Molecular Markers and its Breeding Approaches in Crop Improvement

Divya Chaudhary, Sivendra Joshi, Arushi Arora

Molecular markers have revolutionized crop improvement by enabling the accurate and efficient selection of desirable traits. This chapter provides a comprehensive overview of the role of molecular markers in plant breeding, emphasizing their use in disease resistance, tolerance to abiotic stress, and improving quality traits. The chapter delves into various types of molecular markers, including RFLPs, RAPDs, AFLPs, SSRs, SNPs, and their respective advantages and limitations in breeding programs. It also explores marker-assisted selection and genomic selection as key strategies for accelerating the breeding process. The combination of molecular markers with traditional breeding methods is explored, with case studies illustrating the effective use of these tools in advancing crop improvement. The chapter concludes by emphasizing the potential of molecular markers to enhance crop resilience and productivity in the face of global challenges, such as climate change and food security.

Keywords: *Molecular markers, Genetic diversity, Gene pyramiding, QTL mapping*

Divya Chaudhary[1*], Sivendra Joshi[2], Arushi Arora[3]
[1]ICAR-National Bureau of Plant Genetic Resources, New Delhi, India.
[2]Central Institute of Medicinal and Aromatic Plants, Research Center, Pantnagar, Uttarakhand, India.
[3]Department of Plant Breeding and Genetics, Punjab Agricultural University, Ludhiana, Punjab, India.

[*]Email: divyachaudhary6767@gmail.com

Publisher: Cornous Publications LLP, Puducherry, India.
Advances in Crop Breeding
Editors: Dr. Uday Govinda Reddy, Dr. Sanjeev K Deshpande, Dr. Priyanka K, Dr. Shamarao Jahagirdar
ISBN: 978-81-973154-8-0
DOI: https://doi.org/10.37446/edibook072024/108-127

Introduction

The landmark discoveries of Darwin & Mendel laid the scientific groundwork for plant breeding and genetics as the 20th century began. Similarly, recent advancements in biotechnology, genomics, and the use of molecular markers, when integrated with traditional plant breeding methods, have laid the foundation for molecular plant breeding. This integrated field is transforming crop improvement in the 21st century. As molecular plant breeding techniques advance, they continue to be a central area of interest for plant breeders and crop scientists. The lengthy process of developing a commercial cultivar and the limitations associated with traits that are heavily influenced by the environment or have low heritability necessitate the use of

additional methods to support the breeding process. Advancements in molecular biology have given rise to a new category of markers based on DNA sequence variations, which have broadened the scope of addressing new challenges in plant breeding (Nadeem et al., 2018). DNA-based markers are distributed across the entire genome, remain stable regardless of environmental factors, and can be identified in any tissue and at any stage of development. Since their inception, DNA-based markers have become increasingly utilized in agriculture. Their applications now include creating genetic maps for crop species, associating molecular markers with important agronomic traits, and analyzing quantitative traits. Beyond estimating genetic distances, molecular markers are invaluable for assessing genetic diversity, choosing the best parental lines for breeding programs, managing germplasm collections, and identifying different varieties (Dormatey et al., 2020).

Types of molecular markers

Molecular markers are essential tools in modern plant breeding, facilitating the precise identification and selection of preferred traits. They are generally categorized into three main types: DNA markers, RNA markers, and protein markers. Below is a detailed overview of these marker types, including their characteristics.

1. DNA Markers

DNA markers are DNA fragments that show polymorphism due to insertions, deletions, or substitutions between different individuals. The DNA markers used in molecular breeding are explained as follows.

Restriction fragment length polymorphism (RFLP): RFLP was one of the first DNA markers were developed by Botstein et al., (1980), and used in molecular breeding. With this method, restriction enzymes are used to break down DNA, and the resultant fragments are separated via gel electrophoresis before being hybridized with tagged probes. RFLP markers are co-dominant. However, the technique is labor-intensive and requires large amounts of high-quality DNA, limiting its application in large-scale breeding programs (Collard et al., 2005).

Random amplified polymorphic DNA (RAPD): RAPD markers rely on the amplification of random DNA regions using short primers in polymerase chain reaction (PCR). These are dominant type of markers. RAPD is relatively simple and inexpensive but suffers from poor reproducibility, making it less favorable for high-resolution mapping (Agarwal et al., 2008).

Amplified fragment length polymorphism (AFLP): AFLP combines the principles of RFLP and PCR. The method entails digesting DNA using restriction enzymes and selectively amplifying the resulting fragments (Vos et al., 1995). These are highly polymorphic and reproducible markers, making them suitable for various genetic studies, including diversity analysis, linkage mapping, and marker-assisted selection.

Simple sequence repeats (SSRs or Microsatellites): SSRs are short, tandemly repeated DNA sequences, typically 1-6 bp in length. These markers are highly polymorphic, co-dominant, and distributed throughout the genome (Varshney et al., 2005). SSRs are ideal for genetic applications, such as genetic diversity studies, linkage mapping, and MAS. Their high level of polymorphism makes them especially useful in crop improvement programs.

Single nucleotide polymorphisms (SNPs): SNPs are single base pair variations in DNA sequences and represent the most abundant type of genetic variation in genomes. These markers are highly specific and increasingly popular due to their high density in the genome, making them suitable for fine mapping and genomic selection. Advances in next-generation sequencing (NGS) have made SNP genotyping cost-effective and high-throughput, accelerating their use in crop improvement (Rasheed et al., 2017). RFLPs are hybridization-based markers that use restriction enzymes and hybridization techniques like Southern blotting to detect polymorphisms. In contrast, RAPDs, AFLPs, and SSRs are PCR-based markers that rely on PCR to amplify specific DNA sequences and detect genetic variations. SNPs are sequencing-based markers identified through DNA sequencing to detect single base pair changes. PCR-based markers amplify DNA sequences to detect variations, while hybridization-based markers use the binding of complementary DNA sequences, often with labeled probes, to identify specific alleles or mutations. Other markers utilized in molecular breeding include Cleaved Amplified Polymorphic Sequences (CAPS), Sequence-Characterized Amplified Regions (SCAR), Diversity Arrays Technology (DArT), and Inter-Simple Sequence Repeat (ISSR). While DArT is a hybridization-based marker, CAPS, SCAR, and ISSR are PCR-based markers. On the basis of application and species involved, ideal markers (DNA) should meet the following criteria:

- It should be highly polymorphic
- Evenly distributed throughout the entire genome i.e. should not clustered in certain regions
- Co-dominant expression (to differentiate between heterozygotes from homozygotes)
- Clear distinct allelic features (Enabling easy identification of different alleles)
- Single copy with non-pleiotropic effect
- Cost effective
- Simple detection methods and ease of automation
- Wide availability (unrestricted use) and compatibility with duplication/multiplexing (allowing data to be collected and shared among laboratories)

2. RNA markers

Expressed sequence tags (ESTs): ESTs are short DNA sequences derived from mRNA, representing expressed genes in a particular tissue or developmental stage. ESTs serve as valuable markers for gene discovery, functional genomics, and comparative genomics (proite et al., 2007). they help in identifying traits related to specific genes and developing gene-based markers.

Microarray markers: Microarrays allow the simultaneous analysis of thousands of gene expressions, providing a comprehensive view of transcriptional activity. This technology is used to identify differentially expressed genes under various conditions, such as stress or disease. Microarray markers have been applied in functional genomics, transcriptomics, and gene discovery, although RNA sequencing (RNA-seq) technologies are gradually replacing them (Wang et al., 2020).

3. Protein markers

Isozymes: Isozymes are different forms of enzymes that vary in their amino acid sequences but catalyze the same chemical reaction. They can be separated by electrophoresis and used as markers for genetic diversity and population structure studies (Pollock et al., 1987). These enzymes are produced by different genes at separate loci and react with a specific substrate but have different migration patterns on gels. Although

isozymes were once widely used, their low polymorphism compared to DNA markers has led to a decline in their use in molecular breeding.

