

# Exploring crop genomics: role of molecular markers in identifying genetic diversity and characterization

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## Abstract

This review delves into the forefront of crop genomics, elucidating the pivotal role of molecular markers in unraveling genetic diversity and facilitating germplasm characterization for crop improvement. Understanding and harnessing crop genetic diversity are paramount with the escalating demand for sustainable agricultural practices and the need to address global food security challenges. Molecular markers have emerged as powerful tools enabling precise identification, characterization, and utilization of genetic resources for crop enhancement.

The paper provides an in-depth analysis of various molecular marker techniques, encompassing DNA-based markers such as single nucleotide polymorphisms (SNPs), simple sequence repeats (SSRs), and amplified fragment length polymorphisms (AFLPs), as well as RNA-based markers like expression sequence tags (ESTs) and microRNAs. By elucidating genetic relationships, population structure, and phylogenetic analysis, these markers facilitate the conservation and utilization of crop germplasm. Moreover, the review highlights recent advancements in high-throughput genotyping technologies and bioinformatics tools, which have revolutionized crop genomic research. These advancements enable comprehensive genome-wide analyses, accelerating breeding efforts for the development of improved crop varieties with enhanced traits such as yield, quality, and stress tolerance. Integration of molecular marker-assisted selection (MAS) into breeding programs further enhances the efficiency and precision of crop improvement strategies.

Despite the significant contributions of molecular markers to crop genomics, challenges persist. The review addresses these challenges and discusses potential solutions, emphasizing the importance of collaborative efforts, data sharing, and interdisciplinary research endeavors. By overcoming these hurdles, the agricultural community can fully harness the potential of genomics for sustainable crop improvement, thus addressing global food security challenges in the face of changing environmental conditions and population growth.

In conclusion, understanding the role of molecular markers in crop genomics is crucial for optimizing breeding strategies, conserving genetic resources, and developing resilient crop varieties to meet the demands of a growing population and ensure food security in a changing world.

**Keywords:** *Crop Genomics; Molecular Markers; Genetic Diversity; Germplasm Characterization; Sustainable Agriculture; Global Food Security; SNP Markers; SSRs; AFLPs; High-Throughput Genotyping; and Bioinformatics.*

## 1. Introduction

**Introduction to Crop Genomics and its Importance:** Start by introducing the significance of crop genomics in modern agriculture, highlighting the importance of understanding genetic diversity for crop improvement and food security. Genetic variations within and among plant populations serve as a crucial foundation for the effective utilization of plant genetic resources. Historically, plant breeders have relied predominantly on evaluating agronomic and morphological traits to assess genetic diversity and other essential characteristics. However, these methods have inherent limitations, such as environmental influence and the relatively low resolution in distinguishing closely related genotypes. Contemporary breeders increasingly favor diversity analysis using molecular markers due to their proficiency in uncovering variations among genotypes, which surpasses the constraints of traditional morphological markers.

Molecular markers are segments of DNA associated with a specific location within the genome, and they are invaluable tools in plant genetics and breeding. They provide several advantages over morphological markers, including high polymorphism, stability, and the ability to detect genetic differences at the DNA level, irrespective of environmental conditions. Here are some key roles of molecular markers in identifying genetic diversity and characterization:

**High Resolution in Genetic Variation Detection:** Molecular markers offer high-resolution detection of genetic variations, enabling the precise assessment of genetic diversity within and among populations. Techniques such as Single Nucleotide Polymorphisms (SNPs) and

Simple Sequence Repeats (SSRs) provide detailed insights into genetic differences, facilitating the identification of unique genetic traits that are not discernible through morphological analysis (Agarwal et al., 2008).

**Unbiased and Reliable:** Unlike morphological markers, molecular markers are not influenced by environmental factors. This makes them reliable tools for consistent genetic analysis across different environments and seasons. For instance, DNA-based markers such as SNPs and SSRs can provide consistent results, making them ideal for genetic characterization and diversity studies (Varshney et al., 2005).

**Facilitation of Germplasm Characterization:** Molecular markers are extensively used in the characterization of germplasm collections. They help in cataloging genetic resources, identifying duplicates, and determining the genetic structure of populations. This aids in the efficient management and utilization of genetic resources for breeding programs (Kumar et al., 2009).

**Marker-Assisted Selection (MAS):** Molecular markers are integral to marker-assisted selection, where specific markers linked to desirable traits are used to select plants that carry those traits. This accelerates the breeding process and enhances the accuracy of selection, leading to the development of improved crop varieties with enhanced traits such as yield, quality, and stress tolerance (Collard and Mackill, 2008).

**Genetic Mapping and QTL Analysis:** The use of molecular markers in genetic mapping and quantitative trait loci (QTL) analysis allows breeders to identify regions of the genome associated with important agronomic traits. This knowledge facilitates targeted breeding strategies and the introgression of beneficial alleles into elite cultivars (Xu, 2010).

**Assessment of Genetic Relationships and Population Structure:** Molecular markers are essential for assessing genetic relationships and population structure within and between species. Techniques such as AFLP, SSR, and SNP genotyping provide detailed information on genetic relatedness, aiding in the conservation of genetic diversity and the formulation of breeding strategies (Peakall and Smouse, 2012).

## 1.2. Genetic diversity and its importance in crop improvement

Genetic diversity is a fundamental concept in plant breeding and crop improvement, representing the degree of genetic variation present within a species. It can be defined in various ways by different authors. One definition describes genetic diversity as “the degree of genetic variation seen in individuals within a species because of processes including recombination, mutations, gene flow, and genetic drift. It symbolizes the diversity of alleles and gene combinations among a group of organisms” (Reed and Frankham, 2003). Another definition is “the range and sum of genetic variation within a population or between populations,” with diversity representing the differences among individuals (Hughes et al., 2008). Additionally, it is defined as “the variability of heritable traits presents in a population of a given species” (Lande, 1996) or as “the variety of alleles present in a particular population or among populations, reflected in morphological, physiological, and behavioral differences” (Frankham et al., 2002). Another perspective views it as “any measure that can calculate the magnitude of genetic variability present in a population” (Nei, 1987).

Genetic diversity forms the bedrock of plant breeding and crop improvement initiatives. Understanding and characterizing the existing genetic variation within crop germplasm is essential for identifying desirable traits that can be utilized to develop improved varieties with higher yields, resilience against biotic and abiotic stresses, and superior nutritional profiles (Gepts, 2006; Govindaraj et al., 2015). A vast genetic diversity endows plants with the capacity to adapt to abrupt environmental fluctuations, enhancing their survival and productivity under changing conditions (Hajjar and Hodgkin, 2007).

