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# **Breeding Methods in Mulberry**

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Mulberry (genus *Morus*), a member of the family Moraceae is an economically viable plant primarily raised for its leaves which serve as the primary appetite for the domesticated silkworm (*Bombyx mori* L.). The silk industry, particularly sericulture is heavily dependent on high-quality mulberry leaves. Mulberry is also known for its use in traditional medicine, food and animal fodder. Given its multiple utilities, improving the genetic quality of mulberry plants through selective breeding has become an essential aspect of modern agriculture.

The major objectives of mulberry breeding include enhancing leaf yield, improving leaf quality, increasing resistance to pests and diseases and improving tolerance to environmental stresses such as drought and salinity. This article presents an in-depth discussion on various breeding methods employed in mulberry improvement thereby including both conventional and advanced approaches.

## Importance of Mulberry Breeding

Mulberry breeding is critical to enhancing the productivity and sustainability of sericulture. Key goals of breeding programs include:

- ✤ Increased Leaf Yield: More leaves per plant lead to increased silk production.
- Improved Leaf Quality: Better-quality leaves directly influence silkworm growth and silk quality.
- Pest and Disease Resistance: Resistance to pests such as the whitefly and diseases like powdery mildew ensures better crop health.
- ✤ Abiotic Stress Tolerance: Developing mulberry varieties that can thrive in harsh conditions such as drought or saline soils.
- Sustainability: Improving plant longevity and reducing reliance on chemical inputs to protect the environment.

## **Conventional Breeding Methods**

Conventional breeding in mulberry involves traditional approaches such as selection, hybridization and mutation breeding. These methods have been practiced for centuries and have played a pivotal role in crop improvement.

**Selection:** Selection is the oldest and simplest method of plant breeding. It involves identifying and selecting plants with desirable traits from a natural population or a cultivated variety. There are two types of selection:

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- ✤ Mass Selection: Involves selecting superior plants based on phenotypic characteristics, harvesting seeds and growing the next generation from these seeds. Over several generations, the overall quality of the population improves.
  - **Pure Line Selection:** Involves selecting individual plants and selfing them over multiple generations to create homozygous lines. This method ensures uniformity and stability in the desired traits.

In mulberry, selection has been traditionally used to improve traits such as leaf yield, leaf quality and tolerance to environmental stresses.

Hybridization: Hybridization involves crossing two genetically diverse and different parents to produce offspring with mixed traits. This method is commonly used to create hybrids with improved vigour, higher yield and disease resistance. There are several types of hybridization methods:

- ◆ Intraspecific Hybridization: Crossing two closely relatable varieties of the same species. For instance, crossing different varieties of *Morus alba* to combine high yield and disease resistance.
- \* Interspecific Hybridization: Crossing different species of mulberry such as *Morus alba* and Morus indica, to create hybrids with novel traits such as drought tolerance or resistance to pests.

#### **Steps in Hybridization:**

- \* Selection of Parents: Parents with desirable traits such as high yield and disease resistance are selected.
- **Emasculation:** Removal of anthers from the flowers of the female parent to prevent selfpollination.
- ◆ Pollination: Pollen from the male parent is applied to the stigma of the emasculated flower.
- Seed Collection and Raising Progeny: Seeds from the cross are collected and grown into new plants which are evaluated for desirable traits.

Hybridization in mulberry breeding has resulted in the development of high-yielding and pest-resistant varieties thereby contributing to the overall improvement of sericulture.

Mutation Breeding: Mutation breeding involves inducing genetic variations through physical or chemical mutagens such as gamma rays or ethyl methane sulfonate (EMS). Mutations can result in new characters such as improved disease resistance, leaf yield or drought tolerance.

### **Steps in Mutation Breeding:**

- ★ Mutagen Treatment: Seeds, cuttings or tissue cultures are treated with a mutagen.
- Screening and Selection: After mutagenesis, plants are screened for desirable mutations such as improved leaf yield or stress tolerance.
- **Evaluation:** Selected mutants are evaluated for stability and performance across different environments.

Mutation breeding has been used to develop mulberry varieties with unique traits that are not easily achieved through traditional hybridization.

## **Advanced Breeding Methods**

In recent decades, advancements in biotechnology and molecular genetics have expanded the tools available for mulberry breeding. These methods offer greater precision and efficiency thereby accelerating the development of improved varieties.

Tissue Culture and Micropropagation: Tissue culture techniques involve the in vitro cultivation of plant cells, tissues or organs to produce new plants. Micropropagation, a type of tissue culture is widely used in mulberry breeding to produce genetically identical clones of elite varieties.

#### Advantages of Tissue Culture in Mulberry Breeding:

- \* **Rapid Multiplication:** Large numbers of plants can be produced in a short time.
- **\* Disease-Free Plants:** Plants grown in vitro are free from pathogens and pests.
- Preservation of Elite Varieties: Tissue culture allows the preservation and propagation of elite mulberry varieties with desirable traits such as high yield and pest resistance.

**Somaclonal Variation:** Somaclonal variation refers to the genetic variation that occurs during tissue culture. While this variation is often considered undesirable, it can be a valuable source of new traits for breeding. Somaclonal variation has been used to develop mulberry varieties with improved leaf yield and disease resistance.

#### **Steps in Somaclonal Variation:**

- \* Induction of Callus: Tissue cultures are initiated from leaf, stem or root explants.
- ✤ Selection of Variants: Plants showing desirable traits are selected and propagated for further evaluation.

