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Genomic approaches to ensure a more sustainable and productive future of mulberry for sericulture industry

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Abstract
Over the past few decades, the field of genomics has made significant strides in understanding the molecular basis of plant growth and development, and mulberry (*Morus* spp.) is no exception. Mulberry is a highly valued plant in sericulture due to its nutritional and medicinal properties. With the help of genomics, researchers are uncovering new ways to improve mulberry productivity and quality. One of the key advancements in mulberry genomics has been the identification and characterization of genes involved in key metabolic pathways. By studying these pathways, researchers have gained insights into the molecular mechanisms that regulate mulberry growth and development, and have identified potential targets for genetic improvement. With the advent of high-throughput sequencing technologies, it is now possible to rapidly sequence the entire genome of a plant and identify genetic markers that are associated with desirable traits. By using these markers, breeders can select for specific traits with greater precision and efficiency. Overall, the emergence of genomics in mulberry research holds great promise for improving productivity and quality for such important plant. By continuing to uncover the molecular mechanisms that regulate mulberry growth and using these insights to guide breeding efforts, researchers can ensure a more sustainable and productive future for sericulture.

Keywords: Mulberry, sericulture, genomics, metabolomics, transcriptomics, QTL mapping

Introduction
Silk, commonly referred to as the "Queen of fabrics," was solely used by royalty in ancient China. People have been enthralled by its opulent and exquisite features throughout history (Sarkar *et al*., 2018) [53]. The Moraceae family's deciduous woody perennial tree known as the mulberry is renowned for its quick development. Mulberry carries enormous economic significance due to its application in sericulture in several emerging Asian nations (Dutta *et al*., 2023a) [17]. To ensure the sustained growth of the sericulture business in light of the escalating global climate changes and the scarcity of both land and water, it is essential to create mulberry types suited for various agro-climatic situations. Current biotechnology developments in mulberry have the potential to lead to enhanced technologies for its cultivation, improving sericulture's economy and the lives of individuals who practice it (Dutta *et al*., 2023a) [17]. Mulberry is said to have originated in the lower slopes of the Himalayan range, bordering China and India (Dutta *et al*., 2023a) [17]. Due to its dioecious nature and unusually asynchronous flowering, particularly in tropical variants, the mulberry plant presents a difficulty for breeding efforts (Vijayan *et al*., 2022a) [59]. The *Morus* genus is separated into two sections namely Dolichostylae and Macromorus on the basis of taxonomy of mulberries. Also, based on the type of stigmatic hair, each part is separated into two groups namely Papillosae and Pubescentae (Chang *et al*., 2014) [5]. Around 68 species are currently well known, of which there are 24 recognized species and one subspecies overall in this group. Only a few of these species, *Morus alba*, *Morus indica*, *Morus bombycis*, *Morus latifolia, and Morus multicaulis* are grown for their leaves to feed silkworms, while *Morus nigra* is raised for its fruits. In terms of ploidy levels, mulberry shows a range across the species. The majority of naturally occurring species are diploids (2n=2x=28), but there are also tetraploids (2n=4x=56; *Morus laevigata*, *Morus cathayana*, and *Morus boninensis*), hexaploids (2n=6x=84; *Morus serrata and Morus tiliaefolia*), octoploids (2n=8x=112; *M. cathayana*), and even docosaploids (2n=22x=308; *M. nigra*) and haploids (*M. notabilis*) with 14 chromosomes

that occur naturally (Dutta *et al*., 2023a) [17]. Given that mulberry is a clonally propagated plant, clones are largely used to protect mulberry genetic resources. The collection and characterization of mulberry germplasm, adherence to conservation protocols, and evaluation of genetic and agronomic features are only a few of the efforts that go into its preservation. The Central Sericultural Germplasm Resources Center in India has actively contributed to the preservation and reinforcement of mulberry germplasm with 1109 unique accessions, including 845 native and 264 foreign accessions. Several mulberry germplasm accessions have been preserved using standard cryopreservation techniques, enabling the long-term use of these various resources. There are no differences between the mother plant and *in vivo* regenerated cryo samples as suggested by genetic stability analyses utilizing DNA markers made with ISSR primers. The need for the creation of specific preservation protocols is highlighted by the poor rate of wild species survival (Tikader, A., Dandin, 2008) [56]. Mulberry breeding traditionally takes a long time and is difficult because of the plant's heterozygous nature. Thus, biotechnology techniques present a potentially effective way to enhance mulberry (Vijayan, 2010) [58]. In addition to conventional breeding, modern biotechnological tools including plant tissue culture, r-DNA technology, and molecular markers are being employed to improve the genetics of mulberries. Mulberry tissue culture techniques, including micropropagation, plant regeneration from leaf discs, and stress tolerance testing, have evolved dramatically (Sarkar *et al*., 2018) [53]. Recently, genetic engineering has been used to improve ability to withstand salt and drought. The main goal of these biotechnological methods is to enhance the host plant as a whole in order to exploit and use it in sericulture. These developments have also aided in the isolation of somaclonal variants, screening of germplasm for tolerance to abiotic stresses, production of synthetic seeds, cryopreservation of genetic resources, creation of transgenic plants, characterization of germplasm accessions, and the discovery of markers linked to economically significant traits (Dutta *et al*., 2023a) [17]. The yield of high-quality mulberry leaves is crucial for the sustainability of sericulture because the silkworm, *Bombyx mori* L., only consumes mulberry leaves. Mulberry is an excellent choice for animal food because it is also delicious, edible, and digestible (Dutta *et al*., 2023a) [17]. Mulberry fruits can also be used to make fresh fruit, jam, marmalade, pulp, juice, paste, ice cream, and wine for human consumption. Mulberry leaves are utilized as feed for animal husbandry, making it an integral part of mixed pastoral systems. Mulberry has unique therapeutic substances with antimicrobial, anti-hyperlipidemic, anti-hyperglycemic, anti-diabetic, chemo-preventive, neuroprotective, and anti-oxidative potential in its leaves, fruit, stem, seed, and roots (Dutta *et al*., 2023a) [17]. However, there have not been many attempts to take advantage of recent developments in plant genomics for mulberry genetic improvement (Khurana & Checker, 2011) [28]. Hence, the purpose of this paper is to give a general review of the genomic technologies that can improve the adaptability and productivity of mulberries.

Traditional breeding in mulberry

The main goals of mulberry breeding are to increase the output of high-quality cocoons and to make mulberry leaves more palatable to silkworms. These objectives are dependent on a number of quantitative properties, such as leaf retention capacity, leaf size and weight, total biomass, pest and disease resistance, and tolerance to abiotic stresses like drought, salinity, and cold stress (Rafiqui *et al*., 2022) [43]. The majority of the leaf yield component features are regulated by both additive and nonadditive gene activities in mulberry. Hence, a promising method for increasing mulberry production potential involves utilizing nonadditive genetic variation through clonal propagation (Dutta *et al*., 2023a) [17]. By creating several mulberry varieties with good agronomic qualities, traditional plant breeding techniques significantly contributed to the horizontal expansion of sericulture. Several cultivars have been made available for use in commerce. The ultimate objective is to develop a new mulberry variety that possesses every desirable characteristic in a single genotype and can be utilized to feed the silkworm (*Bombyx mori*) and generate premium cocoons. Salinity-resistant mulberry cultivars have been developed as a result of these efforts (Sarkar *et al*., 2018) [53]. Therefore, it is crucial to produce mulberry varieties that are particular to different agro-climatic zones in order to assist the vertical development of sericulture. There are currently no mulberry cultivars available that are ideal for situations like alkaline, saline, acidic soil, and flooding. Obstacles like intrinsic genetic constraints, the heterozygous and perennial nature of the plant system, the lack of knowledge about how different plant features are inherited, and genetic markers have made traditional breeding methods difficult. Abiotic stressors like salt and drought also have a negative effect on mulberry productivity and are complicated quantitative features that are challenging to breed for and select phenotypically (Sarkar *et al*., 2018) [53]. Mulberry lacks genetic diversity, which makes breeding initiatives to enhance the plant's genetic makeup difficult. Mulberry germplasm has sufficient genetic diversity, but current breeding efforts frequently use the cultivated varieties of mulberry (*M. alba* and *M. indica*), which reduces the variety of genes accessible for breeding. Integrating wild/exotic species like *M. laevigata* and *M. serrata* into current breeding programs can offer unique genes that influence essential features like abiotic and biotic responses, which can help to overcome this constraint. Yet, because it can take up to 15-20 years for mulberries to generate a new variety, it is crucial to comprehend how genetics regulate critical agronomic properties in domesticated species. Moreover, improvements in breeding methods and sericulture practices could be made possible by developments in genomics and molecular biology approaches, increasing mulberry productivity in difficult environmental conditions (Vijayan *et al*., 2009) [60].