Plant genetic diversity is invaluable for breeders as it provides a pool of desirable traits to select from when developing new crop varieties or parental lines for hybridization (Govindaraj et al., 2015). Utilizing genetically divergent parents in breeding programs allows for the improvement of productivity, disease resistance, and other favorable characteristics in agricultural and horticultural crops (Acquaah, 2007). Maintaining high levels of genetic diversity is crucial as it equips breeders with an array of genetic variations to draw upon. By combining diverse genetic backgrounds, breeders can assemble unique gene combinations, leading to the development of superior and adaptable varieties that meet changing environmental conditions and market demands (Govindaraj et al., 2015; Acquaah, 2007).

The success of various crop improvement programs depends on the efficient identification and incorporation of plant genetic resources, such as currently grown cultivars, newly developed varieties, landraces, wild relatives, and germplasm collections (Gepts, 2006). A detailed understanding of genetic diversity and its distribution is important for its effective conservation and utilization, guiding breeders on what to conserve and where to focus efforts (Jarvis et al., 2008). From a plant breeder's perspective, genetic diversity is essential for developing resilient and adaptable crops that can withstand various abiotic and biotic stresses. It is also crucial for improving yield and yield-related traits (Tester and Langridge, 2010).

## 1.3. Role of germplasm characterization in plant breeding

Germplasm characterization is a crucial process in plant breeding, involving the detailed description of plant germplasm through the evaluation of highly heritable traits such as morphology, physiology, agronomic features, seed proteins, oils, and molecular markers. This information is essential for the effective utilization of germplasm in breeding programs. Despite being time-consuming and costly, germplasm characterization can be performed at any stage of conservation (Mace et al., 2013).

Plant breeders employ various methods to characterize germplasm and evaluate genetic diversity, which is fundamental for identifying diverse parents for breeding programs. These methods include the evaluation of phenotypic or morphological traits, molecular approaches, and biochemical or allozyme analysis (Van Hintum et al., 2000). Germplasm is considered a valuable source for the identification of genes related to desirable traits. Once a trait is linked to biosynthetic pathways, the alleles responsible for the expression of that trait are identified, allowing researchers to understand crops on a broader basis. This knowledge is crucial for making specific parental and allelic combinations, leading to the discovery of superior varieties (Singh et al., 2014). By leveraging these characterization methods, breeders can unlock the potential of genetic diversity and develop improved crop varieties with enhanced performance and adaptability.

Germplasm collections comprise trait-specific accessions exhibiting tolerance to stresses such as heat, drought, salinity, and cold, as well as desirable traits like high nitrogen and water use efficiency. These accessions provide valuable genetic resources for crop improvement programs (Hoisington et al., 1999). Through comprehensive characterization, breeders can identify and utilize these genetic resources to develop new crop varieties that are resilient and high-yielding, addressing the challenges posed by environmental stresses and changing agricultural demands.

In finally, germplasm characterization plays a pivotal role in plant breeding by providing detailed information on genetic diversity and trait-specific accessions. This information aids in the selection of diverse parents and the development of improved crop varieties, thereby enhancing the overall efficiency and effectiveness of breeding programs. This structured and detailed explanation, with comprehensive references, highlights the essential role of germplasm characterization in the context of plant breeding and crop improvement.

## 1.4. Overview of molecular marker technologies

Over the last three decades, major advancements in understanding plant genomes and gene functions have revolutionized plant breeding. These advancements have enabled breeders to engineer crops with desired traits such as higher yields and increased resilience, thereby driving more effective crop improvement (Varshney et al., 2005). Central to these advancements are molecular markers, which are DNA sequences that highlight genetic variations such as insertions, deletions, and mutations across individuals. Molecular markers function by identifying and differentiating specific genomic regions, making them invaluable tools for genetic mapping and analysis (Collard et al., 2005).

The ability to detect genetic variations using molecular markers has enabled breeders to pinpoint desirable traits and identify superior plant genetics. This technological advancement has significantly contributed to the development of improved crop varieties with enhanced characteristics (Varshney et al., 2005).

## 2. Molecular marker-based diversity analysis

Molecular markers are invaluable tools in the study of genetic diversity. They play a critical role in analysing phylogenetic relationships, facilitating the selection of elite varieties, and allowing for the comparison of genetic similarities and differences between species (Govindaraj et al., 2015). Various DNA markers have been employed as important molecular tools in plants for studying genetic relationships among individuals, hybrid validation, varietal identification, phylogenetic relationships between species, gene mapping, and quantitative trait loci (QTL) detection over the past few decades (Collard and Mackill, 2008; Mohan et al., 1997).

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Genetic variability within a population can be assessed through the number (and percentage) of polymorphic genes in the population, the number of alleles for each polymorphic gene, and the proportion of heterozygous loci per individual (Weir, 1996).

Types of Molecular Markers

Molecular markers are broadly categorized into two types: hybridization-based markers and PCR-based markers.

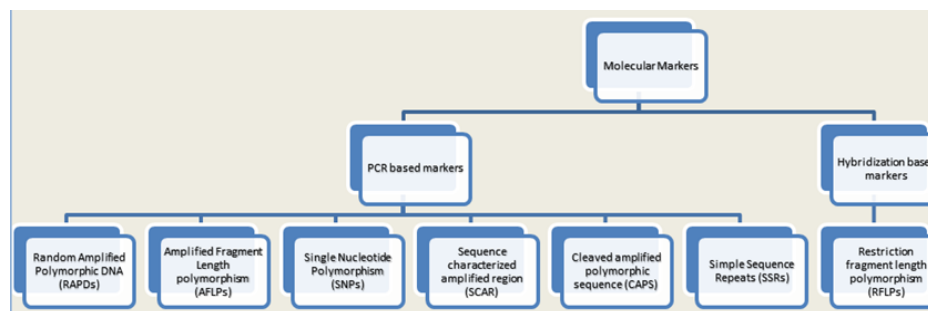


Fig. 1: Diverse Applications of Molecular Markers in Genetic Diversity Studies".

### 2.1. Hybridization-based markers

Hybridization-based markers are used to visualize DNA profiles. This involves digestion of DNA with restriction enzymes followed by the hybridization of the resulting fragments to a radioactive-labelled probe, which is a DNA segment of a known sequence. The first hybridization-based marker developed was Restriction Fragment Length Polymorphism (RFLP) (Botstein et al., 1980). RFLP markers are powerful tools for comparative and synteny mapping studies. They exhibit co-dominant inheritance patterns and are highly specific to loci. One of the key advantages of RFLP genotyping is its high reproducibility and straightforward methodology that does not require specialized equipment (Nei and Kumar, 2000).