**Marker-Assisted Selection (MAS):** Marker-assisted selection is a powerful tool that allows breeders to select plants with desirable traits based on genetic markers linked to those traits. MAS is particularly useful in mulberry breeding for traits that are difficult to assess through traditional phenotypic selection such as disease resistance or drought tolerance.

#### Steps in MAS:

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- Identification of Markers: Molecular markers such as SSRs (simple sequence repeats) or SNPs (single nucleotide polymorphisms) are identified and linked to traits of interest.
- Selection of Plants: Plants are screened for the presence of these markers, allowing breeders to select individuals with the desired traits without waiting for full phenotypic expression.

MAS has been instrumental in improving disease resistance and stress tolerance in mulberry thereby accelerating the breeding process and reducing the reliance on field trials.

**Genetic Engineering and Transgenic Breeding:** Genetic engineering allows the direct manipulation of a plant's DNA to introduce new traits. In mulberry, genetic engineering has been explored for improving traits such as disease resistance, abiotic stress tolerance and leaf quality.

#### **Key Techniques in Genetic Engineering**

- ✤ Agrobacterium-Mediated Transformation: A common method where the bacterium Agrobacterium tumefaciens is used to introduce foreign genes into the mulberry genome.
- CRISPR-Cas9: A modern gene-editing technology that allows precise modifications to the mulberry genome thus offering potential for creating drought-resistant or pestresistant varieties.

#### **Examples of Genetic Engineering in Mulberry**

- ✤ Bt Mulberry: A transgenic mulberry variety containing genes from Bacillus thuringiensis (Bt) has been developed for resistance to pests such as the mulberry caterpillar.
- Drought-Resistant Mulberry: Genetic engineering has been used to develop mulberry plants with improved tolerance to water stress by introducing genes related to water-use efficiency.

Despite its potential, the use of genetic engineering in mulberry breeding faces regulatory challenges and public concerns regarding the safety of genetically modified organisms (GMOs).

### **Breeding for Specific Traits in Mulberry**

The breeding methods discussed above are used to improve specific traits in mulberry plants. Below are some key traits that are the focus of modern mulberry breeding programs: Leaf Yield and Quality: Improving leaf yield and quality is the primary goal of mulberry breeding for sericulture. High leaf yield ensures more food for silkworms, while good leaf quality enhances silkworm growth and silk production.

Breeding strategies for improving leaf yield include:

- **Hybridization:** Creating hybrids with vigour for higher leaf production.
- \* MAS: Using markers linked to high leaf yield to select plants with this trait.

Disease Resistance: Diseases such as powdery mildew, leaf spot and root rot significantly affect mulberry production. Breeding for disease resistance is essential for reducing crop losses.

Strategies for breeding disease-resistant mulberry varieties include:

- \* Interspecific Hybridization: Introducing disease resistance from wild relatives.
- ✤ MAS: Selecting for genetic markers linked to disease resistance.
- Genetic Engineering: Introducing genes that confer resistance to specific pathogens.

Abiotic Stress Tolerance: Abiotic stresses such as drought, salinity and temperature extremes can limit mulberry growth and productivity. Developing stress-tolerant varieties is crucial for maintaining mulberry cultivation in diverse environmental conditions.

Breeding for abiotic stress tolerance includes:

- \* Mutation Breeding: Inducing mutations that confer stress tolerance.
- \* MAS: Using markers linked to drought and salinity tolerance for selection.
- \* Genetic Engineering: Introducing genes related to osmotic balance and water-use efficiency.

Pest Resistance: Pests such as whiteflies, aphids and caterpillars can severely damage mulberry leaves. Breeding for pest resistance is a major focus in mulberry improvement programs.

Breeding strategies for pest resistance include:

- ★ Interspecific Hybridization: Introducing pest resistance from wild species.
- \* Genetic Engineering: Developing transgenic mulberry plants expressing insecticidal proteins such as Bt proteins to protect against pests.

### Challenges need to be addressed in Mulberry Breeding

While significant progress has been made in mulberry breeding, several challenges remain:

- \* Long Breeding Cycles: Mulberry is a perennial plant, and conventional breeding methods require several years to develop new varieties.
- ★ Limited Genetic Resources: The genetic diversity in cultivated mulberry species is limited which restricts the potential for improving certain traits.
- \* **Regulatory Hurdles:** The use of genetically modified mulberry varieties faces regulatory and public acceptance issues in many countries.

### Future Prospects in Mulberry Breeding

- ♦ Genomic Selection: The use of whole-genome information to predict the breeding value of plants thereby speeding up the selection process.
- **Gene Editing:** CRISPR-Cas9 and other gene-editing technologies offer new possibilities for precisely modifying mulberry genomes to improve traits such as disease resistance and stress tolerance.
- **Exploiting Wild Relatives:** Wild species of mulberry possess valuable traits such as drought tolerance and disease resistance which can be harnessed through interspecific hybridization and gene transfer.

### Conclusion

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Breeding methods in mulberry have evolved significantly from traditional approaches such as selection and hybridization to advanced biotechnological techniques like marker-assisted selection and genetic engineering. These methods have been instrumental in improving traits such as leaf yield, pest and disease resistance and tolerance to abiotic stresses thereby contributing to the sustainability and productivity of sericulture. As molecular tools continue to advance, mulberry breeding is likely to become more efficient thereby enabling the development of varieties that can meet the growing demands of the silk industry and adapt to changing environmental conditions.

