

International Journal of Advanced Biochemistry Research



ISSN Print: 2617-4693
 ISSN Online: 2617-4707
 NAAS Rating (2026): 5.29
 IJABR 2026; 10(3): 1003-1013
www.biochemjournal.com
 Received: 05-01-2026
 Accepted: 08-02-2026

K Ranganatha
 Department of Sericulture,
 College of Agriculture, UAS,
 GKVK, Bengaluru,
 Karnataka, India

KS Vinoda
 Department of Sericulture,
 College of Agriculture, UAS,
 GKVK, Bengaluru,
 Karnataka, India

K Nisarga
 Department of Sericulture,
 College of Agriculture, UAS,
 GKVK, Bengaluru,
 Karnataka, India

MN Chethan
 Department of Sericulture,
 College of Agriculture, UAS,
 GKVK, Bengaluru,
 Karnataka, India

MS Varun
 Department of Sericulture,
 College of Agriculture, UAS,
 GKVK, Bengaluru,
 Karnataka, India

KG Banuprakash
 Department of Sericulture,
 College of Agriculture, UAS,
 GKVK, Bengaluru,
 Karnataka, India

Corresponding Author:
K Ranganatha
 Department of Sericulture,
 College of Agriculture, UAS,
 GKVK, Bengaluru,
 Karnataka, India

Molecular markers for disease resistance in Silkworm, *Bombyx mori* L.

K Ranganatha, KS Vinoda, K Nisarga, MN Chethan, MS Varun and K G Banuprakash

DOI: <https://www.doi.org/10.33545/26174693.2026.v10.i31.8026>

Abstract

Molecular marker technologies have greatly improved silkworm genetics by providing precise tools for studying genetic variation, disease resistance, genetic diversity, linkage mapping and marker-assisted breeding in *Bombyx mori* L. Marker systems such as RFLP, RAPD, AFLP, ISSR, SSR and SNPs have been widely used to detect DNA polymorphism, identify resistance genes, evaluate germplasm diversity and locate quantitative trait loci associated with cocoon yield and silk productivity. These markers have been particularly useful in identifying genes linked to resistance against major viral diseases such as *B. mori* nucleopolyhedron virus (*BmNPV*) and *B. mori* densonucleosis virus (*BmDNV*). The availability of the *B. mori* genome sequence has further accelerated the development of high-resolution markers and improved understanding of host-pathogen interactions. This review highlights the major molecular marker systems used in silkworm research and emphasizes their importance in disease resistance studies and molecular breeding programmes.

Keywords: *Bombyx mori* L., molecular markers, genetic diversity, disease resistance, linkage mapping, marker-assisted selection

1. Introduction

Sericulture is an important agro-based enterprise that supports rural livelihoods and cottage industries in many Asian countries, particularly India and China, where the silkworm, *Bombyx mori* L. reared on mulberry serves as the principal silk-producing insect. Sustainable silk production depends largely on the availability of robust silkworm breeds possessing high cocoon yield, superior silk quality and tolerance to biotic and abiotic stress factors. However, silkworm productivity is often constrained by narrow genetic base in breeding stocks, infectious diseases and environmental fluctuations, all of which reduce rearing efficiency and economic returns. A better understanding of the genetic architecture of economically important traits has therefore become indispensable for modern silkworm improvement programmes (Nagaraju, 2000; Nagaraju and Goldsmith, 2002; Xia *et al.*, 2014) [29, 28, 52]. Traditional breeding in silkworms has relied mainly on phenotypic selection, hybridization and evaluation of performance traits such as larval duration, cocoon weight, cocoon shell ratio and filament length. Although these approaches have contributed substantially to silkworm improvement, they are time-consuming and often influenced by environmental conditions, epistatic interactions and genotype × environment effects. In contrast, molecular markers provide direct access to heritable DNA-level variation and enable the identification of polymorphism independent of developmental stage or environmental influence. Consequently, molecular marker systems have become indispensable tools for germplasm characterization, genetic diversity analysis, linkage mapping, quantitative trait locus (QTL) detection and marker-assisted selection (Botstein *et al.*, 1980; Collard *et al.*, 2005; Semagn *et al.*, 2006) [7, 16, 40].

The genetic markers used in biological research are generally grouped into morphological, biochemical and molecular markers among which, molecular markers are considered the most powerful because they detect sequence variation directly within the genome and often show high reproducibility, wide genome coverage and greater discriminatory ability. Early marker systems such as RFLP laid the foundation for genome mapping, while PCR-based markers such as RAPD, ISSR and AFLP expanded the scope of polymorphism detection in

non-model organisms. Later, co-dominant and highly informative markers such as SSRs and SNPs provided greater precision for fine mapping, population analysis and breeding applications (Williams *et al.*, 1990; Vos *et al.*, 1995; Brookes, 1999; Collard and Mackill, 2008) ^[51, 48, 8, 15]. The importance of molecular markers in silkworm research has increased markedly with the advancement of silkworm genomics. The draft genome sequence of *B. mori*, estimated at about 530 Mb with approximately 18,500 predicted genes, represented a major milestone and created new opportunities for high-resolution genetic analysis. Genome information has enabled the development of dense SSR and SNP markers, comparative genomics, positional cloning and functional studies of candidate genes associated with productivity, adaptation and disease resistance (Xia *et al.*, 2004; Xia *et al.*, 2014) ^[53, 52]. In addition, second-generation linkage maps and SNP-based arrays have strengthened the identification of QTLs governing cocoon yield traits and silk-related characters, thereby facilitating more precise breeding strategies (Yasukochi *et al.*, 2006; Fang *et al.*, 2020; Li *et al.*, 2025) ^[57, 18, 23].