Unraveling the genetic diversity using molecular markers

For mulberry varieties to be used in the sericulture sector, they must be genetically characterized. Traditional methods for mulberry genetic diversity analysis have relied on morphological and physiological variables, however, these characteristics can be altered by environmental factors and developmental phases (Khurana & Checker, 2011) [28]. Because of their high polymorphism, repeatability, and independence from tissue and environmental factors, DNA markers have gained widespread acceptance (Das *et al*., 2022 & 2023) [15, 16]. The most popular marker method, ISSRs, is frequently used in conjunction with RAPD and SSRs to identify genetic diversity (Table 1). SNPs are widely distributed across the genome of every living being, making

them a potent tool in marker technology. The fact that no SNPs have been discovered in mulberry, however, emphasizes how critical it is to provide expressed sequence tags (ESTs) in open databases for SNP mining and the creation of additional DNA-based markers. Mulberry SNP discovery may help with genetic research, association studies of potential genes and phenotypic variation, QTL mapping, evaluations of genetic diversity, and synteny-based comparisons with model crop species. The discovery of QTLs for traits connected to biotic and abiotic stressors can also benefit from the use of molecular markers (Khurana & Checker, 2011) [28].

Linkage mapping in mulberry

The discovery of molecular markers that are tightly connected to crucial features through genetic linkage mapping can be a useful method to hasten the creation of mulberry varietals (Vijayan, 2010) [58]. Building a genetic map from a population that segregates for an interest trait is necessary to find QTLs. Various studies sought to determine satellite imaging using drones to track leaf harvesting on mulberry bushes grown for silkworm food (Dutta *et al*., 2023a) [17]. Despite the significance of genetic maps, there are not enough tools and resources available for genetic, genomic, and breeding studies on mulberries. For the majority of plants, segregating populations like F_2, backcrosses, doubled haploids, recombinant inbred lines, and near-isogenic lines are used to create linkage maps. Due to a lengthy generation period and a large genetic load, populations with high genetic diversity are frequently challenged to generate or grow in perennial crops like mulberry that are subject to outbreeding. As a result, linkage maps in tree crops are often produced using a "pseudo-testcross technique" from F_1 progenies, either full or half-siblings. This approach posits that in the progeny, dominant markers that are heterozygous in one parent but null in the other will segregate in a 1:1 ratio. It is possible to create linkage maps utilizing data from such crossings using a variety of software programs. The drawback of this approach is that linkage maps must be created for each elite breeding line because they are individual-specific and cannot be integrated or aligned (Vijayan, 2010) [58]. Microsatellite markers and other multiallelic codominant markers can be integrated into maps; however, this is not always practical. Notwithstanding these drawbacks, pseudo-testcross linkage mapping has been established in various tree taxa, including *Eucalyptus*, *Populus*, and others. A genetic linkage map of the mulberry was created in 2006 by Venkateswarlu *et al*., 2006 [57] utilizing 50 F_1 full-sib descendants, RAPD, ISSR, and SSR markers, and a pseudo-test cross mapping technique (Table 2). They created distinct male and female maps with 94 male-specific and 94 female-specific test cross markers and 100 RAPD, 42 ISSR, and 9 SSR primers that amplified 517 markers. In comparison to the male map, which covered an average distance of 18.78 cm and a maximum distance of 34.7 cm, the female map covered an average distance of 15.75 cm and a maximum map distance of 37.9 cm. Since the markers were scattered at random across the linkage groups, the information produced by this map has minimal utility. A thorough linkage map incorporating data from various mapping populations with enough markers is required to detect QTLs and enable marker-assisted selection (Dutta *et al*., 2023a) [17]. These maps should compare the locations of QTLs and candidate genes of interest across germplasm, determine the relative positions of transferable markers, increase the number of DNA markers that are readily available, and generate saturated maps.

QTL discovery and marker-assisted breeding in mulberry

It is obvious that growth-related features are essential for the production and adaptation of mulberry leaves. It is crucial to understand these features using a genetic approach in order to handle them effectively and efficiently (Rukmangada *et al*., 2020) [48]. Nonetheless, both forward and reverse genomic techniques have shown promise in discovering genes involved in the development of numerous characteristics and chemical pathways in a number of tree species, including *Eucalyptus*, *Pinus*, and *Populus*. (Grattapaglia *et al*., 2009) [21]. To find the underlying genetic variants for phenotypic features, forward genomics analyses the current phenotypic traits (Figure 1). In reverse genomics, particular genes are altered using transgenic techniques such as insertional mutagenesis, gene overexpression, RNA interference (RNAi), and miRNA methods to investigate the association between the gene and the phenotype. To find QTLs, a variety of techniques are used, including the single marker approach, interval mapping, and composite interval mapping. There are numerous software programs that can be used to find QTLs. The pseudo-testcross mapping approach only picks up QTLs that are heterozygous in one or both parents and are unaffected by dominance or the environment in which phenotyping takes place when applied to QTLs in tree species like the mulberry. Selective genotyping may hinder the accuracy of mapping several linked QTLs. Results of QTL mapping are strongly influenced by the number of independent measurements per line and the number of lines used for analysis. Repeated measurements from big line collections are essential since most populations have numerous QTLs with minor effects that can combine to produce a significant effect. The success of QTL mapping in mulberry also depends on accurate phenotyping, which requires accurately identifying attributes, evaluating the environment, planning trials, documenting data, and choosing the right analytical techniques. According to Dutta *et al.* (2023a) [17], since over 70% of the protein in mulberry leaves is required to make silk proteins like fibroin and sericin, the nutritional status of mulberry leaves has a significant impact on the quality of cocoons made by adult silkworms. Using both genetic and metabolic strategies, the nutritional value of mulberry leaf can be improved. This objective can be attained with the help of metabolic profiling, which provides insights into plant metabolic networks. The identification of the genetic factors influencing crop quality has also been successful using pathway-based methods. With the help of these techniques, numerous metabolic QTLs (mQTLs) in crops like tomato, cucumber, and sesame have been effectively discovered. Mass spectrometry and nuclear magnetic resonance are the two methods for metabolic profiling that are most frequently employe (Waseem *et al*., 2022) [63]. With the immense potential of mQTL mapping, it might be used to improve mulberry leaf quality, boosting silk production per unit area and enhancing sericulture sustainability. Molecular markers that are strongly associated with desirable qualities are necessary for marker assisted selection (MAS) for transfer of favorable genes (Alam *et al*., 2021) [1]. As opposed to conventional linkage mapping, which uses limited recombination in biparental mapping populations, association mapping makes use of historical and

evolutionary recombination events. By comparing significant allele frequency differences between individuals with the desired phenotype and a group of unrelated control individuals, LD mapping analyses genetic diversity in natural populations to find molecular markers that are closely linked to complex phenotypic traits. Depending on the type of study, LD mapping can be divided into genome-wide association mapping and candidate gene association mapping. While genome-wide association mapping examines genetic variation throughout the entire genome to find genes or specific regions that have statistically significant relationships to a variety of complex traits, candidate gene association mapping focuses on polymorphisms in selected candidate genes with phenotypic variation for particular traits. Linkage disequilibrium can be viewed using many software tools. In many tree plants, association mapping has previously proven effective in identifying gene-trait connections for crucial growth features such as phenology, disease resistance, drought tolerance, and wood qualities (Grattapaglia *et al*., 2009) [21]. As mulberry is a heterozygous and perennial tree plant, genome wide association study or linkage disequilibrium or association mapping using co-dominant markers like SSRs, SNPs could be a viable option for identification of QTLs and candidate genes associated with climate resilient traits such as drought adaptation, nitrogen use efficiency, foliage as well as other yield-components, and disease resistance (Sarkar *et al*., 2018; Maity *et al*., 2023) [53, 33]. Although no attempts at association mapping in the mulberry plant species have been done.