Allozymes: Allozymes are allelic variants of enzymes that differ slightly in their structure and charge, leading to different migration patterns during electrophoresis. They have been employed in genetic studies to assess genetic variation, population structure, and evolutionary relationships. Like isozymes, allozymes are less polymorphic than DNA markers and have become less common in modern molecular breeding (Richardson et al., 2012). Biochemical markers are influenced by tissue type, developmental stage, and environmental factors, and are less polymorphic compared to DNA markers. Therefore, DNA-based markers are widely used in crop improvement programs.

Dominant and codominant markers

The difference between these two types of DNA markers is explained in Table 1 and illustrated in Figure 1.

Table1. Difference between dominant and codominant markers

Features	**Dominant markers**	**Codominant markers**
Definition	Detect the presence of a specific allele, but do not distinguish between homozygous dominant and heterozygous individuals.	Detect both alleles at a locus, allowing identification of homozygous and heterozygous individuals.
Genetic Interpretation	cannot tell the difference between dominant individuals who are homozygous and heterozygous.	Can distinguish between all genotypes: homozygous dominant, homozygous recessive, and heterozygous.
Information Provided	Less informative; only shows presence or absence of a dominant allele.	More informative; shows both alleles, providing detailed genotype information.
Application	Useful for quick screening, but less precise in genetic analysis.	Preferred for detailed genetic studies and accurate genotyping.
Examples	RAPD, AFLP, ISSR	RFLP, SSR, SNP, CAPS

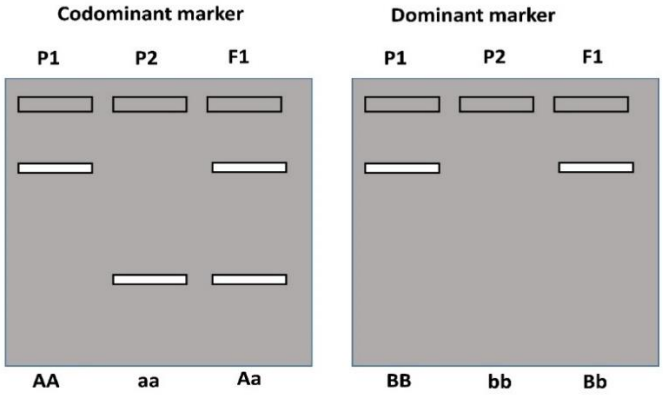

Figure 1. Comparison between codominant and dominant markers. Codominant markers can distinguish between dominant homozygote and heterozygotes, whereas dominant markers do not. P1= Homozygous dominant parent, P2= Homozygous recessive parent, F1= Heterozygous progenies

Application of molecular markers in crop Improvement

Marker-assisted selection: In order to improve efficiency and precise selection of desired traits in crops, marker-assisted selection (MAS), a potent breeding tool, combines molecular biology with conventional plant breeding methods. The idea of MAS is to make selection easier by using molecular markers, which are distinct DNA sequences linked to certain qualities. This method allows researchers in identification and selection of plants that carry desirable genetic traits before these traits expressed phenotypically, thereby accelerating the breeding cycle and improving the overall efficiency of crop improvement programs (Zambelli, 2019). The importance of MAS in modern agriculture cannot be overstated. It addresses several limitations associated with conventional breeding methods, such as the lengthy time required for phenotypic evaluation and the environmental variability that can affect trait expression. By relying on molecular markers, breeders can make more informed selections based on genetic information, which is not influenced by environmental factors (Jiang, 2013). This helps in more rapid accumulation of desirable traits in breeding populations, ultimately resulting in the development of crop varieties that can better withstand biotic and abiotic stresses, improve yield, and enhance nutritional quality. Molecular markers related to desirable characteristics are identified, a genetic linkage map is created, and these markers are then applied in breeding programs as part of the MAS process. Firstly, areas of the genome linked to the desired trait are identified by QTL mapping. Once markers are identified, they can be used in various breeding strategies, such as heterosis breeding, hybridity testing, marker-assisted backcrossing (MABC) and marker-assisted recurrent selection (MARS), to introgress desirable traits into elite breeding lines (Arbelaez et al., 2019). This process not only streamlines the selection of plants with the desired traits but also reduces time and resources needed for traditional breeding methods, which often involve extensive field trials and phenotypic assessments (Milc et al., 2011).

Numerous examples of successful MAS programs in crop breeding illustrate its effectiveness (Table 2.). For instance, in peanut breeding, MAS has been employed to pyramid nematode resistance and high oleic acid traits, significantly reducing the time required for traditional backcrossing methods (Chu et al., 2011). Similarly, in maize, the molecular marker-assisted selection of alleles related to important agronomic traits has demonstrated substantial improvements in breeding efficiency, allowing for the rapid development of varieties with enhanced disease resistance and better yield potential (Chen et al., 2010). In peanut, MAS has been effectively utilized to improve rust resistance, showcasing its applicability across different crops and traits (Varshney et al., 2014). Moreover, the integration of genomic technologies with MAS has further revolutionized crop breeding. The advent of high-throughput sequencing and genotyping technologies has enabled the identification of a vast array of molecular markers, facilitating the mapping of complex traits and enhancing the precision of selection processes. This genomic approach not only accelerates the breeding cycle but also allows for the simultaneous selection of multiple traits, thereby addressing the multifaceted challenges faced by modern agriculture (Patella et al., 2019).

Marker assisted back crossing: In order to improve crop improvement efficiency and precision, Marker-Assisted Backcrossing (MABC) is an advanced breeding approach that incorporates molecular markers into conventional backcrossing methods. This approach is especially useful for eliminating the unwanted genetic baggage sometimes connected with traditional breeding approaches while introducing desired qualities, such disease resistance or abiotic stress tolerance, into elite lines. Using molecular markers associated with desired traits enables breeders to select for these traits early in the breeding process, which speeds up the recovery of the recurrent parent genome and shortens the time needed to create new varieties. This is the basic idea behind MABC.

The application of MABC has been extensively documented in various crops, showcasing its effectiveness in enhancing traits that are critical for agricultural productivity (Table 2.). For instance, in the case of rice, MABC has been successfully employed to improve drought tolerance in the Improved White Ponni variety, which is highly sensitive to drought conditions. This study demonstrated that MABC could significantly speed up the selection process for complex traits, achieving desirable genetic improvements within just two to four backcross generations (Seeli et al., 2024). Similarly, in tobacco, the construction of a high-density genetic map facilitated the identification of quantitative trait loci (QTLs) associated with high yield and disease resistance, further exemplifying the utility of MABC in crop improvement (Tong et al., 2021). The effectiveness of MABC is not limited to a single crop; it has been applied across various species, including common beans, where microsatellite markers were utilized to enhance the precision of backcross selection (Oliveira et al., 2008). The use of multiple markers across chromosomes has been shown to increase the accuracy of trait introgression, thereby improving the overall efficiency of the breeding program. Furthermore, studies have indicated that MABC can recover a significant proportion of the recurrent parent genome, often exceeding 90% within just a few backcross generations, which is a substantial improvement over traditional methods that may require more extensive backcrossing (Kim et al., 2021). In addition to improving individual traits, MABC has also been instrumental in the pyramiding of multiple resistance genes into elite cultivars. For example, in cauliflower, researchers successfully pyramided downy mildew-resistant and black rot-resistant genes into a popular variety using MABC, demonstrating its potential for developing crops with broad-spectrum disease resistance (Saha et al., 2021). This approach not only enhances the resilience of crops against various biotic stresses but also contributes to the sustainability of agricultural practices by reducing the reliance on chemical inputs.

