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REVIEW ARTICLE

ROLE OF MOLECULAR MARKERS IN SILKWORM IMPROVEMENT

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ABSTRACT

Bombyx mori L. (silkworm) is of high commercial importance as a silk producer and is also widely used for implementation of basic and applied research. Silkworm are distributed both in temperate and tropical countries. The tropical genotypes (non diapausing) are hardy and can withstand adverse eco-climatic conditions but produce very small quantities of silk of poor quality while the genotypes of temperate origin (diapausing) produce higher quantities of good quality silk. Evaluation of genetic resources is an essential prerequisite for their effective utilization. The presently available linkage map is based on morphological and bio-chemical markers. Bio-chemical markers resulted in the identification of only one enzyme α-amylase. The development of molecular markers will be extremely useful in silkworm improvement programme. The molecular markers such as RFLP, RAPD, SSR, ISSR and AFLP have been successfully used for identification and genetic diversity in silkworm.

INTRODUCTION

Silkworm, *Bombyx mori* L., domesticated for silk production is an agriculturally important insect and comprises large number of geographical races and inbred lines that are distributed in temperate and tropical countries which shows substantial variation in their qualitative and quantative traits. Currently, it is the major economic resource for nearly 30 million families in countries such as china, India, Vietnam and Thailand. The tropical genotypes (non diapausing) are hardy and can withstand adverse eco-climatic conditions but produce very small quantities of silk of poor quality, while the genotypes of temperate origin (diapausing) produce higher quantities of good quality silk. Jammu and Kashmir region is known for its bivoltine sericulture (Rathore *et al.* 2012; 2011). The classical breeding approaches, although they have increased silk productivity, have not been quite successfully in integrating the high yielding trait of temperate genotypes with the low yielding disease resistant tropical genotype (Goldsmith, 1990). Improvement of high yielding, disease resistant silkworm strains became imminent to increase production of silk, which is major revenue earner for sericulturists. Since environment interacts with phenotype, conventional breeding did not result in commendable yield improvement in synthetic strains of silkworm *Bombyx mori* L. Evaluation of genetic resources is an essential prerequisite for their effective utilization. For the silkworm, the presently available linkage map is based on morphological and bio-chemical markers. The earlier attempts to screen for bio-chemical markers resulted in the identification of only one enzyme, digestive α-amylase which showed polymorphism between diapausing and non-diapausing genotypes (Abraham *et al*. 1992). Advances in molecular biology over the last few years, researchers involved in the study of biology with a range of new tools for addressing issues from gene expression to genetic diversity. Differences in DNA sequences can be directly observed and described to a degree of precision previously impossible to achieve. Many of the developed techniques have already been used to study the extent and distribution of variation in species and to investigate evolutionary and taxonomic questions. They have also shown their value in studies of accession identity and for the detection of novel useful variation.

Silkworm is using widely in basic research in biotechnology and molecular genetics as a model insect. It is worthy to say, due to adaptation of different breeding technologies as well as bio-molecular technologies through utilizing the sequential knowledge of proteomic and genomic trends to fulfill the age old dream of sericulture scientists (scions) to make silkworm become a "molecular model" in the advanced bio-molecular world. Hence, sericulture scientists are efficiently applying the different markers because, the markers have a key role in conservation study of silkworm genetic fingerprinting, variability, diversity and relationships etc, which is helpful in the construction of linkage maps and in the tracking of individuals or lines carrying particular genes. The emergence of marker systems has closely followed RAPD & ISSR application for the molecular genetics in *Bombyx mori* L. developments in biochemistry and molecular biology for the past 40 years (Hubby and Lewontin, 1966). The shortcomings of biochemically derived markers such as Isozymes, drove the development

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of markers based on DNA polymorphism (Kan and Dozy, 1978).

Hence, it is a high time for silkworm conservationists and geneticists to take appropriate measures to conserve the valuables resources available in the world (Table-1) as well as in CSGRI Hosur, India (Table-2), different research centers and Universities from further degradation and extinction. Therefore in this review article it has elaborated the importance of RAPD and ISSR marker techniques application for the conservation of *Bombyx mori* insight into the genetic-diversity, fingerprinting, genetic mapping, genetic polymorphism, identification and relationships of silkworm genotypes and application of these markers system for the conservation of domesticated silkworm *Bombyx mori* L.