Mulberry transcriptomics

Genetic and genomic techniques are preferred in order to speed up the discovery of crucial genomic regions and simplify the laborious process of mulberry breeding (Dutta *et al*., 2023a) [17]. A useful toolkit for investigating new genes and using them in genetic engineering is the mulberry genomics toolbox (Checker *et al*., 2012) [9]. Complete sequencing of the mulberry genome is not currently justified due to its huge size and dearth of relevant genetic and physical maps (Priyadarshan, 2016) [42]. Yet, large-scale EST (Expressed Sequence Tags) sequencing enables the direct identification of a variety of genes and offers a very affordable method for functional genomics in mulberry. Moreover, ESTs provide details on gene structure, alternative splicing, expression patterns, and transcript abundance and are essential for accurate genome annotation (Dutta *et al*., 2020a) [19]. Until to 2009, there was not much information on mulberry functional genomics tools. Nevertheless, a large number of EST experiments have been carried out recently, producing a wealth of data for the study of functional genomics. By examining and sequencing ESTs taken from mature mulberry leaves, researchers are able to start the process of mulberry transcriptomics (Li *et al*., 2022) [32]. Based on their sequence homology with databases that were accessible, these ESTs were divided into functional groups, however, the majority of them did not show any appreciable homology with known proteins. Finding novel genes that have not yet been identified could be especially interesting because they represent difficult genes that need more research. The analysis of genes associated with various categories, such as abiotic and biotic stresses (LEA, RD22, dehydrins, and Hal3) and membrane transporters (vacuolar Na^+/H^+ antiporter and aquaporins) under various stress conditions, can be a starting point for developing various strategies for the genetic enhancement of mulberry (Lal *et al*., 2008) [31]. The researchers examined ESTs from the root tissue of *Morus indica* cv. K-2 and found genes encoding enzymes involved in the generation of secondary metabolites. The researchers were able to learn more about tissue-specific transcriptional activity by contrasting the ESTs from leaf and root tissue. A total of 2,599 unigenes from mulberry leaf and root ESTs were assembled by the researchers, and they determined that EST-SSRs are a practical method for marker-assisted breeding operations. Suppression subtractive hybridization (SSH), in addition to transcriptome analysis, was utilized by the researchers to create cDNA libraries depicting differentially expressed transcripts that are particular to distinct tissues. SSH is an effective method for locating genes that are differentially expressed and can offer useful details for comprehending the functioning of various tissues in the mulberry (Dutta *et al*., 2023a) [17]. Stress-responsive genes that support mulberry drought tolerance were found in the ESTs isolated from the library. Several of the stress-responsive genes were discovered to be involved in the drought stress response using cDNA micro array and northern analysis of the stress-responsive genes. Many cDNA clones encoding remorin genes have been found in numerous plant species since the initial remorin gene was found in the potato. Remorin genes are thought to be present in 16 and 19 copies, respectively, in the *Arabidopsis* and *Oryza* genomes. It was required to examine their expression patterns in order to establish their activities in order to understand the signaling networks in which remorin genes participate (Checker & Khurana, 2013; Dutta *et al*., 2020b) [7]. Several of these proteins are hypothesized to be important in biotic and abiotic stimuli and to be engaged in hormone-mediated reactions and signal transduction. The thylakoid membrane contains enzyme b-carotene hydroxylase, which is in charge of the zeaxanthin biosynthesis pathway by converting b-carotene to zeaxanthin (Dutta *et al*., 2020b). The expression of *bch1* investigation in mulberries revealed that salicylic acid, ABA, high temperatures, and UV radiation all induce its expression (Saeed *et al*., 2014) [49]. Yet, when exposed to UV and high temperature stress, its expression greatly rises. Pan and Lou, 2008 [40] discovered the *1-aminocyclopropane-1-carboxylate oxidase* gene in mulberry and studied how it responded to stress by ethylene production. Proteins called lectins, which bind to carbohydrates, are frequently utilized in scientific studies. The purification of mulberry lectins was initially studied to reveal its antibacterial role against the mulberry tree-damaging bacterium *Pseudomonas syringae* (Ratanapo *et al*., 1998) [46]. Jacalin-related lectins with decreased carbohydrate-binding sites, which dictate their exclusive specificity towards glucose or mannose, are found in the bark of the black mulberry (*M. nigra*) tree. The binding characteristics of these mannose-binding lectins with various ligands were subsequently investigated, which contributed to understanding how lectins recognize cell surface carbohydrates. These results highlight the potential of lectins for biotechnological and clinical research.

Mulberry proteomics

Proteomics is the systematic examination of the proteome found in a tissue, cell, or subcellular compartment of an organism. This method makes it possible to directly examine changes in protein expression patterns, which can offer

crucial information on biological function and the underlying gene products. However, because there are not yet enough data to support functional genomics, the application of proteomics in mulberry is still in its infancy. The lack of a comprehensive sequencing library to match tryptic digest-generated peptides to the proteins that made them is one of the main obstacles to applying proteomics to mulberry. Notwithstanding this disadvantage, a small number of mulberry researchers have used proteomic techniques. For example, Kumari *et al*., (2007) [30] investigated the effects of salinity on proteome alterations in two different mulberry cultivars in an effort to identify genes involved in salinity tolerance that could be targeted for genetic modification to improve salt tolerance in mulberry. Three-month-old plants were exposed to varying salt concentrations over the course of seven days. Two-dimensional electrophoresis was used to evaluate the soluble proteins that were produced. The polypeptide patterns in both plant types were altered in both qualitative and quantitative ways by the application of salt stress. The proteins that were differentially regulated as a result gave researchers a thorough grasp of the biochemical and molecular processes involved in the development of salt shock tolerance. Similar to this, fresh information has been gained through a proteomic investigation of mulberry dwarf responses brought on by phytoplasma in mulberry. A serious infectious illness called mulberry dwarf is brought on by phytoplasma (Ji *et al*., 2009) [24]. The protein profiles of infected and healthy leaves were compared using two-dimensional electrophoresis in order to understand the pathogen of mulberry under stress response. Quantitatively altered spots were found using the mass spectrophotometry technique, which revealed a variety of proteins involved in the body reaction to infection. These proteomic studies are crucial because they uncover novel protein elements involved in growth and stress tolerance.