Molecular markers have been widely used to assess genetic diversity among silkworm races and germplasm collections. Such studies are essential because maintenance of adequate genetic variation is required for long-term breeding success, stress adaptation and conservation of elite and indigenous strains. RAPD, ISSR and SSR markers have revealed substantial polymorphism among silkworm populations and have helped distinguish bivoltine, polyvoltine and mutant races on the basis of genomic variation and evolutionary divergence (Nagaraju *et al.*, 2001; Chatterjee and Tanushree, 2004; Velu *et al.*, 2008) ^[29, 12, 47]. The resulting information has practical relevance for parent selection, hybrid development and germplasm conservation. Identification of genes associated with major viral diseases such as *BmNPV* and *BmDNV* responsible for serious crop loss is a significant application of molecular markers in sericulture. The marker-based studies have successfully identified *nsd-1* and *nsd-2*, the resistance-linked loci while transcriptomic and genomic analyses have further revealed the role of immune-related genes, antimicrobial peptides and signalling pathways in host defence (Abe *et al.*, 2000; Yao *et al.*, 2003; Zhou *et al.*, 2013; Fan *et al.*, 2023) ^[2, 56, 59, 17] strengthening the development of resistant silkworm breeds through marker-assisted introgression and genomic selection.

Linkage mapping and marker-assisted breeding have emerged as key applications of molecular markers in silkworm improvement in addition to diversity analysis and disease resistance studies. The linkage maps developed with RFLP, SSR, AFLP and SNP markers have enabled the localization of genomic regions associated with cocoon weight, cocoon shell ratio, larval growth and silk productivity while Marker-assisted selection has further accelerated the transfer of resistance genes and productivity-related loci into elite backgrounds, reduced the dependence on phenotype alone and improved the breeding efficiency (Nezhad *et al.*, 2011; Hou *et al.*, 2013; Satish *et al.*, 2024) ^[32, 21, 39]. Congregation of all available information regarding application of molecular markers for improvement of silkworm genetics ignites designing of new research ideas for development of robust silkworm hybrids and the parental lines. The present review summarizes and discusses the principles, applications, advantages and limitations of major

molecular marker systems used in the genetic analysis and improvement of *B. mori*. Special emphasis is given to their role in analysis of genetic diversity, constructing linkage maps, identifying resistance genes, locating QTLs and supporting marker-assisted breeding for disease-resistant and high-performing silkworm strains. Recent genomic, transcriptomic and proteomic advances related to host-pathogen interactions are also highlighted in order to provide a comprehensive understanding of the current status and future prospects of molecular marker-assisted silkworm improvement.

Genetic markers

Genetic markers are identifiable DNA sequences with known location on chromosomes that can be used to study genetic variation, gene mapping and inheritance pattern. In biological systems, the markers are broadly classified into phenotypic (morphological), biochemical and molecular markers depending on the type of variation detected (Nagaraju and Goldsmith, 2002; Collard *et al.*, 2005) ^[28, 16].

1. Phenotypic / Morphological markers

Morphological markers were among the earliest genetic markers used in classical genetics and plant or animal breeding. These markers are based on observable traits such as size, shape, colour and structural characteristics of organisms (Collard *et al.*, 2005) ^[16]. In plants, morphological markers include variations in leaf shape, seed colour, plant height and surface texture. In animals, phenotypic markers include characteristics such as body colour, wing shape and eye colour.

Morphological markers are easy to observe and require no specialized laboratory equipment. However, they have several limitations. Their expression may be influenced by environmental conditions and relatively limited number of availability. Consequently, these markers provide lower resolution for genetic analysis compared to molecular markers (Nagaraju and Goldsmith, 2002) ^[28].

2. Biochemical markers

Biochemical markers are based on variations in biochemical compounds such as proteins, enzymes, etc. produced by genes. Examples include isozymes, antigens, antibodies and hormones that show detectable differences among individuals (Avisé, 2012) ^[3]. These markers are often used in disease diagnosis and susceptibility prediction, as certain biochemical compounds may change significantly during disease conditions. For example, enzyme polymorphism detected through electrophoresis have been widely used to study genetic variation.

However, biochemical markers have limitations similar to morphological markers. Their expression can be influenced by environmental conditions, developmental stages and physiological factors. Moreover, the number of detectable biochemical markers is limited because they depend on gene expression at the protein level (Collard *et al.*, 2005) ^[16].

3. Molecular markers

Molecular markers, also known as DNA markers or genetic markers, are variations in DNA sequences that can be detected at the molecular level. These markers are not influenced by environmental factors and are present throughout the genome, making them highly reliable tools for genetic analysis (Nagaraju *et al.*, 2001) ^[29].

A molecular marker may consist of a short DNA sequence variation, such as a single nucleotide polymorphism (SNP), or longer repetitive sequences such as microsatellites. Molecular markers are widely used for genetic mapping, population genetics, disease resistance studies and marker-assisted breeding.

Examples of commonly used molecular markers include:

- Restriction Fragment Length Polymorphism (RFLP)
- Random Amplified Polymorphic DNA (RAPD)
- Amplified Fragment Length Polymorphism (AFLP)
- Simple Sequence Repeats (SSR)
- Inter Simple Sequence Repeats (ISSR)
- Single Nucleotide Polymorphisms (SNP)

The major advantage of molecular markers is that they are stable, not influenced by environmental conditions and can be detected at any developmental stage (Collard *et al.*, 2005) [16].

Types of molecular markers

1. Restriction Fragment Length Polymorphism (RFLP)

Restriction Fragment Length Polymorphism (RFLP) is one of the earliest molecular marker techniques used to detect variations in homologous DNA sequences. The polymorphisms arise when restriction enzymes cut DNA at specific recognition sites, producing fragments of varying lengths depending on sequence variation (Botstein *et al.*, 1980) [7]. RFLP markers are co-dominant, meaning both alleles can be detected in heterozygous individuals. The technique involves digesting genomic DNA with restriction enzymes, separating the fragments using gel electrophoresis and detecting specific fragments using labeled DNA probes through Southern blotting. These markers have been widely used in genome mapping, forensic analysis, paternity testing, and genetic disease diagnostics. Although RFLP markers provide high reliability and locus specificity, the technique is time-consuming and requires large amounts of high-quality DNA. The development of PCR-based methods has therefore reduced the routine use of RFLP markers.