Table 1 The conservation of silkworm races in different countries

S.No.	Country	Bivoltines	Multivoltines	Total
1	Japan	1542	30	1572
2	India	450	150	600
3	China	580	20	600
4	Russia	500	0	500
5	S.Korea	300	6	306
6	N.Korea	281	5	286
7	Bulgaria	183	0	183
8	Brazil	65	10	75
9	France	53	0	53
10	Iran	50	0	50
11	Itlay	30	0	30
12	Thailand	25	5	30
13	Vietnam	20	5	25
Total		4079	231	4310

(**Source**: FAO Manual, 2003)

DIFFERENT TYPES OF MARKERS

Genetic markers

Markers are entities that are heritable as simple Mendelein traits and are easy to secure(Schulman *et al*. 2004). A genetic marker is, a variant allele that is used to label a biological structure or process through the course of experiment,(Griffiths *et al*. 1996). Essentially, it is a "signature" in the DNA that follows diagnostic detection of DNA sequence variation existing between species and varieties. Today, genetic markers are used in both basic research and breeding to characterize Germplasm, for gene isolation, marker- assisted introgression of favorable alleles, production of improved varieties (Henry 2001), and to obtain information about the genetic variation within population. Genetic markers can be divided into three classes; morphological (variation at phenotype level), biochemical (variation at gene product level) and molecular (variation at DNA level).

Morphological Markers

Morphological characters are the easiest of all markers to assay, and their use is as old as breeding and selection itself. Such characters range from organ shape and differences in the pigmentation, gross changes in development (such as vernalization requirement or dwarf nesses), to responses to individual races of a phytopathogen. However, as the board range of phenotypes is visible at different developmental stages, complete characterization can take up the whole growing season. Morphological characters are the predominant descriptors used for the assessment of varieties for stator distinctness, uniformity and stability (DUS testing) (Ardley and Hoptroff 1996).Many of the characters that are observed comprise continuous traits that are subject to the influence of the environment and require that all reference varieties must be grown together with the testing material for a full assessment, thus increasing the cost of DUS testing. There are strong arguments for replacing the testing procedure with a DNA- based system that would be less subjective, independent from environmental factors, season and cheaper to perform (Cooke and Reeves 2003;Donnenwirth *et al*. 2004). Much work has been performed to test the different marker types for the purposes of DUS testing (Lee *et al*. 1996a; Lombard *et al*. 2000; Tommasini *et al*. 2003) and all clearly demonstrate the utility of DNA markers for differentiating varieties. The biggest benefit is that a given race needs to be tested only once and there would not be a need for repeated analyses. Despite their limitations, morphological markers are well-established tools for taxonomy, variety classification and breeding. However, the limitations to the application of morphological markers can be overcome by the application of biochemical and molecular markers.

Table 2 The conservation of silkworm races in CSGRC Hosur, India which obtained from the different countries

S.No.	Country	Bivoltines	Mulvoltines	Total
1	India	207	63	270
2	Japan	64	3	67
3	China	40	4	44
4	Russia	19	0	19
5	France	11	0	11
6	Thailand	4	0	4
7	Bangladesh	0	3	3
8	Brazil	3	0	3
9	Vietnam	3	0	3
10	Poland	3	0	3
11	Ukrain	2	0	2
12	S.Korea	1	0	1
13	Indonesia	1	0	1
14	Iraq	1	0	1
Total		359	73	432

Isozymes markers

Protein markers particularly Isozymes markers, emerged as a promising tool for genetic mapping (Markert and Moller 1959).These were used for development of partial genetic maps in quite a few organisms (Tanksley and Orton 1983). Isozymes have been used in genome analysis to determine evolutionary pathways and phylogenetic relationships and in gene mapping. Isozymes exhibit allelism and for an independent locus alleles can be separated by electrophoresis (Tiselius 1959) on starch or polyacrylamide gels based on peptide size and charge at a set pH. However, Isozymes are also phenotypic markers, that they can be affected by the tissue and growth stages. Tissues need also to be fresh or properly treated before protein extraction or erroneous results may be generated. Protein systems lack adequate polymorphism, genome coverage was low and there was a requirement for the gene to be expressed at the phenotypic level to make detection possible. The number of polymorphic loci is very limited within a gene pool, and polymorphism may be very low.

