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Role of Molecular Markers in Mulberry and Silkworm Improvement

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Abstract
Molecular markers play a significant role in the genetic improvement of both silkworms and mulberry plants. These markers are sequences of DNA that are associated with particular traits, and they provide a powerful tool for identifying and selecting desirable genetic variations. The application of molecular markers in silkworm and mulberry improvement programs offers several advantages, including increased precision in selecting for desired traits, faster breeding cycles, and the ability to combine multiple favourable traits into single strains or varieties. This technology ultimately contributes to more efficient and sustainable sericulture practices, enhancing both silk production and the overall health and productivity of mulberry plants. The various types of molecular markers used in silkworm and mulberry improvement are SNPs, ISSRs, RFLPs, AFLP, RAPD, ISSR, ESTs et cetra. In this article we have given comprehensive overview of molecular markers used in Silkworm and mulberry improvement.

Keywords: DNA, marker, trait, strains, varities, mulberry, silkworm, gene.

Molecular markers for mulberry improvement
The major application of mulberry plants in sericulture is its leaves which are used for silkworm rearing to produce silk. The cost of mulberry cultivation alone accounts for more than 60% of the overall costs associated with the manufacturing of cocoons, according to an estimate of the economic return on sericulture. This makes the creation of mulberry cultivars with high leaf yielding potential even more imperative. Numerous high-yielding mulberry cultivars, including S-1, K-2, Kokuso-13, Shin-Ichinose, V-1, S-13, S-34, S-799, S-30, S-36, S-54, Tr-4, G-9, RC-1, and RC-2, have been developed through traditional mulberry breeding techniques. However, mulberry growers faced a number of challenges in their breeding programs, including incomplete knowledge of mulberry genetics and inheritance patterns, a lack of pure lines, an inability to identify parents, dioeciousness, a decline in inbreeding, and a perennial nature with extended juvenile phase, absence of genetic markers, and effective screening techniques. The majority of the yield, along with related characteristics like leaf quality and yield, efficacy in nutrient uptake capacity, and resistance to biotic and abiotic stressors including root rot, root knot nematode, whitify, and frost. Leaf quality of mulberry is polygenic in nature and impacted strongly by genotype and environment interaction while exhibiting its feature. Thus, the circumstance necessitates the application of contemporary biotechnological methods such as the use of molecular markers, transgenesis to introduce and overexpress desired genes, or RNA interference technology to silence unwanted genes (Vijayan et al 2011). Molecular marker (DNA/RNA) is a specific physical unit on a chromosome whose inheritance pattern can be observed and studied well. Exploration of mulberry genome has created Morus database which is a boon in identifying the vital regions

of mulberry genome for exploiting in breeding process. Morus DB—Mulberry Genome Database (https:// morus.swu.edu.cn/) is one of the sites where all the details of genetic makeup and molecular markers such as SNPs, ISSRs, RFLPs can be accessed. Molecular markers enable fast and accurate identification of breeding lines, hybrids, cultivars and species, facilitate analysis of genetic diversity and also allow establishment of phylogenetic relationship with more precision than that was previously possible with morphological and biochemical techniques. The DNA markers are extensively used for screening genotypes, identification of gene of interest, characterization of germplasm collections and other gene related studies in mulberry (Feng *et a.,* 1996). There are different DNA markers such as restriction fragment length polymorphism (RFLP), random amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), inter simple sequence repeat (ISSR), simple sequence repeats (SSR) and single nucleotide polymorphism (SNP). Marker assisted selection (MAS) has got many advantages over conventional breeding methods as it enables the breeder to select desirable hybrids at the seedling stages, free from environmental interference, time saving, accurate and precise. MAS provide the potential for improving selection effiency by allowing early selection, reduced number of generations and population size. Genetic diversity, molecular characterization of germplasm and varieties, development of linkage and quantitative trait locus (QTL) map, association mapping, parental selection schemes and marker-assisted selection (MAS) are some of the important areas where molecular markers can play a great role for improvement of mulberry plants. There are plenty of reports on application of molecular markers for improvement of mulberry. Studies on molecular profile for genotypes and estimation of the genetic diversity among different germplasm collections of mulberries also resulted identification of SSR markers and SNPs from different mulberry species (Muhonja *et al.,* 2020). When whole-genome shotgun sequencing of the haploid mulberry species (M. notabilis) was reported, the introduction of marker-assisted breeding in mulberries gained even more momentum. The draft genome of 357 Mb included 128 Mb of repetitive sequences with 27,085 high confidence protein coding loci in tandem with complete gene structure (He *et al*.,2013). Using dominant markers like RAPD and ISSR, efforts were made to create QTL (Quantitative Trait Locus) maps that are unique to significant agronomic features like water use effiency (WUE), root attributes, and yield attributing characters in mulberry. These yielded few markers connections in the QTL map regulating the character of interest (Naik *et al*., 2013). However, the discovery of the marker that is closely associated with the specific characteristic in mulberry is still missing.