The methodology of MABC involves several key steps, including foreground selection, background selection, and recombinant selection. Foreground selection focuses on identifying individuals that carry the desired trait, while background selection aims to recover the genetic background of the recurrent parent, minimizing linkage drag associated with the introgressed traits (Chukwu et al., 2020). Selecting backcross progeny with the target gene and recombination events between the target gene and flanking markers is known as recombinant selection. Recombinant selection aims to minimize the size of the target gene-containing donor chromosomal segment. This multi-faceted approach ensures that the resulting progeny retain the beneficial characteristics of the elite parent while incorporating the desired traits from the donor parent. The schematic representation of MABC breeding is illustrated in Figure 2., where Ellur et al., (2016) introduced bacterial blight resistance gene 'Xa38' into Pusa Basmati 1121. Moreover, the integration of genomic tools and high-density markers has further enhanced the capabilities of MABC. For instance, the use of SNP markers derived from transcriptome sequencing has facilitated more precise selection in crops like Capsicum, allowing for the rapid development of improved varieties (Kang et al., 2014). The ability to conduct high-throughput genotyping has transformed MABC into a more efficient and scalable breeding strategy, enabling breeders to handle larger populations and more complex trait combinations. The success of MABC is also evident in its application to rice breeding programs aimed at enhancing cooking and eating quality. By utilizing KASP markers, researchers have been able to select for traits such as amylose content and aroma, significantly improving the overall quality of rice varieties (Kim et al., 2021). This highlights the versatility of MABC in addressing both agronomic and consumer preferences, ultimately leading to more marketable and desirable crop varieties.

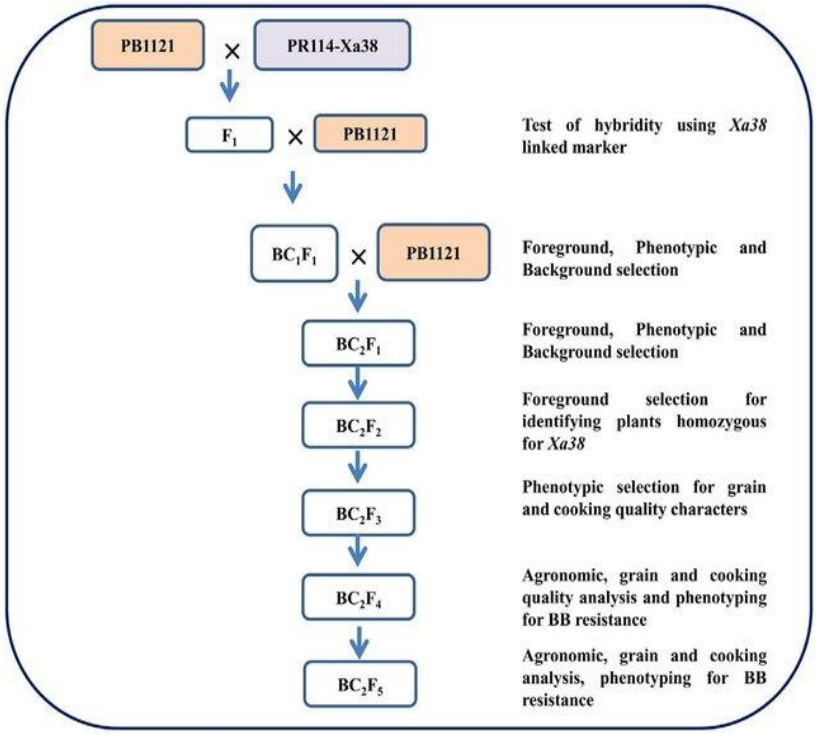

Figure2. Marker assisted backcross breeding scheme adopted for introgression of "*Xa38*" in Pusa Basmati 1121 (Source: Ellur et al., 2016).

Table 2. Crop varieties developed by MAS in India

Crop	**Varieties**	**Trait improved**	**Recurrent parent**	**Donor parent**	**Gene introgressed**	**Year of release**
Rice	Improved Pusa Basmati 1	Bacterial blight resistance	Pusa Basmati 1	IRBB 55	*xa13* and *Xa21*	2007
	Improved Samba Mahsuri	Bacterial blight resistance	Samba Mahsuri	SS1113	*xa5*, *xa13* and *Xa21*	2008
	Improved Lalat	Bacterial blight resistance	Lalat	IRBB 60	*Xa4*, *xa5*, *xa13* and *Xa21*	2013
	Improved Tapaswini	Bacterial blight resistance	Tapaswani	IRBB 60	*Xa4*, *xa5*, *xa13* and *Xa21*	2013
	Pusa Basmati 1718	Bacterial blight resistance	Pusa Basmati 1121	SPS 97	*xa13* and *Xa21*	2017
	CR Dhan 800	Bacterial blight resistance	Swarna	IRBB 60	*xa5*, *xa13* and *Xa21*	2018

	DRR Dhan 59	Bacterial blight resistance	Akshyadhan	FBR1-13	*Xa33*	2021
	DRR Dhan 51	Blast resistance	Swarna	C101A51 (Pi2)	*Pi2*	2018
	Pusa Samba 1850	Blast resistance	Samba Mahsuri	DHMASQ164-2b	*Pi1*, *Pi54* and *Pita*	2018
	DRR Dhan 62	Bacterial blight & blast resistance	Improved Samba Mahsuri	C101A51 and Tetep	*Xa21*, *xa13*, *xa5*, *Pi2* and *Pi-54*	2021
	DRR Dhan 58	Bacterial blight resistance & seedling stage salinity tolerance	Improved Samba Mahsuri	FL478	*Xa21*, *xa13*, *xa5* and *qSaltol*	2021
	Swarna Sub 1	Submergence tolerance	Swarna	IR 49830-7-1-2-3	*qSub1*	2009
	Ranjit Sub 1	Submergence tolerance	Ranjit	Swarna Sub1	*qSub1*	2018
	IR 64 Sub1	Submergence tolerance	IR 64	IR 49830-7-1-2-3 (FR13A)	*qSub1*	2020
	CR Dhan 803 (Trilochan)	Submergence tolerance	Pooja	Swarna Sub1	*qSub1*	2021
	CR Dhan 802 (Subhash)	Submergence & drought tolerance	Swarna Sub1	IR 81896-B-B-195	*qSub1*, *qDTY1.1* and *qDTY2.1*	2019
	Pusa Basmati 1985	Herbicide (Imazethapyr) tolerance	Pusa Basmati 1509	Robin	*AHAS*	2021
Wheat	PBW 761 (Unnat PBW 550)	Stripe rust resistance	PBW 550	Avocet + Yr15	*Yr15*	2019
	PBW 757	Stripe rust resistance	PBW 550	Avocet + Yr15	*Yr15*	2019
	PBW 771	Stripe & leaf rust resistance	DBW 17	*Ae. umbellulata* (Accession no. 3732)	*Yr40* and *Lr57*	2020
Maize	Vivek QPM9	Lysine & tryptophan	CM212 and CM145	CML180 and CML 170	*opaque2*	2008
	Pusa HM4 Improved	Lysine & tryptophan	HKI1105 and HKI323	CML161 and HKI161	*opaque2*	2017