Molecular Markers

Molecular markers are specifically developed to detect variation at the DNA level which can be diagnostics for a genotype, variety or species. Strictly defined, a molecular marker identifies changes in the DNA sequence. Molecular markers allow rapid identification of breeding lines, hybrids, cultivars and species, facilitate genetic diversity and relatedness estimations in Germplasm and they allow phylogenetic relationships to be established with more accuracy than was previously possible with morphological and biochemical techniques. Molecular marker can be classified into two major groups; those base on DNA-DNA hybridization between a DNA or RNA probe and total genomic DNA (e.g. RFLP and dot-blot assay) and those based on the PCR amplification of genomic DNA fragments (e.g. RAPD,SCAR,SSR,AFLP,SNP, etc.). However, some assays combine features from both (e.g. RBIP).

Table 3 Chronological evolution of molecular markers

Acronym	Nomenclature	Reference
RFLP	Restriction Fragment Length Polymorphism	Grodzicker et al.1974
VNTR	Variable Number Tandem Repeats	Jaffreys et al.1985
ASO	Allele specific Oligonucleotides	Saiki et al. 1986
OP	Oligonucleotides Polymorphism	Beckmann 1988
AS-PCR	Allele Specific Polymerase Chain Reaction	Landegren et al.1988
SSCP	Single Stranded Conformational Polymorphism	Orita et al. 1989
STS	Sequence Tagged Site	Olsen et al.1989
STMS	Sequence Tagged Microsatellite Sites	Beckman and Soller 1990
OFLP	Oligo-Amplified Fragment Length Polymorphism	Lee et al.1990
RAPD	Random Amplified Polymorphic DNA	Williams et al.1990
AP-PCR	Arbitrarily Primed Polmerase Chain Reaction	Welsh and McClelland 1990
DAF	DNA Amplification Fingerprinting	Caetano-Anolles et al 1991
RLGS	Restriction Landmark Genome Scanning	Hatada et al. 1991
SSR	Simple Sequence Repeat	Akkaya et al. 1992
CAPS	Cleaved Amplified Polymorphic Sequence	Akopyanz et al. 1992
DOP-PCR	Degenerate Oligonucleotide Primer-PCR	Telenius 1992
MAAP	Multiple Arbitrary Amplicon Profiling	Caetano-Anolles et al. 1993
SCAR	Sequence Characterized Amplified Region	Paran and Michelmore 1993
SNAP	Single Nucleotide Polymorphism	Jordan and Humphries 1994
SAMPL	Selective Amplification Of Microsatellite Polymorphic Loci	Morgante and Vogl 1994
ISSR	Inter-Simple Sequence Repeat	Zietkiewicz et al. 1994
ASAP	Allele Specifie Associated Primers	Gu et al. 1995
AFLP	Amplified Fragment Length Polymorphism	Vos et al. 1995
CFLP	Cleavage Fragment Length Polymorphism	Brow et al. 1996
ISTR	Inverse Sequence –Tagged Repeat	Rohde 1996
DAMD-PCR	Directed Amplification Of Minisatellite DNA-PCR	Bebeli et al.1997
S-SAP	Sequence –Specific Amplified Polymorphism	Waugh et al. 1997
RBIP	Retrotransposon-Basd Insertitional Polymorphism	Flavell et al.1998
IRAP	Inter Retrotransposon Amplified Polymorphism	Kalender et al. 1999
REMAP	Retrotransposon Microsatellite Amplified Polymorphism	Kalender et al. 1999
TE-AFLP	Three Endonuclease AFLP	Vander Wurff et al. 2000
IMP	Inter-MITE Polymorphism	Chang et al.2001
SRAP	Sequence-Related Amplified Polymorphism	Li and Quiros 2001
TRAP	Target Region Amplification Polymorphism	Hu and Vick 2003
TBP	Tubulin –Based Polymorphism	Bardini et al.2004