### 2.2. PCR-based markers

Polymerase Chain Reaction (PCR)-based markers work on the principle of variations in DNA sequences and are considered the second generation of molecular markers (Williams et al., 1990). Major PCR-based markers include Random Amplified Polymorphic DNA (RAPD), Amplified Fragment Length Polymorphism (AFLP), Simple Sequence Repeats (SSRs), and Single Nucleotide Polymorphisms (SNPs).

#### 2.2.1. Random amplified polymorphic DNA (RAPDs)

RAPD is a PCR-based marker system developed independently by Williams et al. (1990) and Welsh and McClelland (1990). The RAPD technique uses short, random oligonucleotide primers (8-15 nucleotides) to amplify multiple complementary sequences scattered across the genome, generating unique banding patterns (Welsh and McClelland, 1990). These patterns are visualized using agarose gel electrophoresis, indicating polymorphisms at or between the primer binding sites (Williams et al., 1990).

#### 2.2.2. Amplified fragment length polymorphism (AFLPs)

AFLP markers combine RFLP and PCR technologies. DNA is first digested using two restriction enzymes a frequent cutter and a rare cutter. Short oligonucleotide sequences are then ligated to the resulting fragments, with one oligonucleotide being specific to the frequent

cutter site and the other to the rare cutter site. PCR amplification is subsequently performed, selectively amplifying only those fragments with both oligonucleotides attached. The banding pattern observed through gel electrophoresis or autoradiography represents different DNA fragments, facilitating genetic variation analysis (Vos et al., 1995).

### 2.2.3. Simple sequence repeats (SSRs)

SSRs, also known as microsatellites, were first discovered by Litt and Luty (1989) and Tautz (1989). They consist of 1-6 base pair tandem repeat motifs that are abundantly present in both coding and non-coding regions of the genome. SSRs are the markers of choice for studying genetic variability due to their high polymorphism, widespread presence throughout genomes, and capability to distinguish between heterozygous and homozygous individuals (Powell et al., 1996).

### 2.2.4. Single nucleotide polymorphisms (SNPs)

SNPs are individual base variations present in DNA, such as substitutions (transitions or transversions) or insertions/deletions (InDels). SNPs are abundant in both coding and non-coding regions of genomes, with plants typically having around one SNP per 100-300 bases (Rafalski, 2002). Common SNP genotyping methods include RFLP, CAPS, ligation, allele-specific hybridization, primer extension, and invasive cleavage. These techniques analyse sequence databases to identify and characterize SNPs within a given genome. For example, in RFLP, if an SNP creates or eliminates a restriction enzyme binding site, digestion will yield fragments of varying sizes, indicating different alleles (Vignal et al., 2002).

## 2.3 Marker selection and optimization

Marker selection and optimization is a critical process in genetic studies and breeding programs, aimed at ensuring that the chosen molecular markers are informative, efficient, and tailored to the specific needs of the application. This process involves several key steps and considerations:

### 2.3.1. Criteria for marker selection

#### 2.3.1.1. Polymorphism

The selected markers must be highly polymorphic to detect a wide range of genetic variations within and between populations. Polymorphic markers provide greater resolution in distinguishing genetic differences (Anderson et al., 1993).

Polymorphism refers to the presence of different forms or variations of a gene or DNA sequence within a population. DNA polymorphism involves genetic variations in the DNA sequence among individuals, including single nucleotide changes, insertions, deletions, or structural modifications. These polymorphisms contribute to genetic diversity, influence traits, and disease susceptibility, and aid in understanding population genetics and evolution. Polymorphic DNA markers, which are regions exhibiting genetic diversity, are major tools for studies such as linkage and association mapping (Schlotterer, 2004).

When selecting genetic markers for research purposes, certain characteristics are preferred to ensure informative and reliable results. Markers with a greater number of alleles tend to be more informative and possess greater discriminatory power (Botstein et al., 1980). Conversely, markers with skewed or unbalanced allele frequencies, where one or a few alleles are highly common while others are rare, tend to be less informative (Nei, 1987; Hedrick, 1999). Additionally, markers with rare or low-frequency alleles may not be as useful, especially in smaller population samples, as these rare alleles may not be represented or could be lost due to genetic drift (Lewontin, 1974).

An even or uniform allele frequency distribution maximizes heterozygosity and provides the highest possible gene diversity or polymorphism information content (PIC) value for a given number of alleles (Botstein et al., 1980; Anderson et al., 1993). This characteristic makes the marker more powerful for distinguishing genotypes and assessing genetic diversity (Lynch and Walsh, 1998). Markers with evenly distributed allele frequencies are more reliable for genetic diversity studies, as they are less affected by sampling effects and genetic drift, especially in smaller populations (Falconer and Mackay, 1996).

- 1) **Reproducibility:** The markers must produce consistent and reproducible results across different experiments and laboratories. High reproducibility is crucial for reliable comparison of data (Jones et al., 1997).
- 2) **Co-dominance:** Co-dominant markers, such as SSRs and SNPs, are preferred because they can distinguish between homozygous and heterozygous individuals. This capability provides more detailed genetic information (Powell et al., 1996).
- 3) **Genome Coverage:** The markers should be distributed evenly across the genome to provide comprehensive coverage. This ensures that all genomic regions are represented in the analysis (Barker et al., 1999).
- 4) **Ease of Use and Cost-effectiveness:** The techniques used for marker analysis should be straightforward, cost-effective, and require minimal specialized equipment. This is essential for practical application in large-scale studies and breeding programs (Rafalski, 2002).

### 2.3.2. Optimization of marker systems

- 1) **Validation and Calibration:** Markers must be validated and calibrated against known standards or reference materials. This step ensures accuracy and reliability in identifying genetic variations (Gupta and Varshney, 2000).
- 2) **Multiplexing Capability:** The ability to analyze multiple markers simultaneously (multiplexing) can significantly enhance efficiency. Techniques like SNP arrays and multiplex PCR enable the concurrent analysis of numerous loci, saving time and resources (Mammadov et al., 2012).
- 3) **Sensitivity and Specificity:** The markers should be sensitive enough to detect low-frequency alleles and specific enough to avoid cross-hybridization or non-specific binding. High sensitivity and specificity are crucial for precise genetic analysis (Karp et al., 1997).
- 4) **Adaptability to Different Species:** Markers should be adaptable to a wide range of species, including those with complex or poorly characterized genomes. This flexibility expands their applicability across various crops and model organisms (Varshney et al., 2005).