Mulberry metabolomics

Mulberry is rich in vitamins, minerals, and useful secondary metabolites, which have huge commercial value (Sarkar *et al*., 2018) [53]. Mulberry latex contains high concentrations of alkaloidal sugar-mimic glycosidase inhibitors (1,4-dideoxy-1,4-imino-D-arabinitol, 1-deoxynojirimycin, and 1,4-dideoxy-1,4-imino-D-ribitol), which are toxic to caterpillars but not to the silkworm *Bombyx mori*, suggesting coevolution between the mulberry tree defense mechanism and silkworms (Konno *et al*., 2006) [29]. Furthermore, the results imply that latex components could be useful sources for the search for novel chemicals. Bae and Suh (2007) [2] examined the anthocyanin composition, concentration, and antioxidant potential of five significant mulberry cultivars after recognizing the relevance of anthocyanin pigments in mulberries. The amount of 1-deoxynojirimycin (DNJ), resveratrol, oxyresveratrol, anthocyanin, and flavonoid was also investigated by Song *et al*., (2009) [54] in 38 cultivars of mulberry fruits and 33 cultivars of mulberry leaves. They discovered that the content of these chemicals varied significantly among various species and cultivars. In addition, six new compounds (cathayanons F–J and cathayanin A) and two known compounds (cathayanins B and C) were isolated and identified as a result of phytochemical studies on the stem bark of *Morus cathayana*, some of which showed marginal efficacy against human cancer cell lines. HPLC was used to separate and isolate the bioactive secondary metabolites that UV-B irradiation also caused to be produced *in vitro*. Five chromatographic peaks with significant modifications were found in the HPLC-DAD fingerprint. The study also advanced the theory that the number of active ingredients present is influenced by the harvesting season. Using northern blot analysis, the expression patterns of the mulberry genes *MiSMT* (*Morus indica*, *Sterol Methyl Transferase*) and MiVR (*Morus indica*, *Vestitone Reductase*) were investigated in ten distinct genotypes under both control and salt stress conditions (Khurana & Checker, 2011) [28]. For both of these genes, notable differences were found in various genotypes. The analysis of these vital bioactive components of metabolic processes and responses to environmental stresses showed that they may play a role in promoting health benefits. This finding has created a number of fresh research opportunities in a range of pharmaceutical and natural product fields. In order to examine gene activities and metabolic status, metabolic profiling of mulberry on a genome-scale would offer compelling findings.

Mulberry plastomics

Plant systematists and taxonomists can swiftly and precisely identify species to provide access to a species-specific database. It is the perfect tool for phylogenetic investigations because of the conservation of gene order, content, and lack of recombination. Using a combination of lengthy PCR and a shotgun method, one of the ground-breaking areas of mulberry research identified the full sequence of the mulberry chloroplast genome (Chen *et al*., 2015) [10]. This is the first full genome from India as well as the first case study from the subfamily Hamamelidae. The 158,484 base pair circular double-stranded DNA of the mulberry chloroplast genome is made up of two identical inverted repetitions of 25,678 base pairs each, which are spaced apart by a large and a small single-copy area of 87,386 and 19,742 base pairs, respectively. Comparing genes from sequenced chloroplast genomes revealed a total of 83 protein-coding genes, including five genes duplicated in the inverted repeat regions, eight ribosomal RNA genes, and 37 tRNA genes (representing 20 amino acids and 30 gene species). The mulberry plastome does not contain the genes *infA*, *sprA*, and *rpl21* but does have the pseudogenes *ycf15* and *ycf68* (Ravi *et al*., 2006) [47]. With the presence of non-coding areas, comparative study at the genome level reveals that Eucalyptus and Cucumis are the closest relatives of *Morus*. This data suggests that the selection pressure on genic and intergenic regions varies during evolution. Since the cpDNA sequence data has proven essential in the phylogenetic and molecular taxonomy of plants, it is imperative to investigate the genetic storehouse of this organelle. The chloroplast genome contains the instructions for many parts of photosystems and metabolic pathways, hence sequencing the plastome is crucial. Because these cpDNA genes have very little possibility of dissemination in this cross-pollinated crop, cpDNA sequences show significant promise for transgenic expression to improve mulberry (Chen *et al*., 2015) [10].

The revolution in tissue culture and transgenic technology in mulberry

Due to competition with other food and cash crops, mulberry is imperative to utilize marginal, problematic soils and non-traditional areas affected by various abiotic stresses such as alkalinity, salinity, and moisture deficit for mulberry

cultivation. Abiotic stress tolerance in plant systems is a quantitative trait that involves interaction among several genes through signal transduction pathways (Vijayan *et al*. 2022) [59]. Successful tissue culture plant regeneration is important for direct cultivar improvement and essential for genetic engineering and transformation studies. Especially for commercially significant cultivars, reliable and efficient regeneration techniques for the mulberry crop have been documented during the past 40 years. Axillary and apical buds are frequently utilized explants for mulberry *in vitro* growth. In addition, leaf, cotyledon, and hypocotyl explants have also proven successful in regenerating mulberry (Dutta *et al*., 2023a) [17] (Table 3). Although temperate and tropical mulberry has shown somatic hybridization by protoplast fusion, its practical application has not yet been accomplished. Despite some early advancements in androgenesis, producing haploid plants in mulberry is likewise difficult and has a low success rate. Similarly, although mulberry gynogenic plants have been recorded, no effective commercialization attempts have been made with them. A thorough assessment of earlier mulberry tissue culture experiments has been conducted. *Morus indica* is a difficult woody plant, but it can be effectively changed using optimal methods using particle bombardment or *Agrobacterium tumefaciens*. These procedures are adaptable to different cultivars and work effectively on the K-2 cultivar. Conventional plant breeding has not significantly improved abiotic stress resistance, which has resulted in financial losses in recent years. As a result, transgenic methods for molecularly customizing crops have gained importance (Dutta *et al*., 2023b) [18]. For targeted gene-based transgenic techniques that can introduce advantageous genes providing stress tolerance in mulberry, genomic technologies can offer useful information on the molecular basis of stress tolerance (Vijayan *et al*., 2022) [59]. A single gene expressing stress-inducible transcription factors has been reported to control numerous stresses at once. In order to develop a potent transgenic strategy, it is necessary to test potential transgenic plants in a variety of pertinent conditions in order to understand how the inserted gene affects the entire plant. Functional data from numerous studies indicate that the expression of *Hva1* has been highly effective in reducing stress response (Sarkar *et al*., 2018) [53] (Table 4). The barley *Hva1* gene was overexpressed in transgenic mulberry plants utilizing *Agrobacterium*-mediated transformation. Comparing transgenic plants to non-transgenic plants, a thorough examination revealed that the transgenic plants performed better under simulated saline and drought conditions. The transgenic plants had superior relative water content, higher photosynthetic yield, better cell membrane stability, and reduced photooxidative damage. Hence, in transgenic plants, the *Hva1* gene confers a wide range of tolerance to various abiotic environments. To determine if these transgenic plants were suitable for raising silkworms, preliminary investigations were carried out. The transgenic mulberry plants containing the barley *Hva1* gene under the control of the *CaMV35S* promoter were discovered to have growth retardation under normal circumstances (Checker *et al*., 2011) [8]. Instead of using the constitutive *CaMV35S* promoter to overexpress *Hva1*, the stress-inducible *rd29A* promoter was used to limit detrimental impacts on plant growth. The findings show that *rd29A* and *Hva1* work well together to promote plant tolerance to a variety of stresses while limiting adverse impacts on growth. In order to assess how transgenic mulberry lines flourish in natural settings, field studies have been started. Moreover, tobacco *Osmotin* regulated by both a constitutive (*CaMV 35S*) and a stress-inducible promoter (*rd29A*) was successfully used to genetically modify mulberry. Several plant species respond to diverse biotic and abiotic stresses by producing osmotin and osmotin-like proteins, which belong to the plant PR-5 group of stress proteins. Transgenic plants were physiologically analyzed under simulated salinity and drought stress as well as fungal challenges to determine the impact of the incorporated gene. In comparison to transgenic plants with the constitutive promoter, those with the stress-inducible promoter were better able to withstand salt and drought stress. However, *CaMV35S: osmotin* transgenic plants performed better in terms of fungus resistance. Hence, *osmotin* gene transformation of mulberry would give tolerance against salt, drought, and fungal diseases. The last consumers of mulberry leaves (silkworms) took to these transgenic plants favorably. The *bch1* (*b-carotene hydroxylase-1*) gene was used to genetically alter *Morus indica* cv K-2, and the resulting transgenic mulberry plants showed better resistance to stress from high temperatures, high light, and UV radiation. Under stress, transgenic mulberry plants were shown to accumulate more xanthophylls than non-transgenic plants, as shown by ultra-performance liquid chromatography. By overexpressing the *b-carotene hydroxylase-1* gene, this represents the first successful attempt to alter the carotenoid production route in mulberry (Sarkar *et al*., 2018) [53]. Yet, transgenesis must be done carefully because mulberry is a plant that is heavily cross-pollinated. Because transgenes in chloroplast DNA have a low possibility of spreading through cross-pollination, chloroplast genetic engineering is thought to be a safer approach to create transgenic plants (Sarkar *et al*., 2018) [53].