More often, molecular markers are classified on a chronological basis(Table-3).The first generation of the DNA markers included RFLP and RAPD and it has not lived up to initial expectations as universal genotyping assays. The technical limitations of RFLP (Karp *et al.*1996) and lack of reproducibility of RAPD assay (Staub *et al.*1996) have directed scientists towards the development of newer molecular markers that are most robust. In the latter half of the 1990s, three PCR-based marker systems gained popularity for harvesting the potential offered by variations at the DNA level in tissues, these were AFLP,SSR and retrotransposon-based markers. Driven by the need to reduce the cost and increase the information content of molecular-based assays, the research community has begun to exploit the large amount of DNA sequence becoming freely available through the databases(www.ncbi.nim.nih.gov) to generate a number of novel, so-called third generation marker assays. The primary focus has fallen on SNPs, each of which represents a defined position at a chromosomal site at which the DNA sequence of two individuals differs by a single base. SNPs that were first described by (Jordan and Humphries, 1994), have become the marker of choice by virtue of their genome coverage and the parallel testing procedures that enables thousands of loci to be assessed within a single experiment. Recently attempts have been initiated to construct the preliminary linkage map using RFLP, which is one of the earliest molecular mapping techniques to be used (Goldsmith *et al*. 1994).

Recently random amplified polymorphic DNAs (RAPDs) have been developed (Welsh and McClelland, 1990), which are based on the amplification of genomic DNA with single primers of arbitrary nucleotide sequences. The amplification products resulting from the RAPD assay vary between genotypes and hence can be used as genetic markers as well as in construct linkage maps (Reiter *et al*. 1992). RAPD markers have been shown to be useful to identify specific regions of chromosomes, markers linked to specific disease resistance gene (Michelmor *et al*. 1991) as a tool for genetic mapping.

Strain identification, systematic and population studies (Black *et al.*1992).

MOLECULAR GENETIC MAPPING, NEW CONCEPTS, TOOLS AND STRATEGIES

The advent of molecular markers dramatically changed the scenario of genetic linkage mapping in the last two decades of the twenty centaury. Owing to several advantages of DNA based markers over morphological or Isozymes markers, complete genome mapping became a reality. The possibility to produce infinite numbers of such markers facilitating complete genome coverage, phenotypic neutrality, absence of inter-allelic interactions, co-dominant molecular phenotype, and independence of the age of the tissue and the stage are type of tissue for detection made them invaluable tools for construction of genetic linkage maps and the concomitant mapping of genes.

The use of molecular markers to framework genetic linkage maps and to position genes on them in any organism came to be known as molecular genetics mapping. It is important to note however that molecular genetics mapping is a potential tool supplementary to classical genetic linkage mapping, not its substitute.

Molecular mapping was a highly promising and powerful tool because of the availability of molecular markers but the development of several other concepts and strategies had immense contribution as well.

Mapping Populations and Genetic Stocks

Classical genetic maps were constructed by using mainly F2 and backcross populations as the mapping populations. Later on, several types of segregating populations were available for mapping including recombinant inbreed (R1), double haploid (DH), and near isogenic (N1) lines. The farmer too mapping populations facilitated growing of the lines over time and space under replicated designs while extricating the precise contribution of the genotypic component towards total phenotypic variation for a quantative trait .This assisted in mapping of the putative chromosomal locations harboring the polygenic clusters controlling quantative traits, known as quantative loci (QTL).The N1 lines were useful in saturation of a targeted chromosomal region and detection of markers tightly linked to a gene co-segregating with it. The concept of bulked segregant analysis, developed by (Michelmore *et al.* 1991) for gene tagging, is basically similar to two N1 lines. Use of aneuploide stocks, substitution lines are addition lines also helped in anchoring molecular linkage maps to the corresponding chromosomes.

