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REVOLUTIONIZING PLANT BREEDING PROGRAMS WITH ADVANCEMENTS IN MOLECULAR MARKER-ASSISTED SELECTION

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Abstract *The molecular marker method has come a long way in the last 30 years. Genetic research has advanced from RFLPs to SNPs. Array technology was also used. Sequencing progress has also led to the creation of low-cost NGS systems with high throughput. Phenotyping has been very important in making this progress possible. Markers for important crops like rice, corn, and potatoes have been worked on, but markers for crops that aren't used much haven't been studied as much. Phylogenetics and molecular ecology are other areas that don't know much about molecular markers. Plant breeding and DNA modification have greatly changed since recent improvements to CRISPR technology. To the contrary, some MAS (marker-assisted selection) methods need to know about the genome beforehand, which makes the work even harder. Researchers in plant science might find the methods discussed in this review piece useful as a database. They could use them alone or with other sequence-level characters from different fields.*

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Introduction

The Green Revolution began in the 1960s, and wheat and rice yields have been much higher than they could have been. This has helped avoid serious food shortages (Conway, 2019). Although funding has decreased, people have always thought plant breeding efforts would continue, leading to better yields. However, the Green Revolution's push for intense farming has led to more pests and diseases farming on land that wasn't as good for crops (Montgomery, 2017). Farmers use high-yielding semi-dwarf types that geneticists and breeders have been able to find new genetics from traditional landraces (Mefleh et al., 2019; Tahir et al., 2020). Marker-assisted selection (MAS) is a method that has been used to find and copy hundreds of genes from different species (Boopathi and Boopathi, 2020). Many cloned genes have big effects on the rice crop, making it a good example of the pros and cons of metabolic engineering. There are many more identified QTLs than this value, making it a useful tool in marker-assisted selection. This new method avoids some problems with traditional breeding and improves the selection criteria of phenotypes by choosing specific genes directly or indirectly. DNA or molecular markers differ from traditional breeding methods because they don't depend on the environment and can be seen at different times of plant growth. Now that there are many molecular markers and genetic maps to choose from, it is possible to use molecular markers to help in the selection of both dominant and quantitative traits. An effective molecular marker can distinguish molecular markers by detecting nucleotide sequence changes. These variations can be identified by RFLP, AFLP, SSR, RAPD, CAPS, SSCP, SNPs, and others (Amiteye, 2021). Multiple elements must work well for molecular markers to work in breeding methods.

1. Genetic map with closely linked markers to agriculturally significant genes or QTLs
2. Markers closely linked to QTLs or main genes
3. Adequate recombination between desirable markers and genome

4. Time and cost-effective analysis for large populations.

Most molecular-assisted selection (MAS) systems that work depend on a certain type of molecular markers. With the help of one or more quantitative trait loci (QTLs), these markers can tell you where a gene is located on a chromosome. So, when choosing genomic regions, at least one marker must be in the QTL region, or two polymorphic markers must be on each side. Using either strategy; the following examples show how to add QTLs into varied genetic systems. MAS enhances features regulated by a few genes, as shown in this study. However, MAS hasn't been very good at finding more complicated traits because of several problems (Bora, 2023). There are some problems with using MAS for quantitative traits, but this review discusses some good examples and offers ways to make MAS work better for QTLs. *Figure 1* shows how molecular marker-assisted selection works in its most basic form.

Marker-assisted selection (MAS) uses different kinds of DNA markers

When using DNA markers in MAS, dependability, quantity, quality, type, amount, specialist marker analysis techniques, degree of polymorphism, and cost must be considered (Amiteye, 2021).

Increasing Reliability

Markers should be within 5 centimetres of genes to accurately predict phenotypes. Adding surrounding or intragenic markers improves marker accuracy and reliability (Hasan et al., 2021).

Quantity and quality of DNA

Certain marker approaches need much money and high-quality DNA, making procurement difficult and raising the procedure's cost (Billerman and Walsh, 2019).

Specialized framework

Concerns about ease and speed are important when doing a treatment. We strongly recommend methods that can handle a lot of work, are simple to use, and work well. SCAR markers—DNA sequences like restriction fragment length polymorphisms (RFLPs) and links to genes or quantitative trait loci (QTLs)—help marker-assisted selection (MAS) (Shimizu et al., 2020).

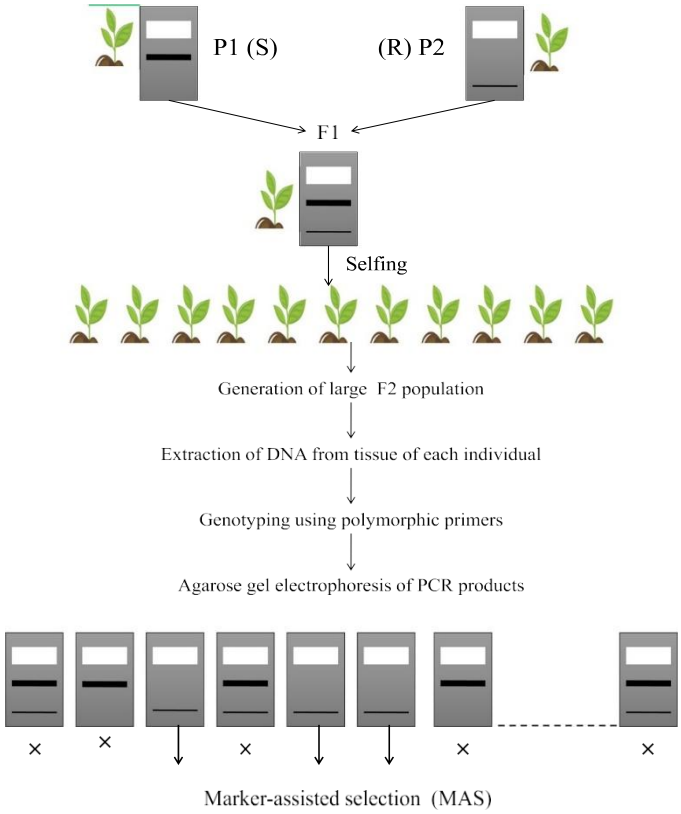

Figure 1 The image shows how marker-assisted selection (MAS) works in its most basic form (Shimizu et al., 2020)