	Pusa Vivek Hybrid-27 Improved (Hybrid)	Provitamin-A	V335 and V345 (parents of maize hybrid, Vivek Hybrid-27)	HP465-30 and HP465-35	*crtRB1*	2020
	Pusa HQPM-1 Improved (Hybrid)	Provitamin-A	HKI193-1 and HKI163 (parents of maize hybrid, HQPM-1)	HP704-23 and HP704-22	*crtRB1* and *lcyE*	2021
Pearl Millet	HHB 67 Improved (Hybrid): Country's first MAS-derived cultivar	Downy mildew resistance	H 77/833-2 (male parent of hybrid, HHB 67)	ICMP 451	*qRSg1* and *qRSg4*	2005
	HHB 67 Improved 2 (Hybrid)	Downy mildew resistance	H 77/833-2-202 (male parent of hybrid, HHB 67)	P1449 and 863B	*qRSg3.1*, *qRSg4.2* and *qRSg6.1*	2021
Chickpea	Super Annigeri-1	Fusarium wilt resistance	Annigeri-1	WR-315 (ICC 8933)	*foc4*	2020
	Pusa Chickpea 20211	Fusarium wilt resistance	Pusa 391	WR 315	*foc1*, *foc3*, *foc4* and *foc5*	2021
	Pusa Chickpea 4005	Drought tolerance	Pusa 362	ICC 4958	QTL hotspot on *LG4*	2021
Soybean	NRC 132	Less beany flavour	JS 97-52// PI 596540	PI 596540	Null allele of *lox2*	2021
	NRC 142	Kunitz trypsin inhibitor free & less beany flavour	JS 97-52	PI 542044 and PI 596540	Null allele of *KTi3* and *lox2*	2021
	NRCSL 1	Yellow Mosaic Virus (YMV) resistance	JS 335	SL525	*Rymv*	2021
Groundnut	Girnar 4	Oleic acid	ICGV-06420//SunOleic 95R	SunOleic 95R	*ahFAD2a* and *ahFAD2b*	2020
	Girnar 5	Oleic acid	ICGV-06420//SunOleic 95R	SunOleic 95R	*ahFAD2a* and *ahFAD2b*	2020

Genetic diversity evaluation: Utilizing DNA markers to measure genetic variety has become a crucial aspect of contemporary genetics, particularly in the domains of plant breeding and conservation biology. Many molecular markers have been employed extensively to assess genetic variability within and between species, such as ISSR, RAPD, and SSR. SSR markers are particularly favored due to their co-dominant inheritance, high polymorphism, and reproducibility. They have been effectively employed in the genetic diversity assessment of crops such as mungbean, where studies have demonstrated significant genetic variation among different genotypes using SSR markers (Das & Baisakh, 2023; Mathivathana et al., 2018). These markers are abundant and uniformly distributed across the genome, making them ideal for evaluating genetic diversity in various species, including cotton and barley (Ferreira et al., 2016). The robustness of SSR markers allows for detailed genetic mapping and the identification of specific traits, which is crucial for breeding programs aimed at improving crop resilience and yield. RAPD markers, on the other hand, are advantageous for their simplicity and speed in generating genetic profiles without prior sequence knowledge. They have been successfully applied in studies assessing genetic diversity in species such as Cannabis sativa and various crops in Nigeria, revealing significant intra-specific variability (Ullah et al., 2023). Although RAPD markers are considered dominant and may have limitations in certain contexts, they provide valuable insights into the genetic structure and diversity of populations, as evidenced by their application in analyzing the genetic diversity of eggplant (Demir et al., 2010).

Heterosis breeding: The phenomenon known as heterosis, or hybrid vigor, occurs when hybrid offspring show superior characteristics than their parents, especially in terms of yield and growth rate. The application of molecular markers, including as SNPs and SSRs, has greatly improved the capacity to choose suitable parental lines for hybridization, optimizing heterosis in a variety of crops. One of the primary advantages of using DNA markers in heterosis breeding is their ability to assess genetic diversity among potential parent lines. Research has shown that a higher genetic distance between parental lines often correlates with increased heterosis in the resulting hybrids. For instance, studies on maize have demonstrated that the selection of inbred lines based on molecular markers can effectively cluster these lines into heterotic groups, facilitating the identification of optimal parental combinations for hybridization (Mukri et al., 2022). This approach not only streamlines the breeding process but also enhances the predictability of hybrid performance based on genetic distance. Moreover, the application of advanced marker technologies, such as DArTseq, has allowed breeders to identify specific markers associated with heterosis-related traits. For example, Tomkowiak et al., (2020) identified several SilicoDArT markers linked to morphological features and yield, underscoring the importance of molecular markers in understanding the genetic basis of heterosis. In crops like upland cotton, the relationship between genetic distance and yield heterosis has been quantitatively assessed using SSR markers, revealing that diverse parental lines contribute to enhanced hybrid vigor (Geng et al., 2021; Li et al., 2018). Furthermore, the exploration of heterosis in clonally propagated crops, such as potatoes, has also benefited from molecular marker applications. The development of inbred line-based hybrid systems has enabled the exploitation of heterosis in tuber crops, showcasing the versatility of DNA markers across different agricultural contexts (Li et al., 2022). This adaptability highlights the broader implications of molecular genetics in enhancing crop productivity and resilience.

Hybrid seed purity testing: The application of DNA markers in hybrid seed purity testing in crop plants has become a pivotal aspect of modern agricultural practices. In order to preserve the quality and efficiency of hybrid seeds, these markers offer a trustworthy way to evaluate genetic purity. In comparison to conventional phenotypic methods, the use of molecular markers, such as Simple Sequence Repeats (SSRs) and Single Nucleotide Polymorphisms (SNPs), offers a number of advantages, including increased accuracy, efficiency, and the capacity to identify genetic variations that are unaffected by environmental factors.

One of the primary benefits of utilizing DNA markers in hybrid seed purity testing is their ability to provide definitive genetic information. Molecular markers can accurately differentiate between hybrid and parental lines, ensuring that the seeds produced are true to type. This is particularly important in hybrid breeding programs where the genetic integrity of the hybrid is crucial for achieving desired traits such as yield, disease resistance, and stress tolerance (Ince & Karaca, 2019; Soriano, 2020). For instance, SSR markers are widely recognized for their high polymorphism and co-dominant inheritance, making them ideal for genetic mapping and purity testing. Moreover, the application of DNA markers extends beyond mere identification of hybrids; they also play a significant role in the assessment of genetic diversity within breeding populations. This is critical for ensuring that hybrid seeds are not only pure but also genetically diverse enough to withstand various environmental challenges (Ahmad & Anjum, 2018). The use of molecular markers facilitates the construction of genetic linkage maps, which can be instrumental in identifying and selecting for desirable traits through marker-assisted breeding strategies. Such approaches have been successfully employed in various crops, leading to improved hybrid varieties with enhanced performance characteristics (Hasan et al., 2021). In addition to SSRs and SNPs, other molecular marker systems such as RAPD and AFLP have also been utilized in hybrid seed purity testing. These markers provide a cost-effective means of evaluating genetic diversity and purity, particularly in resource-limited settings. The ability to utilize a combination of different marker types allows for a more comprehensive assessment of genetic purity, further reinforcing the reliability of hybrid seed production.

Gene pyramiding: Gene pyramiding is an advanced breeding technique that generally involves the incorporation of multiple genes simultaneously into a single genotype. This approach has been applied to numerous traits including yield, abiotic and biotic stress tolerance. Gene pyramiding plays a vital role in plant breeding as single-gene resistance often breaks down mainly due to the evolution of pathogens. However, with the stacking of multiple traits, there occurs a broader spectrum of resistance that ultimately increases the effectiveness and durability of a variety or genotype in any species (Jiang et al., 2019). Though gene pyramiding has been documented in several crops, there are notable achievements towards rice breeding. For example, *Pi46* and *Pita* genes have been pyramided into an elite rice line, HH179 for resistance to rice blast diseases without affecting yield (Peng et al., 2023). Transgenic rice lines were also pyramided with multiple *Pi* genes for improvement of resistance against blast disease. This technique is also applied to numerous other crops like *Brassica rapa* for resistance to clubroot resistance, wheat for rust resistance, potato for late blight resistance, maize for corn borer, tomato for leaf curl virus and others (Dormatey et al., 2020; Liu et al., 2020; Li et al., 2022). Gene pyramiding is a powerful strategy accelerated by the technique of molecular marker-assisted selection (MAS) to develop efficient crop varieties in various crops, cultivar developed by gene pyramiding are illustrated in Table 2.