APPLICATION OF GENETIC MAPPING

Marker-Assisted Crop improvement

The major contribution of molecular genetics mapping is undoubtedly marker assisted crop improvement. The markers closely are flanking genes and QTLs in linkage maps can be used for; introgression of favorable genes and QTLs from a donor genotype into a recipients genetic background; marker assisted selection breeding programs; Germplasm screening for target traits; Uniformity and stability (DUS) of commercial parities. Initially, markers at a distance of >5 cm apart from the trait loci were thought to be useful in breeding. Development of second generation maps, for example saturated and high density maps, increased the efficiency of their use where markers were tightly linked, mostly >1cm apart from the target trait loci. Pyramiding race / biotype specific resistance genes to develop durable resistance in host plants against diseases and insects is a significant contribution of molecular mapping and breeding. Development of high, resolution or fine-scale map of a target chromosomal region containing QTLs through marker assisted backcross breeding paved the way for Mendelisation of QTLs of another significant achievement. Development of sequence converted markers such as sequence tagged site (STS) and sequence characterized amplified region (SCAR) also helped enormously in marker assisted breeding.

Map-Based Cloning of Genes and QTLs

Several strategies are available now for isolation of useful oligogenes and polygene's. However, map based or positional cloning is still the method of choice. Most of the genes to-date have been isolated employing a map based cloning strategies. Information on map position of the target genes and saturation of the chromosomal region flanked by a markers have facilitated chromosome walking and more importantly chromosome landing. Recently, application of comparative mapping has also paved the way for isolation of desirable genes.

RESTRICTION FRAGMENT LENGTH POLYMORPHISM (RFLP)

RFLP is generated by the presence and absence of recognition site for the same restriction endonuclease in the same region of a chromosome from the different individuals of a species. As a result, the concerned restriction enzyme produces fragments of different lengths representing the same chromosome region of different individuals these differences are detected by gel electrophoresis combined with hybridization with a labelled probe specific for that chromosome region. Thus RFLP markers result from combination of a specific restriction endonuclease and a specific DNA sequence used as probe. RFLP studies using PCR-amplified fragment as probe have shown polymorphic pattern of 99-100 per cent sequence identity at nucleotide level among the ecoraces. Phylogenetic relationship of different ecoraces obtained on the basis of RFLP pattern support the phenotypic and geographical relation (Mahendran et al.2006).

RANDOM AMPLIFIED POLYMORPHIC DNAs (RAPDs)

This technique was originally developed by (Williams *et al.* 1990) RAPD polymorphism is detected by using oligonucleotides usually 10 bases long of random sequences as primers in a PCR reaction. In a strain which has in its genomic DNA sequences complementary to primer oligonucleotides, PCR products will be detected in the gel, while in those strains that does not have the complementary sequence, no product will be detected. In practice, some bands do appear in each of the strains tested because the stringency of pairing between primers and the template DNA is reduced to ensure amplification. Some of these bands are present in some and absent in the other strains, these bands constitute the RAPD loci. Thus RAPD is generated by specific oligonucleotides used in a PCR reaction.

RAPD technique was used to study DNA profiling of thirteen silkworm genotypes. These genotypes included six diapausing and seven non diapausing varieties that represent a high degree of divergence with respect to geographic origin and morphological qualitative, quantitative and bio-chemical

characters. Two hundred sixteen amplified products were generated using 40 random primers. Silkworm genotypes were clustered into two groups, one consisting of six diapausing and the other of seven non-diapausing genotypes. Ganachari and Javaregowda (1995).

SIMPLE SEQUENCE REPEATS (SSRs)

Simple sequence repeats (SSRs) for genetic characterisation was examined using 13 diverse silkworm strains. Fifteen SSR loci gave rise to an average of eight alleles each of which 86 per cent were polymorphic (Nagaraju *et al.*2001).

INTERSIMPLE SEQUENCE REPEATS (ISSR)

Inter simple sequence repeats for a genetic characterization was examined using 13 diverse silkworm strains. The ISSR-PCR produced 39 fragments of which 76.98 per cent were polymorphic. The diversity index was observed which is 0.957 per cent (Nagaraju *et al*. 2001). Polymorphic profiles of 147 markers generated with 12 ISSR primers on the genomic DNA of 20 silkworm stocks of diverse yield status were subjected to multiple regression and discriminant function analysis (DFA). This led to the identification of eight markers generated by six primers, which demonstrated high coefficient indices of -0.451 to -0.940 (Chatterjee and Mohandas, 2003). 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 per cent polymorphism among 14 tropical and temperate strains of *Bombyx.mori* with average diversity index of 0.747 (Appukuttannair *et al.* 2007).