- 5) Data Management and Analysis Tools: Effective marker systems are supported by robust data management and analysis tools. Software and bioinformatics resources facilitate the interpretation of complex genetic data, aiding in marker selection and optimization (Collard et al., 2005). By carefully selecting and optimizing molecular markers, researchers and breeders can enhance the accuracy, efficiency, and applicability of genetic studies and breeding programs. This approach enables the identification of desirable traits, accelerates the development of improved crop varieties, and contributes to the overall success of genetic research initiatives.

### 3. Applications of molecular markers in genetic diversity studies

Molecular markers are invaluable tools in the study of genetic diversity, providing detailed insights into genetic relationships, population structure, and phylogenetic analysis in crops. Their applications span across various fields in plant genetics and breeding, offering precise and reliable data essential for crop improvement programs.

#### 3.1. Elucidating genetic relationships

Molecular markers facilitate the assessment of genetic relationships among different crop varieties and species. By analysing genetic variations, researchers can determine the genetic distances between individuals, which is crucial for selecting parental lines in breeding programs.

For example, Simple Sequence Repeat (SSR) markers have been extensively used to study the genetic relationships in rice (*Oryza sativa*). SSR analysis has revealed significant genetic diversity among rice varieties, aiding breeders in selecting genetically distinct parents to enhance hybrid vigor (Xu et al., 2016). Similarly, in wheat (*Triticum aestivum*), SSR markers have helped identify distinct genetic lineages, enabling the development of new varieties with improved traits such as disease resistance and yield (Salem et al., 2015).

#### 3.2. Population structure analysis

Understanding population structure is essential for effective management and conservation of genetic resources. Molecular markers are used to assess population structure by identifying sub-populations and estimating gene flow and genetic drift within and between populations.

Single-nucleotide polymorphisms (SNPs) have been particularly useful in population structure analysis. For instance, in maize (*Zea mays*), SNP markers have been employed to dissect the population structure, revealing the presence of distinct sub-populations adapted to different environmental conditions (Lu et al., 2010). This information is critical for conserving genetic diversity and ensuring the sustainable use of maize genetic resources.

#### 3.3. Phylogenetic analysis

Phylogenetic analysis involves studying the evolutionary relationships among species or varieties. Molecular markers provide the genetic data needed to construct phylogenetic trees, which illustrate the evolutionary history and relatedness of different taxa.

Amplified Fragment Length Polymorphism (AFLP) markers have been successfully used in phylogenetic studies of various crops. In barley (*Hordeum vulgare*), AFLP markers have helped elucidate the evolutionary relationships between wild and cultivated barley, shedding light on the domestication process and the genetic basis of important agronomic traits (Hou et al., 2012). Similarly, AFLP analysis in soybean (*Glycine max*) has provided insights into the phylogenetic relationships between cultivated soybean and its wild relatives, guiding breeding strategies for crop improvement (Zhou et al., 2015).

#### 3.4. Case studies from recent research

##### 1) Rice (*Oryza sativa*):

- A study using SSR markers identified significant genetic diversity among traditional and modern rice varieties in India. This genetic information was used to select parents for hybridization, aiming to develop new rice varieties with higher yield and better stress tolerance (Singh et al., 2018).
- Maize (*Zea mays*): SNP markers were utilized to analyze the genetic structure of maize landraces in Mexico. The study revealed distinct genetic clusters corresponding to different geographic regions, providing valuable insights for the conservation and utilization of maize genetic diversity (Arteaga et al., 2016).
- Soybean (*Glycine max*): AFLP markers were used to study the genetic relationships between wild and cultivated soybean. The results highlighted the genetic bottlenecks during domestication and identified wild soybean accessions with useful traits for breeding programs (Zhou et al., 2015).
- Wheat (*Triticum aestivum*): SSR markers were employed to assess the genetic diversity of wheat landraces in the Fertile Crescent. The study underscored the importance of conserving landraces as a reservoir of genetic diversity for future breeding efforts (van de Wouw et al., 2010).

Molecular markers have revolutionized the study of genetic diversity in crops. Their applications in elucidating genetic relationships, population structure, and phylogenetic analysis have provided profound insights into crop genetics and facilitated the development of improved varieties. As molecular marker technologies continue to advance, their role in genetic diversity studies will become even more pivotal, contributing to sustainable agriculture and food security.

### 4. Advancements in high-throughput genotyping technologies

Recent years have witnessed remarkable advancements in genotyping technologies and bioinformatics tools, revolutionizing crop genomic research and accelerating genetic analyses and breeding efforts. These advancements have led to increased efficiency, accuracy, and scalability in genotyping, enabling researchers and breeders to explore crop genomes at unprecedented levels of detail.

#### 4.1. High-throughput sequencing platforms

High-throughput sequencing platforms, such as next-generation sequencing (NGS) technologies, have significantly transformed crop genomic research. NGS platforms, including Illumina, PacBio, and Oxford Nanopore, offer rapid and cost-effective sequencing of entire genomes, enabling comprehensive characterization of genetic variations within crop species (Mardis, 2013).

The ability to generate massive amounts of sequence data in a relatively short time has revolutionized genetic analyses, including genome-wide association studies (GWAS), marker-assisted selection (MAS), and genomic selection (GS). For example, GWAS studies leveraging NGS data have facilitated the identification of candidate genes associated with important agronomic traits in crops like maize, rice, and wheat (Huang et al., 2010; Xu et al., 2011; Wang et al., 2014).

## 4.2. Genotyping-by-sequencing (GBS)

Genotyping-by-sequencing (GBS) is a cost-effective NGS-based approach for genotyping large populations. GBS enables simultaneous discovery and genotyping of thousands of single nucleotide polymorphisms (SNPs) across the genome, making it ideal for high-resolution genetic mapping and diversity analysis (Elshire et al., 2011). GBS has revolutionized crop breeding by providing breeders with dense genetic markers for trait mapping, marker-assisted selection, and genomic prediction. For example, GBS has been successfully applied in various crops, including soybean, maize, and barley, to accelerate the development of improved cultivars with desirable traits (Sonah et al., 2013; Poland et al., 2012; Hickey et al., 2017).