Other miscellaneous smart methods

For the investigation of complex properties in trees, the use of reverse genomic methods including insertional mutagenesis, gene overexpression, and RNAi is becoming more widespread. By creating insertional mutants with activated or suppressed target genes, insertional mutagenesis is used to discover phenotypes. In transgenic plants, overexpression of dominant genes results in observable phenotypic changes, and this approach has been proven successful in trees. By delivering double-stranded RNA to cause sequence-specific RNA degradation and so effectively silencing the targeted gene, RNAi is another potent method used to verify gene activity in plant development. A particularly promising method for identifying the genes involved in various metabolic pathways is RNAi. Applying these strategies to mulberry requires an effective transformation system. In order to research trait-gene correlations in mulberry utilizing reverse genetics and gene overexpression, breeders now have access to an effective genetic transformation technology (Vijayan, 2010) [58] (Figure 2). Thus, mulberry could be revolutionized as a potential perennial model tree system for genetic research (Dutta *et al*., 2023a) [17].

Drawbacks and future prospects

Although mulberry is a very important plant for the Indian economy, it takes a lot of experimentation across many conditions to create new mulberry kinds by observing physical traits. Also, it takes mulberry bushes at least 3 to 4

years to effectively establish themselves and manifest significant agricultural characteristics. Data collection must span at least three years in all seasons in order to be considered reliable for statistical analysis. It takes a lot of space, time, work, and money to test a lot of mulberry progeny in the field (Vijayan, 2010) [58]. In light of this, phenotypic evaluation may be a more effective, affordable, and non-destructive option than molecular marker-assisted selection (MAS) procedures, particularly for complex features that are expensive to analyze. Current analyses of its genotypic variances have created new opportunities for more effective use of its genetic resources. There has not been much progress made in creating linkage maps for mulberry, even though genetic markers have been utilized to explore its distinctive genotypic variation. Thus, efforts are required to establish a mulberry genomic toolbox that can expedite the generation of better mulberry varieties and offer useful fundamental data for functional genomics. An important factor in reviving research efforts and sparking fresh suggestions for mulberry improvement is biotechnology. Standardized tissue culture research has been utilized to produce stable transgenic plants with a variety of desirable features. Many unique candidate genes have also been made available by the *Morus* genome resources, which have driven efforts to better disease- and stress-resistant plants through genetic engineering (Parmar *et al*., 2017) [41]. Understanding the chloroplast genome opened the door for evolutionary research on mulberries through important findings production. For mining SNPs, creating DNA markers, and mapping expressed genes to a linkage map, redundant sequences found in EST databases can be useful resources. The map is now more advantageous for marker-assisted selection and OTL analysis. An integrated analysis of genomic, transcriptomic, proteomic, and metabolomic data is anticipated to produce favorable results in understanding the mechanisms of increasing mulberry production. Recent sequencing of *Arabidopsis thaliana* and *Populus tricocarpa*, two model dicot angiosperms, offers a great chance to use reference genome systems for comparative genomics. Information transfer regarding gene function, genetic markers, and conserved genomic structures can be facilitated by this method. An international mulberry collaboration would be advantageous to the mulberry community in order to guarantee the widespread acceptance of data.

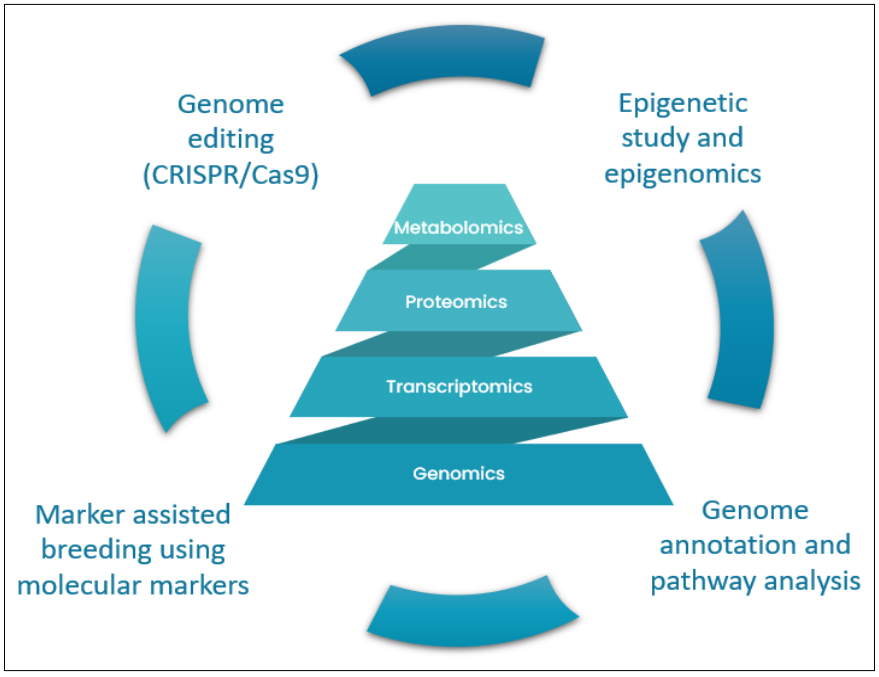

Fig 1: Integrated approaches of genomic and molecular biology tools for improving mulberry leaf productivity.

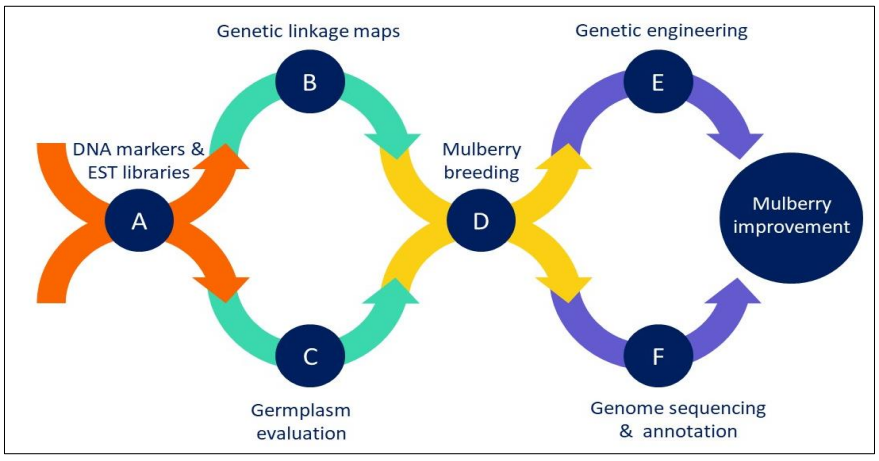

Fig 2: Various genetic methodologies and techniques applied to mulberry genomics.

Table 1: List of molecular markers used for marker-assisted breeding and clonal propagation in *Morus* spp.