Molecular markers for silkworm improvement

In our nation, the practice of raising silkworms for the production of silk has a long history, and sericulture continues to play a crucial role in the rural economy of India (Datta, 1992). The nation possesses an exceptionally abundant genetic foundation for silkworms thanks to mulberry silkworms and wild silk-producing insects. Even though the silkworm genetic basis is so broad, little is known about the distinctive characteristics of many of these genotypes or the degree of genetic variation that exists either within or across the genotypes/races. Up until now, the genetic improvement of these priceless insects has typically been determined by their morphological traits, which are extremely biased and dependent on the environment. For this reason, a genuine technique such as MAS is needed for the characterisation and evaluation of genotypes. As far as mulberry silkworm, *Bombyx mori* L., is concerned, more than 400 visible mutations have been placed in the linkage maps which represent 217 loci consisting of mostly morphological and a few isozyme markers. Research on genetics of silkworm across the world has helped in establishing silkworm genome database called silk base (http://silkb ase.ab.a.u-tokyo.ac.jp/cgi-bin/index. CGI). This has become an integral part in exploring the genome of silkworm for improvement of sericulture industry. Exploring

genome of silkworm has revolutionized the sericulture industry by identifying the molecular markers using linkage mapping. This led in identifying key regions in the genome of silkworm for specific phenotypes that have changed the face of the silkworm breeding. Hence, a lot of work on DNA based genetic markers in silkworm started emanating in the 1990s and a preliminary linkage map of 169 loci using RFLPs and RAPD (Promboon., 1995) was constructed. Studies on PCR-based markers, RAPD markers, and DNA fingerprinting with minisatellite probes in respect to Indian silkworm have also been carried out. Further, thirteen silkworm strains were used for genetic characterization using inter simple sequence repeats (ISSR). The ISSR-PCR produced 39 fragments of which 76.98 per cent were polymorphic and the diversity index was observed to be 0.957 percent. Afterwards, for complete genome analysis of the silkworm, Bombyx mori L., expressed sequence tag (EST) database was constructed covering about 55% of all the genes of silkworm. To identify and mapping of sex-linked traits in the silkworm genetic mapping of Z chromosome and identification of W chromosome- specific markers in the silkworm were carried out (Nagaraja 2005). (Fang *et al.*, 2020) while studying genomic analysis of cocoon and associated traits in silkworm through restriction-site-associated DNA sequencing (RAD-Seq) reported identification of a total of 11 cocoon yield-related QTLs on 7 chromosomes using the composite interval mapping (CIM) algorithm. The research on tasar silkworms also show that molecular markers are consistently superior in detecting the genetic composition, population structure, and relationships between the several Eco races of Antheraea mylitta. When examining the molecular characterization of the Tasar ecoraces Daba and Andhra, (Rao.,2005) noted the presence of specific common bands in both ecoraces, suggesting a potential genetic link between them. Using ISSR markers, (Chatterjee et al., 2004) revealed significant genetic heterogeneity in six distinct wild tasar ecoraces. Significant differences were also found between semi-domesticated Daba bivoltine, Daba-trivoltine, and Daba reared in the wild, as determined by ISSR markers (Kar *et al*,. 2005).

Their report further suggested that semi domesticated population of Daba Eco race was at the threshold level of discriminating themselves. Genetic diversity between and within population of Raily, Daba and Modal was also studied by using 12 ISSR and 10 RAPD primers. Population structures of eight different Eco races using ten amysat microsatellite loci were studied by (Chakraborty *et al*.,2005). Exploration of the silkworm genome has helped to identify specific markers for thermotolerance, no glue eggs, silkworm resistance to viruses, regulation of moulting, regulation of body shape etc. Silkworm research in India by Central Silk Board has made advances by successfully identifying microsatellite markers linked to thermotolerance by Bulk Segregation Analysis (Chandrakanth *et al*.,2014).

Conclusion

Modern biotechnological advancement has assumed greater importance in the development of sericulture and its diversification Molecular markers are critical tools in genetic studies and breeding programs for improving silkworm (*Bombyx mori*) and mulberry (*Morus spp*.) Using these molecular markers, breeders can implement marker-assisted selection (MAS) to accelerate the breeding process and enhance the genetic improvement of silkworm and mulberry. Hence can play imperative role in the sustainable development of sericulture industry.

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