## 4.3. Impact on breeding efforts

The integration of high-throughput genotyping technologies into breeding programs has significantly accelerated the pace of crop improvement. By enabling rapid and precise characterization of genetic variation, these technologies facilitate the identification of superior alleles associated with desirable traits, allowing breeders to develop improved cultivars more efficiently (Varshney et al., 2018). Furthermore, high-throughput genotyping platforms support genomic selection approaches, wherein predictive models are trained using genome-wide marker data to estimate the breeding value of individuals based on their genotypic profiles. This approach enables breeders to select elite individuals for breeding programs at early stages, leading to faster genetic gain and reduced breeding cycles (Heffner et al., 2009). The recent advancements in high-throughput genotyping technologies, such as NGS and GBS, coupled with sophisticated bioinformatics tools, have revolutionized crop genomic research and breeding efforts. These technologies have enhanced our understanding of crop genomes, accelerated genetic analyses, and facilitated the development of improved cultivars with enhanced productivity, resilience, and quality.

## 5. Integration of molecular marker-assisted selection (MAS)

Molecular Marker-Assisted Selection (MAS) is a breeding strategy that integrates molecular markers into conventional breeding programs to facilitate the selection of individuals with desired traits at the molecular level. MAS enables breeders to identify and select plants carrying specific alleles associated with target traits, thus expediting the development of improved crop varieties.

### 5.1. Identification of molecular markers associated with target traits

The first step in implementing MAS is to identify molecular markers associated with target traits of interest. This is typically accomplished through genetic mapping studies, such as quantitative trait loci (QTL) mapping or genome-wide association studies (GWAS). By analyzing the genetic variation within a population, researchers can identify regions of the genome that are linked to traits of agronomic importance (Collard and Mackill, 2008).

### 5.2. Marker validation and development of marker-assisted selection tools

Once molecular markers associated with target traits are identified, they undergo validation to ensure their accuracy and reliability across diverse genetic backgrounds. This involves genotyping many individuals to confirm the association between marker alleles and trait performance (Hospital, 2001).

Subsequently, marker-assisted selection tools are developed based on validated molecular markers. These tools enable breeders to efficiently genotype large populations and select individuals with desired alleles for further breeding purposes (Collard and Mackill, 2008).

### 5.3. Implementation of MAS in breeding programs

In breeding programs, MAS is integrated into the selection process to assist breeders in identifying and selecting individuals with superior trait combinations. Breeders use molecular markers to screen large populations for the presence of target alleles associated with traits such as disease resistance, abiotic stress tolerance, or improved yield potential (Hospital, 2001).

By incorporating molecular information into selection decisions, MAS enhances the efficiency and accuracy of breeding programs. Breeders can select individuals at early stages of development based on their molecular profiles, rather than relying solely on phenotypic evaluations, which are time-consuming and influenced by environmental factors (Collard and Mackill, 2008).

### 5.4. Impact of MAS on crop improvement

The integration of MAS into breeding programs has revolutionized crop improvement efforts by accelerating the development of improved varieties with desirable traits. MAS enables breeders to make more informed and precise selection decisions, leading to the rapid deployment of elite cultivars that exhibit enhanced performance, resilience, and quality (Hospital, 2001).

Furthermore, MAS allows for the introgression of valuable traits from wild relatives or exotic germplasm into elite breeding lines, overcoming barriers to traditional breeding approaches (Collard and Mackill, 2008). This contributes to the diversification and sustainability of crop production systems, particularly in the face of evolving biotic and abiotic challenges.

## 6. Challenges and future perspectives

### 6.1. Challenges and future perspectives in molecular marker utilization for crop genomics

#### 6.1.1. Marker validation

One of the primary challenges in molecular marker utilization is marker validation, which involves confirming the association between molecular markers and target traits across diverse genetic backgrounds. Marker validation is essential to ensure the reliability and accuracy of marker-assisted selection (MAS) in breeding programs (Hospital, 2001).

#### 6.1.2. Data interpretation

The interpretation of molecular marker data can be complex, particularly in the context of genome-wide association studies (GWAS) or genomic selection. Analyzing large datasets generated by high-throughput genotyping platforms requires advanced statistical and bioinformatics techniques, posing challenges for data interpretation and downstream analysis (Varshney et al., 2018).

#### 6.1.3. Cost-effectiveness

While high-throughput genotyping technologies have become more affordable in recent years, the cost of genotyping large populations remains a significant challenge for many breeding programs, especially in developing countries. Cost-effective genotyping strategies and collaborative initiatives are needed to overcome this barrier and ensure equitable access to molecular breeding tools (Varshney et al., 2012).

### 6.2. Future perspectives

#### 6.2.1. Genome editing technologies

Genome editing technologies, such as CRISPR-Cas9, offer promising avenues for precise and targeted genetic manipulation in crops. By enabling the modification of specific DNA sequences, genome editing allows breeders to introduce beneficial alleles or edit genes associated with agronomic traits directly. This technology has the potential to revolutionize crop improvement by accelerating the development of tailored varieties with improved traits (Scheben et al., 2017).

#### 6.2.2. Multi-omics approaches

Multi-omics approaches, which integrate data from genomics, transcriptomics, proteomics, metabolomics, and other omics disciplines, provide comprehensive insights into the complex biological processes underlying trait variation in crops. By combining information from multiple molecular layers, multi-omics approaches enable a holistic understanding of crop biology and facilitate the identification of key genes and pathways associated with target traits. This integrated approach holds great promise for enhancing crop improvement through systems-level analysis and trait prediction (Fernie and Schauer, 2009). Despite the challenges associated with the utilization of molecular markers in crop genomics, ongoing advancements in technology and bioinformatics are expanding the scope and efficacy of molecular breeding strategies. By addressing challenges related to marker validation, data interpretation, and cost-effectiveness, and embracing emerging technologies such as genome editing and multi-omics approaches, researchers and breeders can overcome existing limitations and accelerate crop improvement efforts.

## 7. Conclusion harnessing molecular markers for sustainable crop improvement

In this comprehensive review, we have explored the critical role of molecular markers in enhancing genetic diversity and facilitating crop improvement efforts. Molecular markers have emerged as invaluable tools in crop genomics, enabling researchers and breeders to unravel the genetic basis of important traits, identify desirable alleles, and accelerate the development of improved crop varieties.

## 8. Key findings

- 1) **Enhancement of Genetic Diversity:** Molecular markers play a pivotal role in assessing and characterizing genetic diversity within crop populations. By analyzing DNA variations at the molecular level, researchers can uncover the extent of genetic variation present in germplasm collections, landraces, and wild relatives. This information serves as the foundation for crop breeding programs, allowing breeders to harness the wealth of genetic resources available for trait improvement.
- 2) **Facilitation of Crop Improvement:** Molecular markers facilitate targeted breeding efforts through marker-assisted selection (MAS) and genomic selection (GS). MAS enables breeders to select individuals with desired traits at the molecular level, expediting the development of new crop varieties with improved yield potential, disease resistance, and abiotic stress tolerance. GS, on the other hand, leverages genomic data to predict the breeding value of individuals, enabling more efficient selection decisions and accelerating genetic gain.
- 3) **Challenges and Opportunities:** Despite the immense potential of molecular markers, their widespread adoption in breeding programs faces challenges such as marker validation, data interpretation, and cost-effectiveness. However, ongoing advancements in genotyping technologies, bioinformatics, and genome editing offer promising solutions to overcome these challenges. Collaborative efforts and interdisciplinary research are essential for harnessing the full potential of genomics in sustainable agriculture.