Marker type	Purpose of the study	Country	Reference(s)
RAPD	Genetic variability and phylogenetic relationship among 15 white mulberry genotypes	Turkey	Orhan *et al*., 2007 [39]
RAPD	Molecular characterization of inter and intra-specific hybrids	India	Tikader and Dandin, 2008 [56]
RAPD	Genetic diversity among nine mulberry genotypes with contrasting traits for water use efficiency (WUE) and root	India	Mishra *et al*., 2013 [35]
RAPD	Genetic diversity among 36 genotypes collected from South India	India	Naik *et al*., 2013 [37]
AFLP	Genetic variability among 43 accessions belonging to *M. alba*, *M. nigra* and *M. rubra*	Turkey	Kafkas *et al*., 2008 [25]
AFLP	DNA fingerprinting of ten cultivars	China	Huang *et al*., 2009 [22]
ISSR	Phylogenetic relationship among 18 germplasm collection and association with biochemical parameters	India	Kar *et al*., 2008 [26]
ISSR	Genetic diversity among 73 local mulberry varieties for development of core collection	China	Lin *et al*., 2011
SSR	Genetic diversity among ten accessions belonging to *M. alba* and *M. indica*	Kenya	Wangari *et al*., 2013 [61]
SSR	Phylogenetic relatedness among 17 mulberry genotypes	India	Wani *et al*., 2013 [62]
SSR	Genetic diversity among 72 germplasm accessions belonging to two wild species such as *M. laevigata* and *M. serrata* collected from different eco-geographic regions of India	India	Naik *et al*., 2015 [36]
ISSR & SSR	Genetic diversity among 27 mulberry accessions including 19 cultivated and 8 wild accessions	China	Weiguo *et al*., 2007 [64]
ISSR & RAPD	Association with sprouting and sex expression traits	India	Vijayan *et al*., 2009 [60]
RAPD & ISSR	Genetic diversity among 21 mulberry genotypes collected from 4 geographic regions of Turkey	Turkey	Ipek *et al*., 2011
RAPD & ISSR	Genetic stability of cryo-preserved dormant buds of different *Morus* species belonging to indigenous and exotic collection	India	Choudhary *et al*., 2013 [14]
RAPD & ISSR	Genetic fidelity of *in vitro* regenerated mulberry plants (cv. S1)	India	Saha *et al*., 2016 [50]

Table 2: Linkage maps developed in mulberry.

Molecular map	Pedigree of mapping population	Markers	Hereditary nature of marker	Agronomic trait targeted	Reference (s)
Genetic linkage map	S36 and V1	RAPD, ISSR, and SSR	Dominant, codominant	No	Venkateswarlu *et al*., 2006 [57]
QTL map	V1 and Mysore Local	RAPD and ISSR	Dominant	Yield	Naik *et al*., 2014 [38]
QTL map	Himachal Local and MS3	RAPD and ISSR	Dominant	Water use efficiency	Mishra, 2014 [34]
QTL map	Dudia White and UP	RAPD and ISSR	Dominant	Root traits	Mishra, 2014 [34]

Table 3: Transgenic mulberry (*M. indica* cv. K2 and M5) developed for stress tolerance.

Transgene	Source of transgene	Explant	Performance of transgenic	Promoter used for transgene expression	Selectable marker gene	Agrobacterium Tumefaciens strain	Plant transformation vector	Reference(s)
Hva1	Barley	Hypocotyl and cotyledon	Drought and salinity tolerance	Act1	nptII	LBA4404	pCAMBIA2301	Lal *et al*., 2008 [31]
Hva1	Barley	Hypocotyl and cotyledon	Drought, salinity and cold stress tolerance	rd29A	nptII	Agl1	pBI121	Checker *et al*., 2012 [9]
bch1	Mulberry	Hypocotyl and cotyledon	UV, high temperature and high irradiance stress tolerance	CaMV35S	nptII	-	pCAMBIA2301	Saeed *et al*., 2014 [49]
SHN1	*Arabidopsis thaliana*	Hypocotyl and cotyledon	Efficient leaf moisture retention capacity	CaMV35S	nptII	EHA105	pBI121	Sajeevan *et al*., 2017 [51]

Table 4: List of mulberry (*Morus* spp.) plantlets regenerated from explants through organogenesis since last decade.

Explants	Genotype/cultivar	Species	Country	Type of organogenesis	Reference(s)
Leaf	M5, S13, S36	*M. indica*	India	Direct organogenesis	Chitra and Padmaja, 2005 [13]
Leaf	V1	*M. indica*	India	Direct organogenesis	Raghunath *et al*., 2013 [44]
Leaf, petiole, internode	Chinese White, Kokuso27	*M. alba*	India	Through callus phase	Bhau and Wakhlu, 2001 [4]
Leaf, hypocotyl, cotyledon, petiole, internodal segment	K2, DD	*M. indica*	India	Direct organogenesis	Bhatnagar *et al*., 2001 [3]
Cotyledon	S36, K2, S1	*M. indica*, *M. alba*	India	Direct organogenesis	Thomas, 2003 [55]
Apical bud	S54	*M. indica*	India	Direct organogenesis	Kavyashree, 2007 [27]
Nodal explant	M5, S36, S13, China White	*M. indica*	India	Direct organogenesis	Chitra and Padmaja, 2002 [12]
Nodal segment	S36, V1	*M. indica*	India	Through callus phase	Rao *et al*., 2010 [45]
Nodal segment	S1	*M. alba*	India	Direct organogenesis	Chattopadhyay *et al*., 2011 [6]
Nodal segment	V1	*M. indica*	India	Direct organogenesis	Sajeevan *et al*., 2011 [52]
Shoot meristem	S36	*M. indica*	India	Direct organogenesis	Chitra *et al*., 2014 [11]

Conclusions

Genomic research is developing at an astounding rate as a result of groundbreaking developments in several areas of biology, particularly in DNA sequencing and bioinformatics. This has raised the potential that trait-gene association studies may become as simple to do in a well-equipped laboratory as a PCR amplification reaction. Germplasm assessments, parental choices, progeny studies, introgression of characteristics from unadopted genotypes and species to elite breeding lines, and mulberry improvement using genetic engineering employing the most effective transformation technique available are some of these bottlenecks. Moreover, efforts can be made to evolve effective phenotyping approaches, find and validate SNP markers, develop more ESTs, and develop an adequate number of mapping populations from well-planned crosses. These resources are essential in order to successfully improve the productivity and adaptability of mulberry and to maintain a thriving sericulture sector in Asia.

Contribution of authors

Writing of the manuscript: HD, AS, SD, AP, SY, DP, and BPM; Curation of the table and figure: HD, SD, AS, and BPM; Correction of the manuscript: KKP

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Conflict of Interest

The authors declare that no conflict of interest exists.

References

1. Alam K, Raviraj VS, Chowdhury T, Bhuimali A, Ghosh P, Saha S. Application of biotechnology in sericulture: Progress, scope and prospect. The Nucleus. 2021;65(1):129-150. https://doi.org/10.1007/s13237-021-00355-2
2. Bae SH, Suh HJ. Antioxidant activities of five different mulberry cultivars in Korea. LWT - Food Science and Technology. 2007;40(6):955-962. https://doi.org/10.1016/j.lwt.2006.06.007
3. Bhatnagar S, Kapur A, Khurana P. TDZ-mediated differentiation in commercially valuable Indian mulberry, *Morus indica* cultivars K2 and DD. Plant Biotechnology. 2001;18(1):61–65. https://doi.org/10.5511/plantbiotechnology.18.61
4. Bhau BS, Wakhlu AA. Effect of genotype, explant type and growth regulators on organogenesis in *Morus alba*. Plant Cell Tiss Organ Cult. 2001;66:25-29.
5. Chang LY, Li KT, Yang WJ, Chang JC, Chang MW. Phenotypic classification of mulberry (*Morus* sp) species in Taiwan using numerical taxonomic analysis through the characterization of vegetative traits and chilling requirements. Scientia Horticulturae. 2014;176:208-217. https://doi.org/10.1016/j.scienta.2014.06.042
6. Chattopadhyay S. Comparative micropropagation efficiency of diploid and triploid mulberry (*Morus Alba* cv. S1) from axillary bud explants. African Journal of Biotechnology. 2011, 10(79). https://doi.org/10.5897/ajb10.1474
7. Checker VG, Khurana P. Molecular and functional characterization of mulberry EST encoding remorin involved in abiotic stress. Plant Cell Reports. 2013;32(11):1729-1741. https://doi.org/10.1007/s00299-013-1483-5
8. Checker VG, Chhibbar AK, Khurana P. Stress-inducible expression of barley HVA1 gene in transgenic mulberry displays enhanced tolerance against drought, salinity and cold stress. Transgenic Research. 2011;21(5):939–957. https://doi.org/10.1007/s11248-011-9577-8
9. Checker VG, Saeed B, Khurana P. Analysis of expressed sequence tags from mulberry (*Morus indica*) roots and implications for comparative transcriptomics and Marker identification. Tree Genetics & Genomes. 2012;8(6):1437-1450. https://doi.org/10.1007/s11295-012-0531-6
10. Chen C, Zhou W, Huang Y, Wang ZZ. The complete chloroplast genome sequence of the mulberry *Morus notabilis*. Mitochondrial DNA Part A. 2015;27(4):2856–2857. https://doi.org/10.3109/19401736.2015.1053127
11. Chitra DS, Chinthapalli B, Padmaja G. Efficient regeneration system for genetic transformation of mulberry (*Morus indica* L. Cultivar S-36) using *in vitro* derived shoot Meristems. American Journal of Plant Sciences. 2014;05(01):1-6. https://doi.org/10.4236/ajps.2014.51001
12. Chitra DSV, Padmaja G. Seasonal influence on axillary bud sprouting and micropropagation of elite cultivars of mulberry. Sci Hort. 2002;92(1):55-68.
13. Chitra DSV, Padmaja G. Shoot regeneration via direct organogenesis from *in vitro* derived leaves of mulberry using thidiazuron and 6-benzylaminopurine. Scientia Horticulturae. 2005 Nov 1;106(4):593-602.
14. Choudhary R, Chaudhury R, Malik SK, Kumar S, Pal D. Genetic stability of mulberry germplasm after cryopreservation by two-step freezing technique. African Journal of Biotechnology. 2013 Oct 9;12(41):5983.
15. Das A, Rout BM, Datta S, Singh S, Munshi AD, Dey SS.