RAPD AND ISSR TO UNDERSTAND THE GENETIC POLYMORPHISM

In the molecular marker system, the RAPD & ISSR markers has been efficiently utilizing till even today because mainly to resolve the intricate problems related to genetic polymorphism. The genetic polymorphism is an essential aspect in conservation biology because a fundamental concept of natural selection states that the rate of evolutionary change in a population is proportional to the amount of genetic diversity present in it (Fisher, 1930). In particular ISSR marker is a conceptually simple, easy than RAPD and not required the knowledge of genomic sequences for estimation of genetic polymorphism (Chatterjee *et al*. 2004) and (Kar *et al*. 2005). The detection and exploitation of naturally occurring DNA sequence polymorphisms are among the most significant developments in molecular biology. It is thereby a premier study to understand the genetic polymorphism or variation exist among silkworm genotypes because generally to realize the maximum heterosis of the silkworm races, it is essential to understand the variability among the silkworm genotypes because polymorphism/variability is basic requirement for the genetic improvement of a breed (Siddique, 1992). This kind of higher polymorphism was reported by several sericulture scientists such as (Chatterjee and Datta, 1992) in his investigation utilizing the two races of silkworm *Bombyx mori* to understand the sensitivity of RAPD assay using arbitrary primers in Nistari and NB series and (Nagaraju and Singh, 1997) and also (Nagaraju *et al*. 2001) demonstrated that RAPD is one of the important tool to differentiate each genome without resolving to its physical formation where higher polymorphism exists. This kind of higher polymorphism among closely related insects of non-mulberry silkworms is too reported by (Vijayan *et al.* 2005) in Antheraea mylitta through utilizing both RAPD as well as ISSR markers. Hence, to enrich genetic knowledge of polymorphism, the genetic conservation strategies are key through RAPD & ISSR markers have wide potential applications in silkworm genotypes improvement programmes.

PHYLOGENETIC RELATIONSHIPS

Central Sericulture Germplasm Resource Centre, Hosur is an important silkworm germplasm Bank in India where 432 different types of bivoltines, multivoltines and mutants both from indigenous as well as exotic races conservation is carrying out. It is very important to understand and identify the genetic relationships or back ground genomic knowledge of all the silkworm genotypes, which are conserving/maintaining not only in Indian germplasm but also in the World for different scientific research studies, academic studies, development of new breeds, etc. Therefore, the RAPD and ISSR markers are the potential to resolve the genetic architecture of the races and highly concerned to evolutionary geneticists. Many of these architectural issues can be addressed by analysis of a collection of tightly linked markers and the appropriate experimental design (Walsh, 2001). In plants also several dominant RAPD and ISSR markers on agaroge gel were used to find out the genetic relationships, originality of broad range of silkworm germplasm stocks earlier studies revealed relative advantages of different algorithms based on grouping of maize breeds (Ajmona Marson *et al*. 1992 and Mumm *et al*. 1994). But silkworm strains used in primarily are of Asian origins. It is well know that most of strains were descent from China in the long past and RAPD & ISSR application for the molecular genetics in *Bombyx mori* L. adopted to diverse climates, point to genetic closeness among them. This indicated a necessity of more than one algorithm to examine genetic relationship within the closely related silkworm populations. For instance, Nistari is an original tropical strain of Indian origin and its rearing has been practiced in Ganges River Valley since more than a century (Mukherjee, 1912). On the other hand PM (pure mysore) is a tropical, low yielding Indian strain. Low genetic distance and clustering of PM with Nistari reflected that these strains are genetically closer (Appukuttannair *et al.*2007). Such kinds of investigations have been done by several researchers through utilizing the RAPD & ISSR markers. Apart, the (Dhanikachalam Velu *et al*. 2008) has reported the genetic relationships of 20 mutants and has shown all the strains formed into one major cluster and 6 sub-clusters, showing that all the strains originated from same origin and similar voltinism. Similarly (Reddy *et al*. 1999b) analyzed 13 silkworm strains from different origins and he has classified them into non-diapause and diapauses groups. However, RAPD & ISSR marker techniques have not been used thoroughly in phylogenetic investigations based on relative similarity, in spite of their higher efficiency, cost effective and high reproducibility in silkworm molecular genetics and breeding programmes. In generally these have been effective in resolving problems relating to the phylogeny of Asian cultivated mulberry silkworm races and there is immense scope to use these powerful techniques in resolving domesticated, semi-domesticated and wild silkworm races status in many genus and in deciding the distinctness of different genera within and between the family of mulberry and silkworm genotypes.