## 9. Importance of continued research and collaboration

As we navigate the complexities of modern agriculture and strive to address global food security challenges, continued research and collaboration are paramount. By investing in genomics research and fostering partnerships between academia, industry, and government

agencies, we can accelerate the development and deployment of innovative breeding strategies. These strategies, empowered by molecular markers and genomics tools, hold the key to sustainable crop improvement and resilient food systems in the face of changing environmental conditions and evolving agricultural demands.

In conclusion, molecular markers stand as pillars of modern crop breeding, enabling precision, efficiency, and sustainability in agricultural practices. With concerted efforts and a forward-thinking approach, we can harness the transformative potential of genomics to shape a brighter future for agriculture and ensure food security for generations to come.

## References

- [1] Acquah, G. (2007). Principles of Plant Genetics and Breeding. Blackwell Publishing.
- [2] Adams, M. D., Kelley, J. M., Gocayne, J. D., Dubnick, M., Polymeropoulos, M. H., Xiao, H., ... & Venter, J. C. (1991). Complementary DNA sequencing: expressed sequence tags and human genome project. *Science*, 252(5013), 1651-1656. <https://doi.org/10.1126/science.2047873>.
- [3] Agarwal, M., Shrivastava, N., & Padh, H. (2008). Advances in molecular marker techniques and their applications in plant sciences. *Plant Cell Reports*, 27(4), 617-631. <https://doi.org/10.1007/s00299-008-0507-z>.
- [4] Anderson, J. A., Churchill, G. A., Autrique, J. E., Tanksley, S. D., & Sorrells, M. E. (1993). Optimizing parental selection for genetic linkage maps. *Genome*, 36(1), 181-186. <https://doi.org/10.1139/g93-024>.
- [5] Arteaga, M. C., Moreno-Letelier, A., Mastretta-Yanes, A., Vázquez-Lobo, A., Breña-Ochoa, A., Moreno-Estrada, A., ... & Eguiarte, L. E. (2016). Genomic variation in recently collected maize landraces from Mexico. *Genetic Resources and Crop Evolution*, 63(4), 711-726. <https://doi.org/10.1016/j.gdata.2015.11.002>.
- [6] Barker, J. S. F., Moore, S. S., Hetzel, D. J. S., Evans, D., Tan, S. G., & Byrne, K. (1999). Genetic diversity of Asian water buffalo (*Bubalus bubalis*): Microsatellite variation and a comparison with protein-coding loci. *Animal Genetics*, 28(2), 103-115. <https://doi.org/10.1111/j.1365-2052.1997.00085.x>.
- [7] Botstein, D., White, R. L., Skolnick, M., & Davis, R. W. (1980). Construction of a genetic linkage map in man using restriction fragment length polymorphisms. *American Journal of Human Genetics*, 32(3), 314-331.
- [8] Collard, B. C. Y., & Mackill, D. J. (2008). Marker-assisted selection: An approach for precision plant breeding in the twenty-first century. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 363(1491), 557-572. <https://doi.org/10.1098/rstb.2007.2170>.
- [9] Elshire, R. J., Glaubitz, J. C., Sun, Q., Poland, J. A., Kawamoto, K., Buckler, E. S., & Mitchell, S. E. (2011). A robust, simple genotyping-by-sequencing (GBS) approach for high diversity species. *PLoS One*, 6(5), e19379. <https://doi.org/10.1371/journal.pone.0019379>.
- [10] Falconer, D. S., & Mackay, T. F. C. (1996). Introduction to Quantitative Genetics. Longman Group Ltd.
- [11] Fernie, A. R., & Schauer, N. (2009). Metabolomics-assisted breeding: a viable option for crop improvement? *Trends in Genetics*, 25(1), 39-48. <https://doi.org/10.1016/j.tig.2008.10.010>.
- [12] Foley, J. A., Ramankutty, N., Brauman, K. A., Cassidy, E. S., Gerber, J. S., Johnston, M., ... & Zaks, D. P. (2011). Solutions for a cultivated planet. *Nature*, 478(7369), 337-342. <https://doi.org/10.1038/nature10452>.
- [13] Frankham, R., Ballou, J. D., & Briscoe, D. A. (2002). Introduction to Conservation Genetics. Cambridge University Press. <https://doi.org/10.1017/CBO9780511808999>.
- [14] Gepts, P. (2006). Plant Genetic Resources Conservation and Utilization: The Accomplishments and Future of a Societal Insurance Policy. *Crop Science*, 46(5), 2278-2292. <https://doi.org/10.2135/cropsci2006.03.0169gas>.