Spinach (*Spinacia oleracea* L.) Breeding: From Classical to Genomics-Centric Approach. InSmart Plant Breeding for Vegetable Crops in Post-genomics Era 2023 Jan 1 (pp. 117-142). Singapore: Springer Nature Singapore.
16. Das A, Singh S, Islam Z, Munshi AD, Behera TK, Dutta S, *et al*. Current progress in genetic and genomics-aided breeding for stress resistance in cucumber (*Cucumis sativus* L.). Scientia Horticulturae. 2022;300:111059.
17. Dutta H, Bhattacharya S, Sawarkar A, Pradhan A, Raman RB, Panigrahi KK, *et al*. High yielding mulberry production through controlled pollination for enhanced vegetative growth and early sprouting suitable for tropical agroclimatic regions. The Pharma Innovation. 2023a;12(3):4485-4492. https://doi.org/10.22271/tpi.2023.v12.i3aw.19445
18. Dutta S, Kumar Singh A, Prasad Mondal B, Paul D, Patra K. Perspective chapter: Digital inclusion of the farming sector using drone technology. Intech Open; c2023b. doi: 10.5772/intechopen.108740
19. Dutta S, Muthusamy V, Chhabra R, Zunjare RU, Hossain F. Two-step method for isolation of high-quality RNA from stored seeds of maize rich in starch Biotech. 2020a;3(10):1-8.
20. Sharma A, Gupta RK, Sharma P, Qadir J, Bandral RS, Bali K. Technological innovations in sericulture. International Journal of Entomology Research. 2022;7(1):7-15.
21. Grattapaglia D, Plomion C, Kirst M, Sederoff RR. Genomics of growth traits in forest trees. Current opinion in plant biology. 2009 Apr 1;12(2):148-156. https://doi.org/10.1016/j.pbi.2008.12.008
22. Huang RZ, Yan XP, Li J, Zhang XW. AFLP finger print analysis for 10 mulberry cultivars in Hunan province. Sci Seric. 2009;35(4):837-841.
23. İpek M, Pirlak L, Kafkas S. Molecular characterization of mulberry (*Morus* spp.) genotypes via RAPD and ISSR. Journal of the Science of Food and Agriculture. 2012 Jun;92(8):1633-1637. https://doi.org/10.1002/jsfa.4749
24. Ji X, Gai Y, Zheng C, Mu Z. Comparative proteomic analysis provides new insights into mulberry dwarf responses in mulberry (*Morus alba* L.). Proteomics. 2009 Dec;9(23):5328-539. https://doi.org/10.1002/pmic.200900012
25. Kafkas S, Ozgen M, Doğan Y, Özcan B, Ercişli S, Serçe S. Molecular characterization of mulberry accessions in Turkey by AFLP markers. Journal of the American Society for Horticultural Science. 2008;133(4):593-597. https://doi.org/10.21273/jashs.133.4.593
26. Kar P, Srivastava PP, Awasthi AK, Urs SR. Genetic variability and association of ISSR markers with some biochemical traits in mulberry (*Morus* spp.) genetic resources available in India. Tree Genet Genomes. 2008;4:75-83.
27. Kavyashree R. A repeatable protocol for *in vitro* micropropagation of mulberry variety S54. Indian J Biotechnol. 2007;6(3):385-388.
28. Khurana P, Checker VG. The advent of genomics in mulberry and perspectives for productivity enhancement. Plant Cell Reports. 2011;30(5):825-838. https://doi.org/10.1007/s00299-011-1059-1
29. Konno K, Ono H, Nakamura M, Tateishi K, Hirayama C, Tamura Y, *et al*. Mulberry latex rich in antidiabetic sugar-mimic alkaloids forces dieting on caterpillars. Proceedings of the National Academy of Sciences. 2006 Jan 31;103(5):1337-1341. https://doi.org/10.1073/pnas.0506944103
30. Kumari GJ, Kumar SG, Thippeswamy M, Annapurnadevi A, Naik ST, Sudhakar C. Effect of salinity on growth and proteomic changes in two cultivars of mulberry (*Morus alba* L.) with contrasting salt tolerance. Indian J Biotechnol. 2007;6:508-518.
31. Lal S, Ravi V, Khurana JP, Khurana P. Repertoire of leaf expressed sequence tags (ESTs) and partial characterization of stress-related and membrane transporter genes from mulberry (*Morus indica* L.). Tree Genetics & Genomes. 2008;5(2):359-374. https://doi.org/10.1007/s11295-008-0192-7.
32. Li R, Su X, Zhou R, Zhang Y, Wang T. Molecular mechanism of mulberry response to drought stress revealed by complementary transcriptomic and itraq analyses. BMC Plant Biology, 2022, 22(1). https://doi.org/10.1186/s12870-021-03410-x
33. Maity A, Paul D, Lamichaney A, Sarkar A, Babbar N, Mandal N, *et al*. Climate change impacts on seed production and quality: current knowledge, implications, and mitigation strategies. Seed Science and Technology. 2023;51(1):65-96.
34. Mishra S. Genetic analysis of traits controlling water use efficiency and rooting in mulberry (*Morus* spp.) by molecular markers. Ph.D. Thesis, University of Mysore, Mysuru, India; c2014.
35. Mishra S, Naik VG, Sukumar M, Pinto MV, Sheshshayee MS, Dandin SB. Genetic analysis of parental genotypes for mapping of water use efficiency and root traits in mulberry. Indian Journal of Genetics and Plant Breeding (The). 2013;73(4):405. https://doi.org/10.5958/j.0975-6906.73.4.061
36. Naik VG, Dandin SB, Tikader A, Pinto MV. Molecular diversity of wild mulberry (*Morus* spp.) of Indian subcontinent. Indian J Biotechnol. 2015;14(3):334–343.
37. Naik VG, Subbulakshmi N, Pinto MV, Mishra S, Guruprasad Qadri SMH. Assessment of genetic diversity among mulberry collections from South India using phenotypic and RAPD markers. Indian J Seric. 2013;52(1):34-43.
38. Naik VG, Thumilan B, Sarkar A, Dandin SB, Pinto MV, Sivaprasad V. Development of genetic linkage map of mulberry using molecular markers and identification of QTLs linked to yield and yield contributing traits. *Sericologia*. 2014;54(4):221-229.
39. Orhan E, Ercisli S, Yildirim N, Agar G. Genetic variations among mulberry genotypes (*Morus alba*) as revealed by Random Amplified Polymorphic DNA (RAPD) markers. Plant Systematics and Evolution. 2007;265(3-4):251-258. https://doi.org/10.1007/s00606-007-0525-2
40. Pan G, Lou CF. Isolation of an *1-aminocyclopropane-1-carboxylate oxidase* gene from mulberry (*Morus alba* L.) and analysis of the function of this gene in plant development and stresses response. Journal of Plant Physiology. 2008;165(11):1204-1213. https://doi.org/10.1016/j.jplph.2007.02.012
41. Parmar N, Singh KH, Sharma D, Singh L, Kumar P, Nanjundan J, *et al*. Genetic engineering strategies for biotic and abiotic stress tolerance and quality enhancement in horticultural crops: A comprehensive