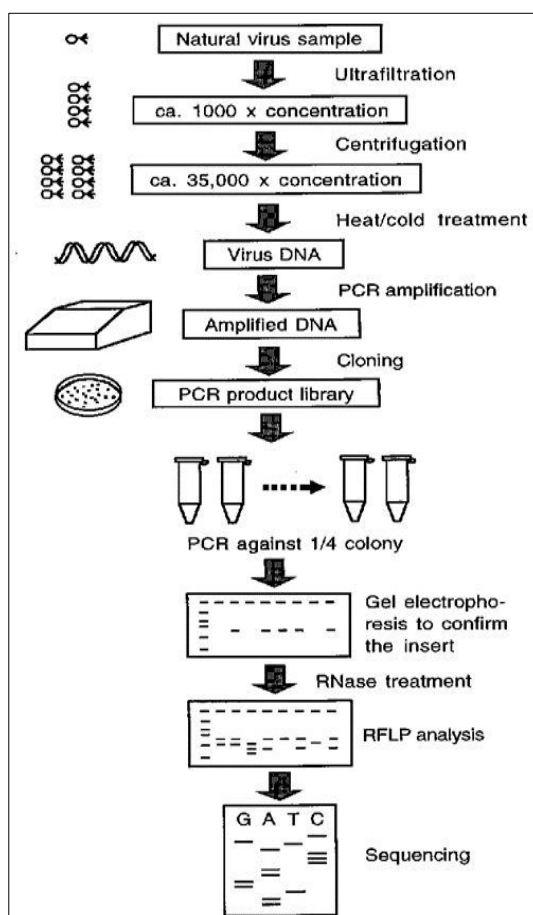


Fig 1: Schematic diagram of molecular analysis of viral DNA using PCR-RFLP technique (Chen *et al.*, 1996) [13].

The PCR-RFLP workflow for viral DNA analysis is an example of application of RFLP in disease detection and confirmation. A natural virus sample is initially concentrated using ultrafiltration and centrifugation, then subjected to heat/cold treatment to facilitate lysis and release of viral DNA. The extracted DNA is subsequently amplified using PCR to obtain sufficient quantities of target fragments. These amplified products are cloned to generate a PCR product library and selected colonies are screened using colony PCR to confirm the presence of inserts, which is further validated by gel electrophoresis. Following the

removal of RNA contamination through RNase treatment, the DNA is digested using specific restriction enzymes in the RFLP analysis, resulting in fragments of varying lengths depending on sequence variation. These fragments are separated by gel electrophoresis to produce distinct banding patterns. The resulting banding profiles are used to detect genetic polymorphism among samples and final confirmation is achieved through DNA sequencing, which provides precise nucleotide information for detailed genetic characterization of the virus (Chen *et al.*, 1996; Yao *et al.*, 2020) [13, 55].

2. Random Amplified Polymorphic DNA (RAPD)

Random Amplified Polymorphic DNA (RAPD) markers are PCR-based markers that use short arbitrary primers (usually 10 nucleotides) to amplify random segments of genomic DNA (Williams *et al.*, 1990) ^[51]. Amplification occurs only when two primer binding sites are located within a suitable distance and orientation. Differences in DNA sequences among individuals may prevent primer binding, resulting in

the absence of specific PCR fragments. This produces different banding patterns on agarose gels, which can be used to identify genetic variation among strains. Though RAPD markers are inexpensive and require no prior sequence information, they are generally dominant markers and may suffer from reproducibility issues due to PCR sensitivity (Mbwana *et al.*, 2006) ^[24].

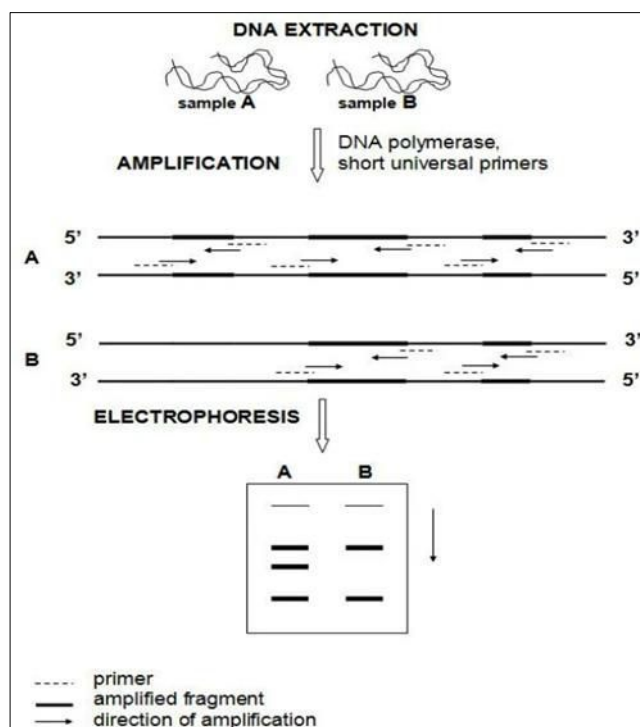


Fig 2: Schematic representation of RAPD marker technique for genetic diversity analysis (Pasqualone, 2013) ^[34].

The work flow of RAPD technique is such that the genomic DNA from two samples (A and B) is first extracted and subsequently subjected to PCR amplification using short, arbitrary primers. The primers anneal randomly at multiple complementary sites across the genome under low-stringency conditions, resulting in the amplification of several DNA fragments of varying sizes depending on the distance between primer binding sites and sequence variations. Different sets of fragments are generated in each sample as the primer binding sites may differ. Distinct banding patterns are obtained upon separation of the amplified products by agarose gel electrophoresis. These banding patterns are considered to represent genetic differences (polymorphisms) between the samples and are widely used for genetic diversity analysis, identification and comparison without requiring prior sequence information (Pasqualone, 2013; Collard *et al.*, 2005) ^[34, 16].