QTL mapping: Quantitative Trait Locus (QTL) mapping is an integral tool in plant breeding and genetics that enables researchers to identify specific regions in genomes that are associated with quantitative traits linked to economically important traits in plants (Asins et al., 2009). QTL mapping begins with the selection of appropriate mapping populations. Populations such as; F2 populations, doubled haploids (DH), Backcross (BCs) and Recombinant Inbred Lines (RILs) can be used depending upon the resources and objective of the programmed (Nadeem et al., 2018). The next step is to measure the phenotypic traits accurately and efficiently followed by genotyping of the selected mapping population using molecular markers like SNPs. The distribution and density of these markers across the genomes are very important for an effective strategy of QTL mapping. Based on the data obtained, a linkage map that represents the genetic distances between the markers is constructed. It helps in the identification of regions associated with the trait of interest. Mapping techniques such as simple interval mapping, composite interval mapping or Inclusive Composite

Interval Mapping (ICIM) are generally used for the construction of these maps (Rocha et al., 2007). Utilizing these maps, certain statistical methods like ANOVA, regression analysis, Bayesian approaches or mixed models are used to identify associations between the trait of interest and the markers. This is often followed by the characterization and validation of QTLs for an effective outcome (Zhang et al., 2005; Gasbarra et al., 2009).

The steps involved in the QTL mapping approach are represented in Figure 3.

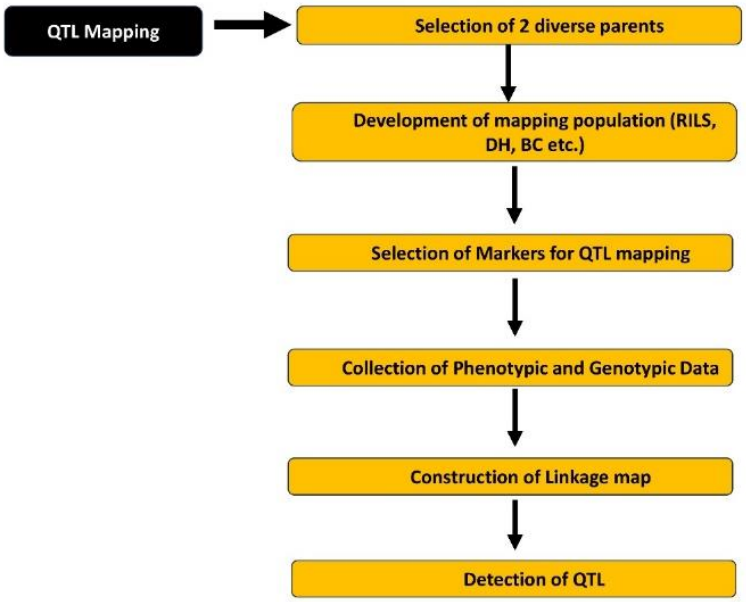

Figure 3. Procedure of QTL mapping

High-density mapping techniques have refined the detection of QTLs in several species. For example, the high-density mapping using single nucleotide polymorphism (SNP) markers in durum wheat has identified major QTLs for disease resistance (Kumar et al., 2018). Moreover, genome wide association study (GWAS) emerged as a complementary approach to traditional techniques involved in QTL mapping like in flax crop to identify multiple QTLs for oil quality and seed yield. GWAS method allows the dissection of the natural variation within populations for the identification of the associations between traits and genetic markers in order to detect QTLs across diverse genetic backgrounds. QTL mapping has been dedicated to the improvement of disease resistance in several crops like wheat, rice, maize, chickpea and others. These QTLs are also utilized further in breeding programmed (Deokar et al., 2018; Pundir et al., 2022). In rice, several QTLs for grain protein have also improved the nutritional content of the crop (Wu et al., 2020). Additionally, in maize, recent studies have focused on yield-related QTLs that are involved in increasing yield in maize (Zhao & Su, 2019). Similarly, QTL mapping is a promising technique to increase yields, resistance to biotic or abiotic stresses and to increase nutritional profiles in crops. Recent advances such as the integration of high-throughput phenotyping and genotyping by sequencing have allowed for a comprehensive analysis of the QTLs especially in diverse backgrounds and across several environments (Wu et al., 2018). Moreover, for the detection of small and linked QTLs, multi-locus models are efficient in uncovering the genetic architecture of the traits involved (Wen et al., 2019). The evolution of QTL mapping technologies has combined the traditional techniques of genetic mapping with novel genomic technologies ultimately contributing to food security and sustainable practices in agriculture.

Association mapping: Association mapping (AM) involves identifying significant correlations between molecular markers and phenotypic traits (Jannink & Walsh, 2002). Statistically, AM measures the covariance between the genetic polymorphism at the marker and the trait of interest. Compared to linkage mapping, AM is more time-efficient and offers higher mapping resolution by utilizing a greater number of recombination events. AM based on linkage disequilibrium (LD) concept. LD refers to the non-random association of alleles at different loci. LD describes the unequal frequency of haplotypes in a population, where certain combinations of alleles occur more or less frequently than expected. AM involves selecting individuals from a natural population that exhibit a broad spectrum of genetic diversity. Thorough and accurate phenotyping is conducted for various traits of interest, ideally across multiple locations and environmental conditions over several years. Following genotyping with appropriate markers, the population structure and kinship relationships are determined. Statistical measures such as D, D', or r^2 are used to quantify LD (Nadeem et al., 2018). Finally, phenotypic and genotypic data are analyzed and correlated using specialized statistical software, with TASSEL being one of the most commonly used tools for AM.

Genomic selection: The concept of Genomic Selection (GS) was proposed by Meuwissen in 2001. It is a relatively modern strategy that utilizes genomic information to predict breeding values of individuals and accelerate the process and efficiency of selection in plant breeding. The approach relies on the early selection of superior genotypes based on their genomic data rather than the phenotypic traits. This technique of integrating genomic data into breeding programs has improved prediction accuracy and reduced time duration in developing novel crop varieties. Thereby, GS has the ability to enhance genetic gain per unit time, especially by utilization of a high-density molecular marker system and identification of favourable alleles in diverse germplasm allowing researchers to make better decisions in genotype selection, especially for improvement for traits with lower heritability (Hoffstetter et al., 2016; Veerendrakumar, 2024). The training population (TP), which is made up from individuals who need to be both genotyped and phenotyped in order to assess the marker effects across the genome, is the central idea of genomic selection. Such information is used for prediction of genomic estimated breeding values (GEBVs) used for individuals in the separate populations consisting of only genotypic data, the breeding populations (BP) (Wang et al., 2020). The factors influencing the training populations include; genetic diversity, size of training population, relationship between training and breeding population. These factors along with other criterias help in selection of a suitable training population while performing GS. Though, this approach is popular in animal breeding, it is recently being utilized in crop breeding as well (Michel et al., 2021). This technique has successfully improved yield, disease resistance or drought tolerance in several crops such as wheat, maize, rice, soybean and others. However, a major challenge is the requirement of extensive training populations that accurately represent the true genetic diversity of the target population. The size and quality of training data play a major role in the effective execution of genomic selection. Additionally, there may be complications in this process due to the high dimensionality of genomic data and the usage of complex statistical methods for prediction. Moreover, the reliability of genomic data overlooks the crucial phenotypic traits that could potentially evaluate the populations precisely. Although, despite of challenges, GS offers significant advantages in aspects of accuracy and efficiency.