review. 3 Biotech, 2017, 7(4). https://doi.org/10.1007/s13205-017-0870-y
42. Priyadarshan PM. Breeding vis-à-vis genomics of tropical tree crops. Advances in Plant Breeding Strategies: Agronomic, Abiotic and Biotic Stress Traits; c2016. p. 203-255. https://doi.org/10.1007/978-3-319-22518-0_6
43. Rafiqui AR, Ayoub OB, Ganie AH, Wani SA, Khan IL. Polyploidy breeding in mulberry - a review. SKUAST Journal of Research. 2022;24(3):285–294. https://doi.org/10.5958/2349-297x.2022.00048.4
44. Raghunath MK, Nataraja KN, Meghana JS, Sajeevan RS, Rajan MV, Hussaine Qadri SM. *In Vitro* plant regeneration of *Morus indica* L. Cv. V1 using leaf explant. American Journal of Plant Sciences. 2013;4(10):2001–2005. https://doi.org/10.4236/ajps.2013.410249
45. Rao JS, Nuthan D, Krishna KS, Basavaraja MK. *In vitro* propagation of irrigated mulberry varieties using nodal explants. Current Biotica. 2010;3(4):555-64.
46. Ratanapo S, Ngamjunyaporn W, Chulavatnatol M. Sialic acid binding lectins from leaf of mulberry (*Morus alba*). 1998139(2):141-148.
47. Ravi V, Khurana JP, Tyagi AK, Khurana P. The chloroplast genome of mulberry: Complete nucleotide sequence, gene organization and comparative analysis. Tree Genetics & Genomes. 2006;3(1):49-59. https://doi.org/10.1007/s11295-006-0051-3
48. Rukmangada MS, Sumathy R, Kruthika HS, Naik VG. Mulberry (*Morus* spp.) growth analysis by morpho-physiological and biochemical components for crop productivity enhancement. Scientia Horticulturae. 2020;259:108819. https://doi.org/10.1016/j.scienta.2019.108819
49. Saeed B, Das M, Khurana P. Overexpression of β-carotene hydroxylase1 (bch1) in Indian mulberry, M*orus indica* CV. K2, confers tolerance against UV, high temperature and high irradiance stress induced oxidative damage. Plant Cell, Tissue and Organ Culture (PCTOC). 2014;120(3):1003-1014. https://doi.org/10.1007/s11240-014-0654-6
50. Saha S, Adhikari S, Dey T, Ghosh P. RAPD and ISSR based evaluation of genetic stability of micropropagated plantlets of Morus alba L. variety S-1. Meta gene. 2016 Feb 1;7:7-15. https://doi.org/10.1016/j.mgene.2015.10.004
51. Sajeevan RS, Nataraja KN, Shivashankara KS, Pallavi N, Gurumurthy DS, Shivanna MB. Expression of Arabidopsis SHN1 in Indian mulberry (*Morus indica* L.) increases leaf surface wax content and reduces post-harvest water loss. Frontiers in Plant Science, 2017, 8. https://doi.org/10.3389/fpls.2017.00418
52. Sajeevan RS, Singh SJ, Nataraja KN, Shivanna MB. An efficient *in vitro* protocol for multiple shoot induction in mulberry, *Morus alba* L variety V1. Int. Res. J. Plant Sci. 2011;2(8):254-61.
53. Sarkar T, Mogili T, Gandhi Doss S, Sivaprasad V. Tissue culture in Mulberry (*Morus* spp.) intending genetic improvement, micropropagation and secondary metabolite production: A review on current status and future prospects. Biotechnological Approaches for Medicinal and Aromatic Plants; c2018. p. 467–487. https://doi.org/10.1007/978-981-13-0535-1_21
54. Song W, Wang HJ, Bucheli P, Zhang PF, Wei DZ, Lu YH. Phytochemical profiles of different mulberry (*Morus* sp.) species from China. Journal of agricultural and food chemistry. 2009 Oct 14;57(19):9133-9140. https://doi.org/10.1021/jf9022228
55. Thomas T. Thidiazuron induced multiple shoot induction and plant regeneration from cotyledonary explants of mulberry. *Biologia Plantarum*. 2003;46(4):529–533. https://doi.org/10.1023/a:1024807426591
56. Tikader A, Dandin SB. DNA fingerprint of inter and intraspecific hybrids from *Morus* species using RAPD. Geobios. 2008;35(2/3):113-120.
57. Venkateswarlu M, Urs SR, Nath BS, Shashidhar HE, Maheswaran M, Veeraiah TM, *et al*. A first genetic linkage map of mulberry (*Morus* spp.) using RAPD, ISSR, and SSR markers and pseudo testcross mapping strategy. Tree Genetics & Genomes. 2006;3(1):15-24. https://doi.org/10.1007/s11295-006-0048-y
58. Vijayan K. The emerging role of genomic tools in Mulberry (*Morus* sp.) genetic improvement. Tree Genetics & Genomes. 2010;6(4):613-625. https://doi.org/10.1007/s11295-010-0276-z
59. Vijayan K, Arunakumar GS, Gnanesh BN, Sangannavar PA, Ramesha A, Zhao W. Genomic designing for biotic stress resistance in mulberry. Genomic Designing for Biotic Stress Resistant Technical Crops; c2022. p. 285–336. https://doi.org/10.1007/978-3-031-09293-0_8
60. Vijayan K, Nair CV, Chatterjee SN. Diversification of mulberry (*Morus indica* var. S36), a vegetatively propagated tree species. Casp J Env Sci. 2009;7(1):23-30.
61. Wangari P. Use of SSR markers for genetic diversity studies in mulberry accessions grown in Kenya. International Journal of Biotechnology and Molecular Biology Research. 2013 Jun 30;4(3):38-44.
62. Wani SA, Bhat MA, Malik GN, Zaki FA, Mir MR, Wani N, *et al*. Genetic diversity and relationship assessment among mulberry (*Morus* spp) genotypes by simple sequence repeat (SSR) marker profile. African Journal of Biotechnology. 2013;12(21):3181-3187.
63. Waseem M, Nkurikiyimfura O, Niyitanga S, Nyimbo WJ, Shaheen I, Aslam MM. Role of metabolomics and next-generation sequencing for sustainable crop production. Principles and Practices of OMICS and Genome Editing for Crop Improvement; c2022. p. 123-147. https://doi.org/10.1007/978-3-030-96925-7_5
64. Weiguo Z, Zhihua Z, Xuexia M, Yong Z, Sibao W, Jianhua H, *et al*. A comparison of genetic variation among wild and cultivated *Morus* species as revealed by ISSR and SSR markers. Biodiv Conserv. 2007;16:275-290.
65. Dutta S, Muthusamy V, Hossain F, Baveja A, Chhabra R, Jha SK, *et al*. Analysis of genetic variability for retention of kernel carotenoids in sub-tropically adapted biofortified maize under different storage conditions. Journal of Cereal Science. 2020;93:102987.