3. Amplified Fragment Length Polymorphism (AFLP)

A highly sensitive DNA fingerprinting technique that combines restriction enzyme digestion and selective PCR amplification to detect polymorphisms in genomic DNA is explained as Amplified Fragment Length Polymorphism (AFLP) (Vos *et al.*, 1995) ^[48].

The AFLP technique is a highly sensitive PCR-based molecular marker method in which genomic DNA is first digested using specific restriction enzymes such as *EcoRI* and *MseI*. The resulting DNA fragments are then ligated with double-stranded adaptors at their sticky ends, providing known sequences for primer binding. These adaptor-ligated fragments are subsequently subjected to pre-selective PCR amplification using primers complementary to the adaptor and restriction site sequences. This is followed by selective PCR amplification, in which additional selective nucleotides are included at the 3' end of the primers to amplify only a subset of fragments. The selectively amplified products are then separated by gel electrophoresis, resulting in distinct banding patterns. These banding profiles are used to detect genetic polymorphism and are widely applied in genetic diversity analysis, fingerprinting and molecular characterization (Vos *et al.*, 1995; Meudt and Clarke, 2007) ^[48, 25].

AFLP markers generate large numbers of polymorphic fragments, making them useful for genetic mapping and population genetics studies. However, AFLP markers are usually dominant and require specialized laboratory protocols.

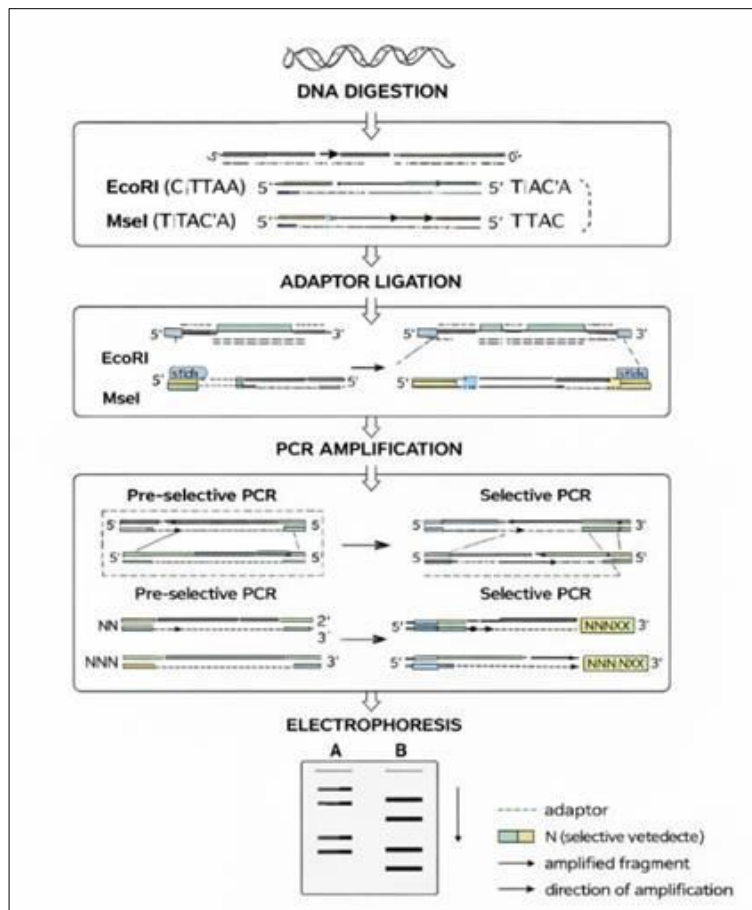


Fig 3: Schematic representation of AFLP technique

4. Simple Sequence Repeats (SSR / Microsatellites)

Simple Sequence Repeats (SSRs), also known as microsatellite markers, are short tandem repeats consisting of 1–6 base pair motifs repeated multiple times in the genome (Tautz and Renz, 1984) [45]. These markers are highly polymorphic due to variations in the number of repeat units.

SSR markers are widely used in genetic mapping, population genetics, paternity testing and forensic analysis. Because they are co-dominant markers, both alleles can be detected in heterozygous individuals. However, SSR analysis relies on PCR amplification and may sometimes produce null alleles due to mutations in primer binding sites.

5. Inter Simple Sequence Repeats (ISSR)

Inter Simple Sequence Repeats (ISSR) markers amplify DNA fragments located between adjacent microsatellite regions using primers based on microsatellite core sequences (Zietkiewicz *et al.*, 1994) [60]. ISSR markers typically generate 10–60 fragments from multiple loci, which can be separated using gel electrophoresis and scored as presence or absence of bands. These markers are useful for genetic diversity analysis, population studies and gene mapping.

The major advantage of ISSR markers is that no prior sequence information is required. However, as multilocus markers, ISSRs may sometimes produce fragments that are not homologous despite having similar sizes.

6. Single Nucleotide Polymorphisms (SNPs)

Single nucleotide polymorphisms (SNPs) represent single base pair variations in DNA sequences and are the most

abundant type of genetic variation in genomes (Brookes, 1999) [8]. SNPs occur approximately once in every 1,000 nucleotides, resulting in millions of SNPs across a genome. Hence, these markers are widely used in genome-wide association studies, disease gene mapping and pharmacogenomics. SNP markers enable high-throughput automated genotyping and provide very high resolution for genetic studies. Although most SNPs have no functional effects, some occur within genes or regulatory regions and can influence disease susceptibility or gene expression.