- [15] Govindaraj, M., Vetriventhan, M., & Srinivasan, M. (2015). Importance of Genetic Diversity Assessment in Crop Plants and Its Recent Advances: An Overview of Its Analytical Perspectives. *Genetics Research International*, 2015, 431487. <https://doi.org/10.1155/2015/431487>.
- [16] Gupta, P. K., & Varshney, R. K. (2000). The development and use of microsatellite markers for genetic analysis and plant breeding with emphasis on bread wheat. *Euphytica*, 113(3), 163-185. <https://doi.org/10.1023/A:1003910819967>.
- [17] Hajjar, R., & Hodgkin, T. (2007). The use of wild relatives in crop improvement: a survey of developments over the last 20 years. *Euphytica*, 156(1-2), 1-13. <https://doi.org/10.1007/s10681-007-9363-0>.
- [18] Hedrick, P. W. (1999). Highly variable loci and their interpretation in evolution and conservation. *Evolution*, 53(2), 313-318. <https://doi.org/10.2307/2640768>.
- [19] Heffner, E. L., Sorrells, M. E., & Jannink, J. L. (2009). Genomic selection for crop improvement. *Crop Science*, 49(1), 1-12. <https://doi.org/10.2135/cropsci2008.08.0512>.
- [20] Hickey, J. M., Chiurugwi, T., Mackay, I., Powell, W., & Mwangi, L. (2017). Genomic prediction unifies animal and plant breeding programs to form platforms for biological discovery. *Nature Genetics*, 49(9), 1297-1303. <https://doi.org/10.1038/ng.3920>.
- [21] Hoisington, D., Khairallah, M., & González-de-León, D. (1999). Laboratory Protocols: CIMMYT Applied Molecular Genetics Laboratory. CIMMYT. Hospital, F. (2001). Marker-assisted breeding. In *Plant Breeding Reviews* (pp. 145-217). John Wiley & Sons, Inc.
- [22] Hou, J., Jiang, Q., Hao, C., Wang, Y., Zhang, H., & Zhang, X. (2012). Global selection on sucrose synthase haplotypes during a century of wheat breeding. *Plant Physiology*, 160(3), 986-997.
- [23] Huang, X., Wei, X., Sang, T., Zhao, Q., Feng, Q., Zhao, Y., ... & Li, W. (2010). Genome-wide association studies of 14 agronomic traits in rice landraces. *Nature Genetics*, 42(11), 961-967. <https://doi.org/10.1038/ng.695>.
- [24] Hughes, A. R., Inouye, B. D., Johnson, M. T., Underwood, N., & Vellend, M. (2008). Ecological consequences of genetic diversity. *Ecology Letters*, 11(6), 609-623. <https://doi.org/10.1111/j.1461-0248.2008.01179.x>.
- [25] Jarvis, D. I., Brown, A. H., Cuong, P. H., Collado-Panduro, L., Latournerie-Moreno, L., Gyawali, S., ... & Sadiki, M. (2008). A global perspective of the richness and evenness of traditional crop-variety diversity maintained by farming communities. *Proceedings of the National Academy of Sciences*, 105(14), 5326-5331. <https://doi.org/10.1073/pnas.0800607105>.
- [26] Jones, C. J., Edwards, K. J., Castaglione, S., et al. (1997). Reproducibility testing of RAPD, AFLP, and SSR markers in plants by a network of European laboratories. *Molecular Breeding*, 3(5), 381-390. <https://doi.org/10.1023/A:1009612517139>.
- [27] Jones-Rhoades, M. W., Bartel, D. P., & Bartel, B. (2006). MicroRNAs and their regulatory roles in plants. *Annual Review of Plant Biology*, 57, 19-53. <https://doi.org/10.1146/annurev.arplant.57.032905.105218>.
- [28] Karp, A., Seberg, O., & Buiatti, M. (1997). Molecular techniques in the assessment of botanical diversity. *Annals of Botany*, 78(2), 143-149. <https://doi.org/10.1006/anbo.1996.0106>.
- [29] Kumar, L. S. (2009). DNA markers in plant improvement: An overview. *Biotechnology Advances*, 17(2-3), 143-182. [https://doi.org/10.1016/S0734-9750\(98\)00018-4](https://doi.org/10.1016/S0734-9750(98)00018-4).
- [30] Lande, R. (1996). Statistics and partitioning of species diversity, and similarity among multiple communities. *Oikos*, 76(1), 5-13. <https://doi.org/10.2307/3545743>.
- [31] Lewontin, R. C. (1974). The Genetic Basis of Evolutionary Change. Columbia University Press.
- [32] Litt, M., & Luty, J. A. (1989). A hypervariable microsatellite revealed by in vitro amplification of a dinucleotide repeat within the cardiac muscle actin gene. *American Journal of Human Genetics*, 44(3), 397-401.
- [33] Lu, Y., Yan, J., Guimarães, C. T., Taba, S., Hao, Z., Gao, S., ... & Xu, Y. (2010). Molecular characterization of global maize breeding germplasm based on genome-wide single nucleotide polymorphisms. *Theoretical and Applied Genetics*, 120(1), 93-115. <https://doi.org/10.1007/s00122-009-1162-7>.
- [34] Lynch, M., & Walsh, B. (1998). Genetics and Analysis of Quantitative Traits. Sinauer Associates.