Molecular breeding for biotic and abiotic stress tolerance: Due to the increase in adversities of climate change in recent years, molecular breeding for abiotic and biotic stress tolerance has paced and gained attention for the immediate need for sustainable agricultural practices. Recent advances in molecular techniques involving the integration of molecular tools with traditional breeding methods have allowed for the development of several stress-tolerant genotypes for crop plants (Table 2.). Several studies have utilized techniques such as transcriptomic analysis, genome-wide association studies (GWAS) for the identification

of QTLs associated with drought tolerance in maize and rice, revealing several SNPs that can be effectively targeted in breeding programmed (Yang et al., 2022). A combination of conventional plant breeding and modern molecular techniques has accelerated the development of drought-tolerant varieties in rice crop (Banoth, 2023; Kumar et al., 2023). Another critical abiotic stress factor, heat stress is affecting yields in crops grown in regions experiencing a rise in temperatures. Molecular breeding techniques have successfully identified key genes involved in the heat tolerance of various crops like wheat and chickpea (Gaur et al., 2019; Patil & Ram, 2024). The powerful technique of genomic selection has shown promising results in the identification of heat-tolerant genotypes in major crop plants (Ayenan et al., 2019). Another significant concern, especially in coastal regions is the salinity stress. Utilizing a combination of technologies, such as high-throughput phenotyping and omics techniques several molecular markers have been linked to the salinity tolerance for the development of resistant varieties in salinity-sensitive crop plants (Schmidt Hoffer et al., 2018; Mohammed, 2024). The Crop wild relatives (CWRs) have played a crucial role as a component of molecular breeding for biotic stress tolerance as they are a source of diverse genes that are currently introrse into plants of several species successfully to develop disease-resistant varieties (Mammodov et al., 2018). Molecular markers have played a pivotal role in the introgression of disease resistance genes and the improvement of existing cultivars (Table 2.). Thus, the integration of molecular breeding tools with conventional techniques has significantly advanced the development of efficient varieties resistant to abiotic and biotic stresses.

Molecular breeding for quality traits: The molecular breeding techniques integrated with conventional breeding techniques have successfully enhanced shelf life, quality traits, nutritional quality and marketability in several crop species to fulfil the growing demands around the globe (Table 3.)

Table 3. Role of molecular breeding for improvement of quality traits

Crop	Trait improved	Technique/ methods used	Reference
Tomato	Shelf life and marketability	Marker assisted backcross breeding (MABB)	Kwabena Osei et al., 2022
Rapeseed	Oil Quality	Molecular markers linked to oil quality traits	Sachan et al., 2024
Rice	Nutritional Quality	Genomic Selection	Xiao et al., 2021
Maize	QPM (Quality Protein Maize) Vitamin A, Zinc and Iron	MABB and multi-omics approaches	Prasanna et al., 2020; Gedil et al., 2024
Lettuce	Shelf life	Molecular markers linked to post-harvest traits identified	Belisle et al., 2021; Chase et al., 2024

There is still ongoing research in several crops using novel molecular breeding methods aiming to improve public health and meet market demands.

Conclusion

Molecular marker technology has revolutionized plant breeding and agriculture since the 1980s. Numerous molecular marker types have been applied to genetic mapping, MAS, genetic resource characterization, and hybrid seed purity testing. With the use of these techniques, breeding programs have become much more accurate and effective leading to developing crops with desired characteristics like increased yield, stress

tolerance, and disease resistance. For instance, MAS has been used to develop bacterial blight-resistant rice varieties like “Improved Pusa Basmati 1” and “DRR Dhan 59,” as well as nutritionally enhanced maize varieties like “Vivek QPM9” (high in lysine and tryptophan) and “Pusa HQPM-1 Improved” (enriched with provitamin A). The integration of molecular markers with traditional breeding methods has accelerated crop improvement, offering significant benefits in a shorter timeframe. As the field continues to evolve, advancements in next-generation sequencing and bioinformatics are further expanding the possibilities for molecular marker applications. These developments could open up new options for the identification and application of markers in agricultural crop improvement. In the future, molecular markers will become even more significant as agriculture deals with issues like food security and climate change on a global scale. The future of agriculture will continue to be greatly influenced by molecular markers as they facilitate the development of resilient and productive crops.

References

Agarwal, M., Shrivastava, N., & Padh, H. (2008). Advances in molecular marker techniques and their applications in plant sciences. *Plant cell reports*, *27*, 617-631.

Ahmad, R., & Anjum, M. A. (2018). Applications of molecular markers to assess genetic diversity in vegetable and ornamental crops-a review. *Journal of Horticultural Science and Technology*, *1*, 1-7.

Arbelaez, J. D., Tandayu, E., Reveche, M. Y., Jarana, A., van Rogen, P., Sandager, L., ... & Cobb, J. (2019). Methodology: ssb-MASS: a single seed-based sampling strategy for marker-assisted selection in rice. *Plant Methods*, *15*, 1-11.

Asins, M. J., Bernet, G. P., Villalta, I., & Carbonell, E. A. (2009). QTL analysis in plant breeding. *Molecular Techniques in Crop Improvement: 2nd Edition*, 3-21.

Ayenan, M. A. T., Danquah, A., Hanson, P., Ampomah-Dwamena, C., Sodedji, F. A. K., Asante, I. K., & Danquah, E. Y. (2019). Accelerating breeding for heat tolerance in tomato (Solanum lycopersicum L.): an integrated approach. *Agronomy*, *9*(11), 720.

Banoth, M., Nunavath, U. S., Bhimireddy, S., Konne, D., Govada, V., & Lavudya, S. (2023). Conventional and molecular breeding strategies for improvement of drought tolerance cultivars in rice: Recent approaches and outlooks. *Environment Conservation Journal*, *24*(4), 367-381.

Belisle, C. E., Sargent, S. A., Brecht, J. K., Sandoya, G. V., & Sims, C. A. (2021). Accelerated shelf-life testing to predict quality loss in romaine-type lettuce. *HortTechnology*, *31*(4), 490-499.

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.

Chase, K., Belisle, C., Ahlawat, Y., Yu, F., Sargent, S., Sandoya, G., ... & Liu, T. (2024). Examining preharvest genetic and morphological factors contributing to lettuce (Lactuca sativa L.) shelf-life. *Scientific Reports*, *14*(1), 6618.

Chen, F., Zhu, S. W., Xiang, Y., Jiang, H. Y., & Cheng, B. J. (2010). Molecular marker-assisted selection of the ae alleles in maize. *Genetics and Molecular Research*, *9*(2), 1074-1084.

Chu, Y., Wu, C. L., Holbrook, C. C., Tillman, B. L., Person, G., & Ozias-Akins, P. (2011). Marker-assisted selection to pyramid nematode resistance and the high oleic trait in peanut. *The plant genome*, *4*(2).

Chukwu, S. C., Rafii, M. Y., Ramlee, S. I., Ismail, S. I., Oladosu, Y., Muhammad, I. I., ... & Yusuf, B. R. (2020). Recovery of recurrent parent genome in a marker-assisted backcrossing against rice blast and blight infections using functional markers and SSRs. *Plants*, *9*(11), 1411.

Collard, B. C., Jahufer, M. Z. Z., Brouwer, J. B., & Pang, E. C. K. (2005). An introduction to markers, quantitative trait loci (QTL) mapping and marker-assisted selection for crop improvement: the basic concepts. *Euphytica*, *142*, 169-196.

Demir, K. Ö. K. S. A. L., Bakir, M. E. L. İ. K. E., Sarikamis, G. Ö. L. G. E., & Acunalp, S. (2010). Genetic diversity of eggplant (Solanum melongena) germplasm from Turkey assessed by SSR and RAPD markers. *Genetics and Molecular Research*, *9*(3).

Deokar, A., Sagi, M., Daba, K., & Tar'an, B. (2019). QTL sequencing strategy to map genomic regions associated with resistance to ascochyta blight in chickpea. *Plant Biotechnology Journal*, *17*(1), 275-288.

Dormatey, R., Sun, C., Ali, K., Coulter, J. A., Bi, Z., & Bai, J. (2020). Gene pyramiding for sustainable crop improvement against biotic and abiotic stresses. *Agronomy*, *10*(9), 1255.

Ellur, R. K., Khanna, A., Bhowmick, P. K., Vinod, K. K., Nagarajan, M., Mondal, K. K., ... & Singh, A. K. (2016). Marker-aided incorporation of Xa38, a novel bacterial blight resistance gene, in PB1121 and comparison of its resistance spectrum with xa13+ Xa21. *Scientific Reports*, *6*(1), 1-8.

Ferreira, J. R., Pereira, J. F., Turchetto, C., Minella, E., Consoli, L., & Delatorre, C. A. (2016). Assessment of genetic diversity in Brazilian barley using SSR markers. *Genetics and molecular biology*, *39*(1), 86-96.