Genome mapping

The silkworm *Bombyx mori* is an economically important insect and also well known excellent model genomic system. However, its genome analysis has been initiated since long period with emphasis objectives of obtaining genetic maps using different markers even today. The RAPD & ISSR markers were too utilized in the genetic mapping programme for conservation of molecular genetics in *Bombyx mori* by several genetic conservationists are (Promboon *et al*.1995), he has used RAPDs markers and (Yasukochi, 1998) has used RAPD double primers and ISSR markers by(Reddy *et al.* 1999a).Furthermore, the RAPD & ISSR markers were also utilized and utilizing efficiently in mulberry and other plant species. The ISSRs have also been used along with AFLP and RAPD markers in the mapping of Japanese and European genomes by (Aracade *et al*. 2000). According to an investigation by (Wang *et al*. 1998), 58 ISSR makers were mapped onto 18 RAPD linkage groups in soybean. The genetic linkage map of citrus was further saturated using 75 ISSR markers, which were dispersed among all the linkage groups (Sankar and Moore, 2001). However, also it was shown that the level of segregation distortion of ISSRs is lower compare to RAPDs.

Genome fingerprinting

The genetic fingerprinting, is also called as genetic profiling, the main aim of this studies is identifying individual by DNA pattern through RAPD & ISSR and other markers, which are used to run on Electrophoresis/Agarose-gel electrophoresis. The DNA fingerprinting unlike the usual fingerprinting which is based on the morphological features and primarily restricted to humans is revealing the identity of an organism at the molecular level. In fact this is the technique of finding the genetic identity. This is primarily based on the variation occurring at the molecular level that is on the base sequences of the genome. The fundamental techniques involved in genetic fingerprinting were discovered serendipitously in 1984 by geneticist Alec J. Jeffreys of the University of Leicester in Great Britain. The technique crossed the arena of the scientific frontiers mainly with the application in the forensics. With advent of time, development of various techniques paved way for the use of this technique in different fields giving newer dimensions to this Technique. The DNA profiling has been using in silkworms for conservation of bio-molecular genetics, identifying markers for traits, identification of gene diversity and variation etc. The most popular or widely used techniques used with relevant to silkworms are RFLP, RAPD, ISSR, SSR etc. Among all the markers, the RAPD & ISSR are potentially optimistic for the study on fingerprinting/profiling of silkworm genome. Several researchers have been made foundational investigations on genome fingerprinting of silkworms are (Nagaraja and Nagaraju, 1995), (Sharma *et al*. 1999), (Vijayan *et al*. 2005), (Pradeep *et al*. 2006); etc.

SEQUENCE TAGGED SITES (STSs)

A sequence tagged site (STS) is any site on the genome that is unambiguously defined in terms of flanking primers that are used for PCR amplification of this site. Thus when a pair of PCR primers amplifies only a single sequence within a haploid genome, the amplified region of the genome is called a sequence tagged site.