Advantages of molecular markers

- 1. High level of polymorphism detection:** Molecular markers detect variations directly at the DNA level, allowing identification of even minor genetic differences among closely related individuals. This high resolution makes them highly reliable for diversity and phylogenetic studies (Powell *et al.*, 1996; Collard *et al.*, 2005; Gupta *et al.*, 2010) [36, 16, 19].
- 2. Independence from environmental influence:** Unlike morphological traits, molecular markers are not influenced by environmental factors or developmental stages. This ensures stable expression across different conditions and improves the accuracy of genetic analysis (Nagaraju and Goldsmith, 2002; Semagn *et al.*, 2006) [28, 40].
- 3. Early detection of traits:** Markers allow identification of desirable traits at early developmental stages, such as seedlings, without waiting for phenotypic expression. This significantly reduces breeding cycles and

accelerates selection (Mohan *et al.*, 1997; Varshney *et al.*, 2000; Collard and Mackill, 2008) [27, 46, 15].

4. **High reproducibility and reliability:** Many marker systems such as SSRs, AFLPs and SNPs provide consistent and reproducible results across laboratories. This reliability is crucial for: studies and long-term breeding programs (Semagn *et al.*, 2006; Jones *et al.*, 2009) [40, 22].
5. **Genome-wide coverage:** Molecular markers are distributed throughout the genome, enabling comprehensive analysis of genetic variation. This allows effective mapping of genes and quantitative trait loci (QTLs) (Varshney *et al.*, 2000; Collard *et al.*, 2005) [46, 16].
6. **Detection of heterozygosity (Co-dominant markers):** Co-dominant markers such as SSRs and SNPs can distinguish between homozygous and heterozygous genotypes. This is particularly useful in population genetics and breeding programs (Powell *et al.*, 1996; Gupta *et al.*, 1999; Collard *et al.*, 2005) [36, 20, 16].
7. **No prior phenotypic information required:** Markers can be used even when phenotypic traits are not expressed or are influenced by multiple genes. This is especially useful for studying complex traits and cryptic variation (Mohan *et al.*, 1997; Collard *et al.*, 2005; Semagn *et al.*, 2006) [27, 16, 40].
8. **Wide range of applications:** Molecular markers are widely applied in genetic mapping, diversity analysis, phylogenetics, marker-assisted selection and disease diagnostics, making them versatile tools in modern biology (Mohan *et al.*, 1997; Collard *et al.*, 2005) [27, 16].

Limitations of molecular markers

1. **High cost of advanced techniques:** Techniques such as SNP genotyping and sequencing require expensive instruments, reagents and software, limiting their use in low-resource laboratories (Varshney *et al.*, 2000; Semagn *et al.*, 2006) [46, 40].
2. **Requirement of skilled personnel:** Molecular marker analysis involves complex procedures such as PCR optimization, electrophoresis and data interpretation, requiring trained personnel and technical expertise (Mohan *et al.*, 1997; Collard *et al.*, 2005) [27, 16].
3. **Reproducibility issues in some markers:** Certain markers like RAPD are sensitive to reaction conditions, which may result in inconsistent banding patterns across experiments and laboratories (Powell *et al.*, 1996; Gupta *et al.*, 1999; Jones *et al.*, 2009) [36, 20, 22].
4. **Limited application of some marker systems in the comparative genomic study of heterozygotes:** Dominant markers such as RAPD and AFLP cannot distinguish between homozygous and heterozygous individuals, reducing their informativeness in genetic studies (Gupta *et al.*, 1999; Collard *et al.*, 2005) [20, 16].
5. **Limited transferability across species:** Some markers are species-specific and may not work effectively across different species, limiting their application in comparative studies (Collard *et al.*, 2005; Semagn *et al.*, 2006) [16, 40].
6. **Dependence on DNA quality and quantity:** High-quality, intact DNA is essential for accurate results. Degraded or contaminated DNA can lead to poor amplification and unreliable data (Mohan *et al.*, 1997; Semagn *et al.*, 2006) [27, 40].

7. **Limited information content in some markers:** Markers like RAPD and AFLP provide only presence/absence data, which may not fully capture genetic variation at the sequence level (Powell *et al.*, 1996; Varshney *et al.*, 2000) [36, 46].
8. **Complex data analysis in high-throughput systems:** Large datasets generated from SNP and sequencing platforms require advanced computational tools and bioinformatics expertise for analysis and interpretation (Semagn *et al.*, 2006; Collard and Mackill, 2008) [40, 15].

Application of molecular marker systems in silkworm (*Bombyx mori* L.) genomics

Molecular marker technologies have significantly improved the understanding of genetic variation and disease resistance in the mulberry silkworm, *B. mori*. Reliable tools for the identification of resistance genes, assessment of genetic diversity, construction of linkage maps and support of marker-assisted breeding programmes have been provided by these markers. Several molecular marker systems, including RAPD (Random Amplified Polymorphic DNA), ISSR (Inter Simple Sequence Repeat), SSR (Simple Sequence Repeat), AFLP (Amplified Fragment Length Polymorphism) and SNP (Single Nucleotide Polymorphism) markers, have been widely used in silkworm genetic research (Nagaraju and Goldsmith, 2002; Buhroo *et al.*, 2016) [28, 9]. Detection of polymorphisms at the DNA level and analysis of genetic relationships among silkworm populations have been facilitated by these markers.

Molecular Marker Systems with special reference to the silkworm, *B. mori*, various DNA marker techniques and their applications in silkworm genetics and breeding have been documented here. It was explained that RAPD markers are particularly useful for the rapid detection of polymorphisms without the requirement of prior genome sequence information, making them suitable for preliminary genetic studies (Buhroo *et al.*, 2016) [9]. However, higher reproducibility and co-dominant inheritance patterns have been provided by microsatellite (SSR) markers and SNP markers, owing to which greater suitability for genetic mapping, quantitative trait locus (QTL) analysis and marker-assisted selection in silkworm breeding programmes has been achieved (Nagaraju *et al.*, 2001; Xia *et al.*, 2014) [29, 52].

