70.91 and 74.70%) revealed by ISSR and RAPD markers. The dendrogram generated, using unweighted pair group method using arithmetic average from these markers, grouped the 14 silkworm races into two major groups, which corroborates the differences in cocoon characteristics. Discriminant function analysis of ISSR and RAPD markers identified three functions for cocoon weight and two functions for shell weights, respectively. Step-wise multiple regression analysis identified six ISSR markers (834_{500}, 884_{1700}, 884_{1850}, 827_{1500}, 840_{1500} and 789_{1250}) and seven RAPD markers (834_{500}, 885_{900}, 810_{1400}, 884_{900}, 836_{1500}, 789_{1250} and 621_{700}) significantly associated with cocoon and shell weights. The authors suggested that genetically divergent parents, identified through this study, can be used for the preparation of hybrids for commercial utilization. Similarly, the DNA markers identified can be utilized for marker assisted selection along with those identified through quantitative trait locus mapping by others. Further, the information generated by this study is useful in silkworm breeding programmes in sericulture industry.

To Identify RAPD and SCAR markers associated with yield traits in the Indian tropical tasar silkworm *Antheraea mylitta* Dutta *et al.* (2012) reported that multiple regression analysis identified five markers associated with cocoon and shell weights. However, RAPD marker $OPW16_{905bp}$ and the SCAR marker $SCOPW16_{826bp}$, which were highly reproducible, showed the most significant association with cocoon and shell weights. They reported that ability of $OPW16_{905bp}$ and $SCOPW16_{826bp}$ to discriminate between the high cocoon shell weight (HCSW) and low cocoon shell weight (LCSW) groups makes them very useful diagnostic markers for these individuals and potential molecular tools for improving the yield of tasar silk through marker-assisted selection aimed at developing highly productive *A. mylitta*strains as such further experiments are required to identify the genes located close to these markers for future breeding programmes. Li *et al.* (2013) carried out genetic analysis of quantitative trait loci for cocoon and silk production in *B. mori* and identified a total 14 QTL's for the economically significant silk quality characteristics viz., cocoon filament length, whole cocoon weight, pupal weight, silk filament weight and cocoon shell weight. These fourteen QTL's were found to be distributed on 5 linkage groups (linkage group 1,14,18,23 and 25). Their findings highlight that QTL's for the same characters are linked which indicate the quantitative inheritance of these characters. The results of their study provide an excellent foundation for the map-based cloning of major genes that control silk production and marker assisted selection for improving silk quality of economically important silkworm strains.

Conclusion

Though silkworm has been domesticated for hundreds of generations, based on available literature, it is speculated that it has not experienced any major reduction of genetic diversity due to phenotypic selection and breeding. But there is concern that bottlenecks may restrict breeding flexibility and slow response to new opportunities, pests, pathogens, and other practices in the future. To broaden the genepool of silkworm, exotic elite strains were required to be introduced from various countries. The genomes of introduced exotic germplasm will broaden the genepool; thereby diversity can be maintained. The original genetic composition of genetic resources should be maintained by avoiding genetic drift and selection process. Maintaining adequate population size can prevent the loss of genetic variability due to genetic drift. Study on effect of inbreeding on inbreeding coefficients in silkworm populations is limited. Hence understanding the effects of inbreeding for various traits can be very crucial points in the management of germplasm.

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