AMPLIFIED FRAGMENT LENGTH POLYMORPHISM (AFLP)

AFLP shares some features of both RFLP and RAPD analysis. It uses restriction enzyme digested genomic DNA as template for PCR amplification using primers that contain the restriction enzymes recognition sites plus a number of usually 2-3 arbitrary nucleotides. This approach allows the simultaneous screening of a large number of anonymous markers randomly distributed throughout the genome. AFLPs are faster, less labour intensive and provide more information than RFLPs and they are highly reproducible, which is a great advantage over RAPDs. An AFLP is detected as a differential mobility of an amplified DNA fragment, AFLP therefore behave as co-dominant markers during inheritance. A genetic linkage map using 204 amplified fragment length polymorphism (AFLP) markers was developed. Twenty Pst^1/Taq^1 primer combinations were used to genotype 78 progenies from an F_2 population of the P107 x Khorasan Lemon cross. Each primers combination generated an average of 10.2 AFLP markers qualified for linkage mapping. The number of markers in the linkage groups ranged from 2 to 53. There were seven major linkage groups with 13-53 markers and five small linkage groups with 2-6 markers. The 12 linkage groups varied in length varied in length from 12.3 to 938.4 cM and total length of linkage map was 42620 cM, giving an average marker resolution of 20.89 cM (Syed *et al*. 2009).

AFLP markers are used to determine QTL(s) affecting cocoon weight trait, 20 selected primer combinations from among 81 primers combinations of Pst^1/Taq^1 at the level of three F_2 populations including 33, 36 and 34 offspring's sample, respectively. These populations were obtained by crossing two lines of Lemon Khorasan (as maternal) and 107 (as paternal). The parental lines, F_1 and F_2 individuals DNA were extracted with phenol chloroform method. Then they were digested by two restriction enzymes (Taq^1 and Pst^1) and amplified by using of appropriate adaptors. There amplified samples are transferred on annealed six per cent polyacrylamide gels. After genotyping of individuals, the linkage maps of population were drawn by map manager/QTX and QTL cartographer ver. 2.5 software's. Number primer combinations in each population were 930, 944, 810 and 142. 171, 178 bands respectively. Therefore polymorphic frequencies were 15.27, 18.11 and 21.97 per cent. The obtained linkage maps were included in 16, 18 and 24 linkage groups. The total lengths of this linkage maps and average distance between two markers were 2186.40, 2582.50 and 2392.60 cM and 18.37, 16.45 and 14.95 cM, respectively. The detection of QTLs numbers of cocoon weight character in each F2 populations also showed 1, 6 and 1 loci in LRs>17 (LoD> 3.7) by compound interval mapping methods respectively (Mirhosseini *et al*.2010).

APPLICATION OF RAPD AND ISSR MARKERS IN FUTURE FOR DVELOPMENT OF THE SILKWORM BOMBYX *MORI*.L

We note here several additional applications currently under investigations for advanced development of silkworm *Bombyx mori*.

1. **Sex determination**: In silkworm molecular (as well as agricultural insects and other) applications it would be convenient to have available markers that were sex-specific. We expect that little difficulty will be encountered in developing RAPD and ISSR markers with this characteristic.

2. **Generation of specific PCR primers for anonymous genomes**: A major limitation in the application of RAPD & ISSR-PCR to molecular problems is the absence of sequence information for the vast majority of genotypes. We suggest that this difficulty may be overcome for many applications by using a RAPD & ISSR-based strategy for developing 'designed PCR primers. Specifically, RAPD & ISSR primers with an embedded restriction site may be used to detect fragments showing the desired properties (e.g. detecting a particular taxon). These fragments may then be cloned and sequences used to develop specific 'designed' PCR primers for diagnostic markers.
3. **Quantitative analysis of mixed biosamples:** Analogous to the analysis of mixed paternity samples in silkworm, analysis of field samples of different genotypes/climate may be performed.
4. **Phylogeny**: RAPD & ISSR markers may prove to be useful characters for cladistic analysis, etc.

CONCLUSION

The domesticated silkworm *Bombyx mori* is of high commercial importance as a silk producer. Improvement of high yielding, disease resistant silkworm strain becomes imminent to increase production of silk. Since environment interacts with phenotype, conventional breeding did not result in commendable yield improvement in synthetic strains of silkworms. Thus it is concluded that identification of DNA markers associated with different economically biomass traits and its interogression could assist molecular breeding and expression of stabilized high yielding characters.

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