Gasbarra, D., Pirinen, M., Sillanpaa, M. J., & Arjas, E. (2009). Bayesian quantitative trait locus mapping based on reconstruction of recent genetic histories. *Genetics*, *183*(2), 709-721.

Gaur, P. M., Samineni, S., Thudi, M., Tripathi, S., Sajja, S. B., Jayalakshmi, V., ... & Dixit, G. P. (2019). Integrated breeding approaches for improving drought and heat adaptation in chickpea (Cicer arietinum L.). *Plant Breeding*, *138*(4), 389-400.

Gedil, M., Mengesha, W., Ilesanmi, O., & Menkir, A. (2024). Advances in Genetic Enhancement of Nutritional Quality of Tropical Maize in West and Central Africa. *Agriculture*, *14*(4), 577.

Geng, X., Qu, Y., Jia, Y., He, S., Pan, Z., Wang, L., & Du, X. (2021). Assessment of heterosis based on parental genetic distance estimated with SSR and SNP markers in upland cotton (Gossypium hirsutum L.). *BMC genomics*, *22*, 1-11.

Hasan, N., Choudhary, S., Naaz, N., Sharma, N., & Laskar, R. A. (2021). Recent advancements in molecular marker-assisted selection and applications in plant breeding programmes. *Journal of Genetic Engineering and Biotechnology*, *19*(1), 128.

Hoffstetter, A., Cabrera, A., Huang, M., & Sneller, C. (2016). Optimizing training population data and validation of genomic selection for economic traits in soft winter wheat. *G3: Genes, Genomes, Genetics*, *6*(9), 2919-2928.

Ince, A., & Karaca, M. (2019). Sequence data characterization and development of DNA markers for sesame (Sesamum indicum L.). *International Journal of Agriculture Environment and Food Sciences*, *3*(4), 240-244.

Jannink, J. L., & Walsh, B. (2002). Association mapping in plant populations, editor. Quantitative genetics, genomics and plant breeding. Oxford: CAB International; p. 59–68.

Jiang, G. L. (2013). Molecular markers and marker-assisted breeding in plants. *Plant breeding from laboratories to fields*, *3*, 45-83.

Jiang, H., Li, Z., Liu, J., Shen, Z., Gao, G., Zhang, Q., & He, Y. (2019). Development and evaluation of improved lines with broad-spectrum resistance to rice blast using nine resistance genes. *Rice*, 12, 1-11. https://doi.org/10.1186/s12284-019-0292-z

Kang, J. H., Yang, H. B., Jeong, H. S., Choe, P., Kwon, J. K., & Kang, B. C. (2014). Single nucleotide polymorphism marker discovery from transcriptome sequencing for marker-assisted backcrossing in Capsicum. *Horticultural Science & Technology*, *32*(4), 535-543.

Kaur, G., Joshi, A., & Jain, D. (2018). SSR-Marker assisted evaluation of Genetic Diversity in Mungbean (Vigna radiata (L.) Wilcezk) genotypes. *Brazilian Archives of Biology and Technology*, *61*, e16160613.

Kim, M. S., Yang, J. Y., Yu, J. K., Lee, Y., Park, Y. J., Kang, K. K., & Cho, Y. G. (2021). Breeding of high cooking and eating quality in rice by marker-assisted backcrossing (MABc) using KASP markers. *Plants*, *10*(4), 804.

Kumar, S., Knox, R. E., Singh, A. K., DePauw, R. M., Campbell, H. L., Isidro-Sanchez, J., ... & Fedak, G. (2018). High-density genetic mapping of a major QTL for resistance to multiple races of loose smut in a tetraploid wheat cross. *PLoS One*, *13*(2), e0192261.

Kwabena Osei, M., Danquah, A., Adu-Dapaah, H., Danquah, E., Blay, E., Massoudi, M., & Maxwell, D. (2022). Marker assisted backcrossing of alcobaca gene into two elite tomato breeding lines. *Frontiers in Horticulture*, *1*, 1024042.

Li, C., Yu, H., Li, C., Zhao, T., Dong, Y., Deng, X., ... & Zhu, S. (2018). QTL mapping and heterosis analysis for fiber quality traits across multiple genetic populations and environments in upland cotton. *Frontiers in plant science*, *9*, 1364.

Li, D., Lu, X., Zhu, Y., Pan, J., Zhou, S., Zhang, X., ... & Zhang, C. (2022). The multi-omics basis of potato heterosis. *Journal of Integrative Plant Biology*, *64*(3), 671-687.

Li, X., Wei, Y., Ma, Y., Cao, G., Ma, S., Zhang, T., ... & Piao, Z. (2022). Marker-assisted pyramiding of CRa and CRd genes to improve the clubroot resistance of Brassica rapa. *Genes*, *13*(12), 2414.

Liu, R., Lu, J., Zhou, M., Zheng, S., Liu, Z., Zhang, C., ... & Zhang, L. (2020). Developing stripe rust resistant wheat (Triticum aestivum L.) lines with gene pyramiding strategy and marker-assisted selection. *Genetic Resources and Crop Evolution*, *67*, 381-391.

Mammadov, J., Buyyarapu, R., Guttikonda, S. K., Parliament, K., Abdurakhmonov, I. Y., & Kumpatla, S. P. (2018). Wild relatives of maize, rice, cotton, and soybean: treasure troves for tolerance to biotic and abiotic stresses. *Frontiers in plant science*, *9*, 886.

Mathivathana, M. K., Jagadeeshselvam, N., Madhumitha, B., Karthikeyan, A., Pandiyan, M., Karthikeyan, G., ... & Sudha, M. (2018). Screening and identification of SSR markers for genetic diversity for mungbean (Vigna radiata (L.) Wilczek). *Journal homepage: http://www. ijcmas. com*, *7*(04), 2018.

Meuwissen, T. H., Hayes, B. J., & Goddard, M. (2001). Prediction of total genetic value using genome-wide dense marker maps. *genetics*, *157*(4), 1819-1829.

Michel, S., Löschenberger, F., Ametz, C., & Bürstmayr, H. (2021). Genomic selection of parents and crosses beyond the native gene pool of a breeding program. *The Plant Genome*, *14*(3), e20153.

Milc, J., Sala, A., Bergamaschi, S., & Pecchioni, N. (2011). A genotypic and phenotypic information source for marker-assisted selection of cereals: the CEREALAB database. *Database*, *2011*, baq038.

Mohammed, S. B., Ongom, P. O., Togola, A., & Boukar, O. (2024). Enhancing cowpea tolerance to elevated temperature: achievements, challenges and future directions. *Agronomy*, *14*(3), 513.

Mukri, G., Patil, M. S., Motagi, B. N., Bhat, J. S., Singh, C., Jeevan Kumar, S. P., ... & Simal-Gandara, J. (2022). Genetic variability, combining ability and molecular diversity-based parental line selection for heterosis breeding in field corn (Zea mays L.). *Molecular Biology Reports*, *49*(6), 4517-4524.

Nadeem, M. A., Nawaz, M. A., Shahid, M. Q., Doğan, Y., Comertpay, G., Yıldız, M., ... & Baloch, F. S. (2018). DNA molecular markers in plant breeding: current status and recent advancements in genomic selection and genome editing. *Biotechnology & Biotechnological Equipment*, *32*(2), 261-285.

Oliveira, L. K., Melo, L. C., Brondani, C., DEL PELOSO, M. J., & Brondani, R. P. V. (2008). Backcross assisted by microsatellite markers in common bean.

Patella, A., Palumbo, F., Galla, G., & Barcaccia, G. (2019). The molecular determination of hybridity and homozygosity estimates in breeding populations of lettuce (Lactuca sativa L.). *Genes*, *10*(11), 916.

Patil, A., & Ram, M. (2024). Breeding for heat stress tolerance in wheat: A review. *Journal of Advances in Biology & Biotechnology*, *27*(7), 227-237.