- [35] Mace, E. S., Phong, D. T., Upadhyaya, H. D., Chandra, S., & Prasad, S. (2013). A global view of genetic diversity in cultivated sorghums using a core SNP marker set. *Genome Biology*, 14(12), R134.
- [36] Mammadov, J., Chen, W., Ren, R., et al. (2012). Development of highly polymorphic SNP markers from the complexity-reduced portion of maize (*Zea mays* L.) genome for use in marker-assisted breeding. *Theoretical and Applied Genetics*, 124(3), 527-543.
- [37] Mardis, E. R. (2013). Next-generation sequencing platforms. *Annual Review of Analytical Chemistry*, 6, 287-303. <https://doi.org/10.1146/annurev-anchem-062012-092628>.
- [38] Mohan, M., Nair, S., Bhagwat, A., et al. (1997). Genome mapping, molecular markers and marker-assisted selection in crop plants. *Molecular Breeding*, 3(2), 87-103. <https://doi.org/10.1023/A:1009651919792>.
- [39] Nei, M. (1987). *Molecular Evolutionary Genetics*. Columbia University Press. <https://doi.org/10.7312/nei-92038>.
- [40] Nei, M., & Kumar, S. (2000). *Molecular Evolution and Phylogenetics*. Oxford University Press. <https://doi.org/10.1093/oso/9780195135848.001.0001>.
- [41] Paterson, A. H., Lander, E. S., Hewitt, J. D., Peterson, S., Lincoln, S. E., & Tanksley, S. D. (1988). Resolution of quantitative traits into Mendelian factors by using a complete linkage map of restriction fragment length polymorphisms. *Nature*, 335(6192), 721-726. <https://doi.org/10.1038/335721a0>.
- [42] Peakall, R., & Smouse, P. E. (2012). GenAEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research—an update. *Bioinformatics*, 28(19), 2537-2539. <https://doi.org/10.1093/bioinformatics/bts460>.
- [43] Poland, J. A., Brown, P. J., Sorrells, M. E., & Jannink, J. L. (2012). Development of high-density genetic maps for barley and wheat using a novel two-enzyme genotyping-by-sequencing approach. *PLoS One*, 7(2), e32253. <https://doi.org/10.1371/journal.pone.0032253>.
- [44] Powell, W., Machray, G. C., & Provan, J. (1996). Polymorphism revealed by simple sequence repeats. *Trends in Plant Science*, 1(7), 215-222. [https://doi.org/10.1016/1360-1385\(96\)86898-1](https://doi.org/10.1016/1360-1385(96)86898-1).
- [45] Powell, W., Morgante, M., Andre, C., Hanafey, M., Vogel, J., Tingey, S., & Rafalski, A. (1996). The comparison of RFLP, RAPD, AFLP and SSR (microsatellite) markers for germplasm analysis. *Molecular Breeding*, 2(3), 225-238. <https://doi.org/10.1007/BF00564200>.
- [46] Rafalski, J. A. (2002). Applications of single nucleotide polymorphisms in crop genetics. *Current Opinion in Plant Biology*, 5(2), 94-100. [https://doi.org/10.1016/S1369-5266\(02\)00240-6](https://doi.org/10.1016/S1369-5266(02)00240-6).
- [47] Reed, D. H., & Frankham, R. (2003). Correlation between fitness and genetic diversity. *Conservation Biology*, 17(1), 230-237. <https://doi.org/10.1046/j.1523-1739.2003.01236.x>.
- [48] Salem, K. F. M., El-Zanaty, A. M., & Esmail, R. M. (2015). Assessing wheat (*Triticum aestivum* L.) genetic diversity using morphological characters and microsatellites. *World Applied Sciences Journal*, 33(9), 1445-1452.
- [49] Scheben, A., Wolter, F., Batley, J., Puchta, H., & Edwards, D. (2017). Towards CRISPR/Cas crops—bringing together genomics and genome editing. *New Phytologist*, 216(3), 682-698. <https://doi.org/10.1111/nph.14702>.
- [50] Schlotterer, C. (2004). The evolution of molecular markers—just a matter of fashion? *Nature Reviews Genetics*, 5(1), 63-69. <https://doi.org/10.1038/nrg1249>.
- [51] Singh, N., Choudhury, D. R., Singh, A. K., Kumar, S., Srinivasan, K., Tyagi, R. K., ... & Singh, N. K. (2018). Genetic diversity trend in Indian rice varieties: An analysis using SSR markers. *BMC Genetics*, 19(1), 1-10.
- [52] Singh, R., Singh, D., & Singh, R. (2014). *Molecular Breeding for Sustainable Crop Improvement*. Springer.
- [53] Sonah, H., Bastien, M., Iqura, E., Tardivel, A., Légaré, G., Boyle, B., ... & Belzile, F. (2013). An improved genotyping by sequencing (GBS) approach offering increased versatility and efficiency of SNP discovery and genotyping. *PLoS One*, 8(1), e54603. <https://doi.org/10.1371/journal.pone.0054603>.
- [54] Tanksley, S. D. (1993). Mapping polygenes. *Annual Review of Genetics*, 27(1), 205-233. Tanksley, S. D., & McCouch, S. R. (1997). Seed banks and molecular maps: unlocking genetic potential from the wild. *Science*, 277(5329), 1063-1066. <https://doi.org/10.1126/science.277.5329.1063>.
- [55] Tautz, D. (1989). Hypervariability of simple sequences as a general source for polymorphic DNA markers. *Nucleic Acids Research*, 17(16), 6463-6471. <https://doi.org/10.1093/nar/17.16.6463>.
- [56] Tester, M., & Langridge, P. (2010). Breeding technologies to increase crop production in a changing world. *Science*, 327(5967), 818-822. <https://doi.org/10.1126/science.1183700>.
- [57] Tester, M., & Langridge, P. (2010). Breeding technologies to increase crop production in a changing world. *Science*, 327(5967), 818-822. <https://doi.org/10.1126/science.1183700>.
- [58] Van de Wouw, M., van Hintum, T., Kik, C., van Treuren, R., & Visser, B. (2010). Genetic diversity trends in twentieth-century crop cultivars: A meta-analysis. *Theoretical and Applied Genetics*, 120(6), 1241-1252. Van Hintum, T. J. L., Brown, A. H. D., Spillane, C., & Hodgkin, T. (2000). Core collections of plant genetic resources. IPGRI. <https://doi.org/10.1007/s00122-009-1252-6>.
- [59] Varshney, R. K., Bohra, A., Yu, J., Graner, A., & Zhang, Q. (2018). Sorrells, M. E. genomic applications in agriculture: Next generation sequencing, genotyping, and beyond. *Molecular Breeding*, 35(1), 1-13.
- [60] Varshney, R. K., Graner, A., & Sorrells, M. E. (2005). Genic microsatellite markers in plants: features and applications. *Trends in Biotechnology*, 23(1), 48-55. <https://doi.org/10.1016/j.tibtech.2004.11.005>.
- [61] Varshney, R. K., Nayak, S. N., May, G. D., & Jackson, S. A. (2009). Next-generation sequencing technologies and their implications for crop genetics and breeding. *Trends in Biotechnology*, 27(9), 522-530. <https://doi.org/10.1016/j.tibtech.2009.05.006>.
- [62] Vignal, A., Milan, D., SanCristobal, M., & Eggen, A. (2002). A review on SNP and other types of molecular markers and their use in animal genetics. *Genetics Selection Evolution*, 34(3), 275-305. <https://doi.org/10.1186/1297-9686-34-3-275>.
- [63] Vos, P., Hogers, R., Bleeker, M., et al. (1995). AFLP: a new technique for DNA fingerprinting. *Nucleic Acids Research*, 23(21), 4407-4414. <https://doi.org/10.1093/nar/23.21.4407>.
- [64] Wang, Q., Tian, F., Pan, Y., Buckler, E. S., & Zhang, Z. (2014). A SUPER powerful method for genome-wide association study. *PLoS One*, 9(9), e107684. <https://doi.org/10.1371/journal.pone.0107684>.
- [65] Weir, B. S. (1996). *Genetic Data Analysis II: Methods for Discrete Population Genetic Data*. Sinauer Associates.
- [66] Welsh, J., & McClelland, M. (1990). Fingerprinting genomes using PCR with arbitrary primers. *Nucleic Acids Research*, 18(24), 7213-7218. <https://doi.org/10.1093/nar/18.24.7213>.
- [67] Williams, J. G. K., Kubelik, A. R., Livak, K. J., Rafalski, J. A., & Tingey, S. V. (1990). DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. *Nucleic Acids Research*, 18(22), 6531-6535. <https://doi.org/10.1093/nar/18.22.6531>.
- [68] Xu, X., Liu, X., Ge, S., Jensen, J. D., Hu, F. Y., Li, X., ... & Li, J. (2011). Resequencing 50 accessions of cultivated and wild rice yields markers for identifying agronomically important genes. *Nature Biotechnology*, 30(1), 105-111. <https://doi.org/10.1038/nbt.2050>.
- [69] Xu, X., Liu, X., Ge, S., Jensen, J. D., Hu, F., Li, X., ... & Wang, W. (2016). Resequencing 50 accessions of cultivated and wild rice Xu, Y. (2010). *Molecular Plant Breeding*. CABI. <https://doi.org/10.1079/9781845933920.0000>.