Approximately 530 megabases (Mb) of DNA and around 18,500 predicted genes have been reported in the silkworm genome, thereby providing a large number of potential molecular markers for genetic analysis (Xia *et al.*, 2004) [53]. The development of high-resolution genetic markers such as SSR and SNP markers has been greatly facilitated by the availability of the complete genome sequence. Identification of genomic regions associated with disease resistance, productivity and other economically important traits in silkworm breeding programmes can be achieved through the use of these marker systems (Fang *et al.*, 2020; Li *et al.*, 2025) [18, 23].

Genetic diversity analysis among silkworm populations

Genetic diversity among silkworm populations has been recognized to play a crucial role in determining adaptability, productivity, disease tolerance and environmental stability. Understanding of genetic variation among silkworm strains is considered important for the identification of superior breeding lines and conservation of valuable germplasm

resources. Molecular markers such as RFLP, RAPD, ISSR and SSR markers have been widely used for the evaluation of genetic diversity in *B. mori*. Detection of DNA polymorphisms among silkworm strains and assessment of their genetic relationships have been facilitated by these markers.

Fifteen races silkworm, *B. mori* obtained from different geographical regions were analysed using RFLP, RAPD and ISSR markers and the effectiveness of different marker systems for analysing genetic diversity among the silkworm populations was evaluated by Nagaraju *et al.*, 2001 [29]. The RAPD analysis generated 210 DNA bands, of which about 60 per cent were polymorphic, whereas slightly higher polymorphism levels (65–70 per cent) were shown by ISSR markers, indicating greater sensitivity for the detection of genetic variation. The cluster analysis of these polymorphic markers grouped the silkworm races according to their geographical origin and breeding history, demonstrating that molecular markers are effective tools for the study of genetic relationships and population structure in silkworm germplasm. Similarly, Pradeep *et al.*, 2005 employed RAPD and ISSR markers for analysing genetic differentiation among silkworm strains. The RAPD analysis produced 65 per cent polymorphism while, ISSR markers recorded about 75 per cent polymorphism, indicating that ISSR markers are highly sensitive and generate reproducible genetic variation in the diversity analysis.

Genetic variability among silkworm races has also been widely assessed using RAPD markers. Several Indian silkworm strains were analysed using 18 RAPD primers that produced 198 amplified DNA fragments with about 72 per cent polymorphism, indicating considerable genetic variation among the strains. Genetic similarity values ranging from 0.55 to 0.88 were recorded, suggesting moderate to high genetic divergence (Chatterjee and Pradeep, 2003) [11]. Similarly, 65–70 per cent polymorphism among different silkworm races was reported using RAPD markers, indicating high levels of genetic variability that may influence economically important traits such as larval growth rate, cocoon weight and disease resistance (Talebi *et al.*, 2011). High DNA polymorphism among silkworm populations collected from different regions was also reported, highlighting the presence of valuable genetic diversity within silkworm germplasm (Bajwa *et al.*, 2017) [5].

Significant genetic differentiation between bivoltine and polyvoltine silkworm races has also been revealed through comparative molecular studies. Distinct DNA banding patterns observed between bivoltine and polyvoltine strains during diversity analysis using RAPD markers indicated a clear genetic divergence. Higher cocoon productivity and superior silk quality were associated with bivoltine strains, whereas better adaptability to tropical climatic conditions was shown by polyvoltine strains (Chatterjee and Tanushree, 2004) [12]. Similarly, distinct RAPD banding patterns between these two groups were reported, showing that greater genetic stability and productivity are exhibited by bivoltine races, whereas higher tolerance to environmental stress is demonstrated by polyvoltine races (Awasthi *et al.*, 2008) [4].

Velu *et al.* (2008) [47] utilized ISSR marker system for studying the genetic relationship among silkworm races maintained in different sericulture research institutes and identified more than 80 polymorphic DNA fragments,

indicating substantial genetic variation among those strains. The genetic similarity analysis revealed both closely related and genetically divergent strains of silkworms confirming that ISSR markers are reliable tools for the evaluation of genetic diversity and identification of suitable parental lines for silkworm breeding programmes aimed at improving productivity, disease resistance and environmental adaptability.

Identification of resistance genes for viral diseases

Viral diseases such as *Bombyx mori* nucleopolyhedrovirus (*BmNPV*) and *Bombyx mori* densovirus (*BmDNV*) have been recognized among the most destructive pathogens affecting silkworm rearing. Larval mortality of 40–90 per cent can be caused by these viruses, resulting in severe economic losses in sericulture.

Resistance to the virus *BmDNV* type-1 (*BmDNV*-1) was investigated by Abe *et al.* (1998) [1] using RAPD markers. Genomic DNA from resistant and susceptible strains was amplified with 150 RAPD primers producing about 450 DNA fragments, of which 35 per cent were found polymorphic. Through linkage analysis, markers associated with the *nsd-1*, resistance gene for *BmDNV*-1 was identified on chromosome 21. In the infection experiments, 90–95 per cent survival was exhibited by strains carrying the *nsd-1* gene, whereas susceptible strains showed 80–90 per cent mortality. Prevention of viral replication in midgut epithelial cells was attributed to the *nsd-1* gene. In a subsequent study, another resistance gene, *nsd-2*, located on chromosome 17, was identified by Abe *et al.* (2000) [2] and it is known to confer resistance to *BmDNV* type-2 (*BmDNV*-2). Higher resistance was shown by silkworm strains possessing both *nsd-1* and *nsd-2* genes, indicating that resistance to *BmDNV* is controlled by multiple genetic loci. Minimal viral replication and maintenance of normal physiological functions were observed in resistant strains, whereas severe midgut damage was developed by susceptible larvae within 48–72 hours after infection.