Peng, P., Jiang, H., Luo, L., Ye, C., & Xiao, Y. (2023). Pyramiding of multiple genes to improve rice blast resistance of photo-thermo sensitive male sterile line, without yield penalty in hybrid rice production. *Plants*, *12*(6), 1389. https://doi.org/10.3390/plants12061389

Pollock, C. J., Stoddart, J. L., Thomas, H., & JOHNES, T. (1987). Protein electrophoresis: Isoenzymes as genetic markers. *1BPGR Lecture Series-1. Collection, Characterization and Utilization of Genetic Resources of Temperate Forage Grass and Clover*, 41-53.

Prasanna, B. M., Palacios-Rojas, N., Hossain, F., Muthusamy, V., Menkir, A., Dhliwayo, T., ... & Fan, X. (2020). Molecular breeding for nutritionally enriched maize: status and prospects. *Frontiers in genetics*, *10*, 1392.

Proite, K., Leal-Bertioli, S. C., Bertioli, D. J., Moretzsohn, M. C., da Silva, F. R., Martins, N. F., & Guimarães, P. M. (2007). ESTs from a wild Arachis species for gene discovery and marker development. *BMC plant biology*, *7*, 1-10.

Pundir, S., Sharma, R., Kumar, D., Singh, V. K., Chaturvedi, D., Kanwar, R. S., ... & Sharma, S. (2022). QTL mapping for resistance against cereal cyst nematode (Heterodera avenae Woll.) in wheat (Triticum aestivum L.). *Scientific reports*, *12*(1), 9586.

Rasheed, A., Hao, Y., Xia, X., Khan, A., Xu, Y., Varshney, R. K., & He, Z. (2017). Crop breeding chips and genotyping platforms: progress, challenges, and perspectives. Molecular plant, 10(8), 1047-1064.

Richardson, B. J., Baverstock, P. R., & Adams, M. (2012). *Allozyme electrophoresis: a handbook for animal systematics and population studies*. Academic press.

Rocha, R. B., Barros, E. G., Cruz, C. D., Rosado, A. M., & Araújo, E. F. D. (2007). Mapping of QTLs related with wood quality and developmental characteristics in hybrids (Eucalyptus grandis x Eucalyptus urophylla). *Revista Arvore*, *31*, 13-24.

Sachan, D. S., Naimuddin, S. K., Patra, D., Subha, L., Senthilkumar, T., Chittibomma, K., ... & Prasad, S. V. (2024). Advancements in enhancing oil quality in rapeseed and mustard: A Comprehensive Review. *Journal of Experimental Agriculture International*, *46*(5), 181-193.

Saha, P., Ghoshal, C., Saha, N. D., Verma, A., Srivastava, M., Kalia, P., & Tomar, B. S. (2021). Marker-assisted pyramiding of downy mildew-resistant gene Ppa3 and black rot-resistant gene Xca1bo in popular early cauliflower variety pusa meghna. *Frontiers in Plant Science*, *12*, 603600.

Schmidthoffer, I., Szilák, L., Molnár, P., Csontos, P., & Skribanek, A. (2018). Drought tolerance of European barley (Hordeum vulgare L.) varieties. *Agriculture (Pol'nohospodárstvo)*, *64*(3), 137-142.

Soriano, J. M. (2020). Molecular marker technology for crop improvement. *Agronomy*, *10*(10), 1462.

Tomkowiak, A., Bocianowski, J., Kwiatek, M., & Kowalczewski, P. Ł. (2020). Dependence of the heterosis effect on genetic distance, determined using various molecular markers. *Open Life Sciences*, *15*(1), 1-11.

Tong, Z., Xiu, Z., Ming, Y., Fang, D., Chen, X., Hu, Y., ... & Xiao, B. (2021). Quantitative trait locus mapping and genomic selection of tobacco (Nicotiana tabacum L.) based on high-density genetic map. *Plant Biotechnology Reports*, *15*, 845-854.

Ullah, M., Akram Babar, H. M., Malik, and M. F. (2023). Assessing Intraspecific Genetic Variability in Cannabis sativa Populations of Punjab Pakistan Using RAPD and DNA Barcode Markers. *Phytopathogenomics and Disease Control*, 2, 111–118.

Varshney, R. K., Graner, A., & Sorrells, M. E. (2005). Genic microsatellite markers in plants: features and applications. *TRENDS in Biotechnology*, *23*(1), 48-55.

Varshney, R. K., Pandey, M. K., Janila, P., Nigam, S. N., Sudini, H., Gowda, M. V. C., ... & Nagesh, P. (2014). Marker-assisted introgression of a QTL region to improve rust resistance in three elite and popular varieties of peanut (Arachis hypogaea L.). *Theoretical and Applied Genetics*, *127*, 1771-1781.

Veerendrakumar, H. V., Barmukh, R., Shah, P., Bomireddy, D., Jamedar, H. V. R., Roorkiwal, M., ... & Pandey, M. K. (2024). Genomic Selection in Crop Improvement. In *Frontier Technologies for Crop Improvement* (pp. 187-222). Singapore: Springer Nature Singapore.

Vos, P., Hogers, R., Bleeker, M., Reijans, M., Lee, T. V. D., Hornes, M., ... & Zabeau, M. (1995). AFLP: a new technique for DNA fingerprinting. *Nucleic acids research*, *23*(21), 4407-4414.

Wang, N., Wang, H., Zhang, A., Liu, Y., Yu, D., Hao, Z., ... & Zhang, X. (2020). Genomic prediction across years in a maize doubled haploid breeding program to accelerate early-stage testcross testing. *Theoretical and Applied Genetics*, *133*, 2869-2879.

Wang, Z., Gerstein, M., & Snyder, M. (2009). RNA-Seq: a revolutionary tool for transcriptomics. *Nature reviews genetics*, *10*(1), 57-63.

Wen, Y. J., Zhang, Y. W., Zhang, J., Feng, J. Y., Dunwell, J. M., & Zhang, Y. M. (2019). An efficient multi-locus mixed model framework for the detection of small and linked QTLs in F2. *Briefings in bioinformatics*, *20*(5), 1913-1924.

Wu, J., Zhao, Q., Zhang, L., Li, S., Ma, Y., Pan, L., ... & Sun, D. (2018). QTL mapping of fiber-related traits based on a high-density genetic map in flax (Linum usitatissimum L.). *Frontiers in Plant Science*, *9*, 885.

Wu, Y. B., Li, G., Zhu, Y. J., Cheng, Y. C., Yang, J. Y., Chen, H. Z., ... & Ying, J. Z. (2020). Genome-wide identification of QTLs for grain protein content based on genotyping-by-resequencing and verification of qGPC1-1 in rice. *International Journal of Molecular Sciences*, *21*(2), 408.

Xiao, N., Pan, C., Li, Y., Wu, Y., Cai, Y., Lu, Y., ... & Li, A. (2021). Genomic insight into balancing high yield, good quality, and blast resistance of japonica rice. *Genome Biology*, *22*, 1-22.

Yang, Y., Wang, B., Wang, J., He, C., Zhang, D., Li, P., ... & Li, Z. (2022). Transcription factors ZmNF-YA1 and ZmNF-YB16 regulate plant growth and drought tolerance in maize. *Plant Physiology*, *190*(2), 1506-1525.

Zambelli, A. (2019). The impact of molecular genetics in plant breeding: realities and perspectives. *BAG, J. basic appl. genet. (Online)*, 11-15.

Zhang, M., Montooth, K. L., Wells, M. T., Clark, A. G., & Zhang, D. (2005). Mapping multiple quantitative trait loci by Bayesian classification. *Genetics*, *169*(4), 2305-2318.

Zhao, Y., & Su, C. (2019). Mapping quantitative trait loci for yield-related traits and predicting candidate genes for grain weight in maize. *Scientific reports*, *9*(1), 16112.