Genes associated with resistance to *BmNPV* have also been identified through molecular marker studies. RAPD markers were screened by Yao *et al.* (2003) [56] using a resistant strain NB and a susceptible strain 306. A 700 bp fragment amplified by primer OPA-18 was identified as being strongly associated with a dominant resistance gene and a 400 bp fragment produced by primer OPY-11, linked with susceptibility. In the analysis of F₂ populations, the amplification of OPA-18 marker was represented by 21 out of 24 resistant individuals, confirming its linkage with NPV resistance. Similarly, commercial silkworm hybrids were analyzed by Ribeiro *et al.* (2009) [38] using RAPD markers, and 145 DNA bands with 61 per cent polymorphism were generated. In infection experiments, viral symptoms were developed within 48–72 hours by susceptible strains, whereas delayed viral replication and lower viral load were exhibited by resistant strains.

More recent studies have focused on the molecular and genomic mechanisms underlying viral resistance. Zhou *et al.* (2013) [59] reported 2–4 fold higher expression of antiviral immune genes, including those encoding antimicrobial peptides among the resistant silkworm strains. Similarly, increased production of antimicrobial peptides such as defensins and cecropins that suppress viral replication and enhance host defense, was observed by Nesa *et al.* (2020) [31]. Wang *et al.* (2015) [50] identified 1,287 differentially

expressed genes through transcriptome analysis of *BmNPV* infected silkworms, of which approximately 650 genes were upregulated, many being involved in immune signalling pathways and antimicrobial peptide synthesis. More recently, several genomic regions associated with antiviral immune responses were identified by Fan *et al.*, 2023^[17] through genome sequencing and SNP marker analysis in which more than 10,000 SNP markers were examined. These studies indicate that viral resistance in silkworms is a complex trait regulated by multiple genes and immune mechanisms.

Development of linkage maps and QTL identification in *Bombyx mori* L.

Genetic linkage mapping has been considered as an important approach in silkworm genetics for the identification of genomic regions controlling economically important traits such as cocoon weight, larval growth rate, silk yield and disease resistance. Linkage maps are constructed through the analysis of recombination frequencies between molecular markers across the genome. Identification of quantitative trait loci (QTLs) associated with complex traits and facilitation of marker-assisted breeding in silkworm improvement programmes have been enabled by these maps.

One of the most detailed genetic linkage maps of *B. mori* was developed using 1,018 molecular markers, including RFLP, SSR and EST markers. A mapping population derived from crosses between genetically distinct silkworm strains was used for the analysis of recombination events among markers. The markers were assigned into 28 linkage groups corresponding to the 28 chromosomes of the silkworm genome, with a total map length of about 2,000 centimorgans (cM). Important information on the distribution of genes across the silkworm genome was provided by this linkage map, and a valuable resource for the identification of genomic regions associated with economically important traits such as disease resistance and silk productivity were established (Yasukochi *et al.*, 2006)^[57]. Similarly, a high-density linkage map was developed using 518 SSR markers and a mapping population of more than 200 F₂ individuals. All silkworm chromosomes were covered by the map, and identification of genomic regions associated with traits such as larval growth rate, cocoon weight and silk productivity were enabled, indicating that these traits are controlled by multiple genetic loci (Miao *et al.*, 2008)^[26].

The molecular marker systems have also been used for identification of genes associated with important biological traits on the linkage maps of silkworm, *B. mori*. A linkage map was constructed using 208 polymorphic AFLP markers that covered approximately 1,360 cM of the silkworm genome and the genomic regions associated with traits such as larval weight, cocoon weight and silk productivity were identified through this map, demonstrating the usefulness of AFLP markers in genetic mapping studies (Nezhad *et al.*, 2011)^[32]. Similarly, bulked segregant analysis with 240 SNPs identified markers linked to cocoon traits in the two *B. Mori* strains, Pure Mysore and CSR2. Among these primers, 48 were found to be polymorphic, and one SNP marker (No. 04124) located on linkage group 4 was shown to be closely associated with cocoon and shell weight, indicating its usefulness for marker-assisted breeding (Sreekumar *et al.*, 2013)^[41].

The recent genomic studies have further improved the resolution of genetic mapping in *B. mori*. A high-density linkage map was constructed using 9,632 SNP markers derived from a cross between a domestic silkworm strain and its wild relative *B. mandarina*. The QTL analysis identified 11 loci associated with cocoon yield traits such as cocoon weight, shell weight and pupal weight, each explaining about 9–14 per cent of the phenotypic variation (Fang *et al.*, 2020)^[18]. Later, a genome-wide Silk_40K SNP array containing 41,887 SNP markers was developed to study silk yield traits in *B. mori*. Using this high-density marker system, 61 loci associated with cocoon shell weight and cocoon shell ratio were identified through QTL analysis. Candidate genes such as *BmOCIAD1* and *BmCOPB2* were detected within these QTL regions and, the role of *BmOCIAD1* in the regulation of silk yield traits was confirmed through functional validation using CRISPR/Cas9 (Li *et al.*, 2025)^[23]. These studies demonstrated that SNP-based genomic tools are highly effective for the identification of genes controlling economically important traits and for supporting marker-assisted breeding in silkworm improvement programmes.

Marker-assisted breeding for disease-resistant silkworm strains

Marker-assisted selection (MAS) is a modern breeding strategy that utilizes DNA markers linked to desirable traits to accelerate the selection process in breeding programs. MAS has been used to develop strains with enhanced resistance to viral diseases, higher cocoon productivity and better silk quality in silkworm improvement programs. Unlike conventional breeding methods that rely on phenotypic evaluation, MAS enables the identification of desirable genotypes at early developmental stages by detecting DNA markers linked to the target genes.

Nagaraju and Goldsmith (2002)^[28] described the potential applications of molecular marker technologies in silkworm breeding and emphasized that DNA markers such as RAPD, SSR and AFLP can be used to identify genomic regions associated with economically important traits including disease resistance, cocoon weight and silk yield. MAS enables early identification of individuals carrying desirable alleles without waiting for phenotypic expression, thereby accelerating the breeding programme and improving breeding efficiency. Similarly, Hou *et al.* (2013)^[21] demonstrated the practical application of marker-assisted breeding for developing virus-resistant silkworm lines by transferring the densovirus resistance gene (*nsd-2*) from a resistant strain into a commercial line through marker-assisted backcross breeding. DNA markers linked to *nsd-2* were used to select resistant individuals in each generation. The resulting hybrids showed about 25–30 percent higher survival rate after viral infection compared to healthy silkworms.

Molecular markers have also been widely used to identify productive silkworm strains and improve breeding efficiency. Bindroo *et al.* (2014)^[6] used RAPD markers to screen breeding populations and identify genetically superior individuals possessing desirable traits, leading to development of silkworm hybrids with improved productivity and better adaptability to tropical climatic conditions. Similarly, Chandrakanth *et al.* (2021)^[10] applied SSR markers to analyze genetic diversity among silkworm hybrids and identified loci associated with the economic

traits such as cocoon weight, larval growth rate and silk productivity. These studies demonstrated that molecular markers are valuable tools for selecting superior breeding materials and improving economically important traits in silkworms.

Recent studies have further emphasized the importance of MAS in sericulture improvement programs. Satish *et al.* (2024) ^[39] highlighted that advances in silkworm genomics have enabled identification of various DNA markers such as RFLP, RAPD, AFLP, SSR and SNP that are linked to genes responsible for disease resistance. Documented literature has reflected that the resistance genes such as *nsd-1* and *nsd-2* can be efficiently introduced into productive silkworm strains through MAS, enabling the development of hybrids resistant to major viral diseases including *BmNPV*, *BmDV* and *BmIFV*. Therefore, the integration of molecular markers with conventional breeding strategies has become an important approach for improving disease resistance, productivity and sustainability in the sericulture industry.

Genomic studies on host–pathogen interactions

Recent advances in genomics have greatly improved the understanding of host–pathogen interactions in silkworms. Genes and molecular pathways involved in immune responses against viral, bacterial and fungal pathogens have been identified through these studies. Valuable insights into the molecular mechanisms underlying disease resistance in *B. mori* have been provided by genomic tools such as genome sequencing, transcriptomics and proteomics.

The draft genome sequence of *B. mori* was reported as a major advancement in silkworm genomics. The genome was estimated to be about 530 Mb in size with approximately 18,510 predicted genes. Numerous genes involved in immune responses, including those encoding antimicrobial peptides, pattern recognition receptors and signalling molecules, were revealed through genome annotation. The identification of molecular markers and genes associated with disease resistance in silkworms was greatly facilitated by the availability of the complete genome sequence (Xia *et al.*, 2004) ^[53]. Similarly, genes related to resistance against *BmNPV* were investigated using fluorescent differential display analysis. Twenty differential expression bands were identified, of which 16 were sequenced and 10 candidate genes were detected. Higher expression of two genes, G12532 and G13313, was observed in resistant strains compared with susceptible strains, suggesting their possible role in antiviral defense mechanisms (Xu *et al.*, 2005) ^[54].

The studies on immune gene regulation have deepened the understanding of silkworm defense mechanisms against pathogens. Antimicrobial peptide genes such as *Cecropin* and *Attacin* were demonstrated to be strongly induced by DAP-type peptidoglycan from bacteria due to the higher activity of the transcription factor *BmRelish1* (Tanaka *et al.*, 2009) ^[44]. Several genes involved in pathogen recognition and immune signalling that activate immune pathways leading to antimicrobial peptide production were also identified (Tanaka and Yamakawa, 2011) ^[43]. Similarly, key role of Toll and Imd signalling pathways in regulating immune responses in silkworm through synthesis of antimicrobial peptides that help eliminate invading pathogens was reported by Zhang *et al.*, 2018 ^[58].

Advanced genomic and transcriptomic approaches have been used to study host–virus interactions in silkworms. Transcriptomic changes in *B. mori* cells infected with

BmNPV and *Autographa californica* multiple nucleopolyhedrovirus (AcMNPV) were analysed using next-generation sequencing, and many differentially expressed genes associated with immune response and cellular pathways were identified. Immune genes such as *Cecropin A* and *Cationic peptide 8* were shown to possess antiviral activity (Chen *et al.*, 2018) ^[14]. Through proteomic analysis, several proteins related to stress response, metabolism and immune signalling were identified as being significantly upregulated during viral infection (Wang *et al.*, 2021) ^[49]. Similarly, integrated genomic and proteomic analyses were applied to identify candidate genes associated with resistance to *BmNPV* infection and the viral resistance in silkworms was demonstrated to be regulated by a complex network of immune-related genes (Ouyang *et al.*, 2023) ^[33].

Molecular marker techniques have also been used to study the genetic diversity of silkworm viruses. Five *BmNPV* isolates were analysed using AFLP markers, and 235 DNA bands were detected, of which 202 (87 per cent) were polymorphic. Through cluster analysis, genetic similarity values ranging from 0.28 to 0.47 were recorded, indicating significant genetic variation among viral isolates (Peter *et al.*, 2016) ^[35]. Collectively, the importance of genomic, transcriptomic and proteomic approaches in understanding silkworm immune responses and identifying genes involved in resistance to viral diseases has been highlighted by these studies.

Summary

Molecular marker systems have become essential tools in silkworm research and breeding by providing accurate and high-resolution information on genetic variation. In *B. mori*, molecular markers such as RFLP, RAPD, ISSR, AFLP, SSR and SNPs have been widely used for genetic diversity analysis, identification of disease resistance genes, linkage mapping and QTL detection. These approaches have improved understanding of viral resistance against *BmNPV* and *BmDNV*, revealed diversity among silkworm races and identified genomic regions associated with cocoon yield and silk productivity. The availability of the silkworm genome sequence along with recent advances in genomics, transcriptomics and proteomics, has further strengthened molecular breeding through high-density marker development and functional validation of candidate genes. Thus, molecular markers have played a major role in modernizing silkworm improvement programmes and will continue to be valuable in future sericulture research and breeding.

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