The categorization of molecular markers

There are different groups for DNA or genetic markers based on different factors. First, we need to know how genes work and whether they are dominant or co-dominant traits. Second, the way the markers were found, like genetic markers based on PCR or hybrid markers. Third, the way the markers are passed on, maternal organelle inheritance, paternal nuclear inheritance, or biparental nuclear inheritance (Hasan et al., 2021; Wang et al., 2017).

Breeding and genetic work for many types of crops worldwide has successfully used molecular markers for a long time. This review will give you an overview of what you need to know about molecular markers, especially when it comes to how to find them. *Table 1* shows a study comparing the main parts of the most common genetic markers.

***Table 1* A Comparison of Plant Research's Most Common DNA Markers**

Characteristics	Restriction Fragment Length Polymorphism	Random Amplified Polymorphic DNA	Amplified Fragment Length Polymorphism	Simple sequence repeats	Single nucleotide polymorphisms
Genomic abundance	High	High	High	Moderate to high	Very high
Genomic coverage	Low copy coding region	Complete genome	Complete genome	Complete genome	Complete genome
Expressions	Co-dominant	Dominant	Dominant/co-dominant	Co-dominant	Co-dominant

Number of loci	Small (< 1000)	Small (< 1000)	Moderate (1000s)	High (1000s–10,000s)	Very high (> 100,000)
Individual variability	Moderate	High	High	High	High
Types of variability	Single base change, indel	Single base change, indel	Single base change, indel	Changes in length repeat	Single base change, indel
sequencing	Yes	No	No	Yes	Yes
Type of primers	Low-copy DNA or cDNA clones	10 bs random nucleotides	Specific sequence	Specific sequence	Allele-specific PCR primer
PCR-based	Usually no	Yes	Yes	Yes	Yes
Radioactivity detection methods	Usually yes	No	Yes or no	Usually no	No
Guaranteeing Consistency and Dependability	High	Low	High	High	High
DNA quantity needed	Large (5–50 µg)	Small (0.01–0.1 µg)	Moderate (0.5–1.0 µg)	Small (0.05–0.12 µg)	Small (> 0.05 µg)
High-throughput genotyping	Low	Low	High	High	High
The cost	Moderate to high	Low	Moderate	Moderate to high	High
Marker index	Low	Moderate	Moderate	Moderate to high	Moderate
Time demanding	High	Low	Moderate	Low	Low
Number of polymorphic per loci	1.0–3.0	1.5–5.0	20.1	1.0–3.0	1
Primary application	Genetic	Diversity	Diversity and genetic	All purposes	All purposes

DNA Marker

Molecular markers are unique groups of nucleotides that can be used to study differences between people with these markers. They cause the polymorphisms seen in the population, but they don't change how genes work. The changes they cause include deletions, insertions, gene mutations, duplications, and translocations. A good DNA marker should be able to find a lot of different types of DNA, have co-dominance, and be spread out evenly across the genome (Adhikari et al., 2017).

Based On Hybridization

Genetic markers, also called RFLPs (restriction fragment length polymorphisms), need a properly labelled DNA probe to identify the genes in which they are interested in DNA data. Digestion and plant crossing are ways to do this. The initial usage of RFLPs was as genetic markers, requiring hybridization. Gene alterations, insertions/deletions (InDels), duplications, translocations, and inversions can cause changes within a species (Jeffares et al., 2017). RFLP begins with pure DNA from the intended source. We next add restriction enzymes from bacteria or human cells to the DNA. These enzymes can cleave DNA at certain nucleotide sequences.

DNA Markers Based On PCR

A probe hybridization step is not needed for PCR-based genetic markers. Molecular markers like RAPD, AFLP, microsatellites or SSRs, SNPs, RAMP, SRAP, ISSR, SCAR, ESTs, cDNA Restriction Fragment Length Polymorphism (RFLP), Restriction, and other types of markers have led to the creation of handy and simple new generation markers(Ramesh et al., 2020). With the help of primer pairs—short strings of nucleotides linked to DNA to make dsDNA—these atomic markers can focus on certain parts of DNA to study genetic variation (Amiteye, 2021). These primers are the first step to making a copy of a certain piece of DNA. Once the DNA has been copied, it is broken up and put on a gel so that different band shapes can be seen. After that, if needed, the DNA pieces can be sequenced to find any changes in the order that might explain differences between species (van der Loos and Nijland, 2021). Isolating DNA from its source and checking its quality and amount using gel electrophoresis are necessary for PCR to study molecular markers (Amiteye, 2021). Sequencing usually involves cleaning the amplified products, putting them through sequencing PCR, and then cleaning them again before sequencing (Pomerantz et al., 2022). SNP testing is possible with next-generation sequencing (Silvia et al., 2017), as shown in *Figure 2*.

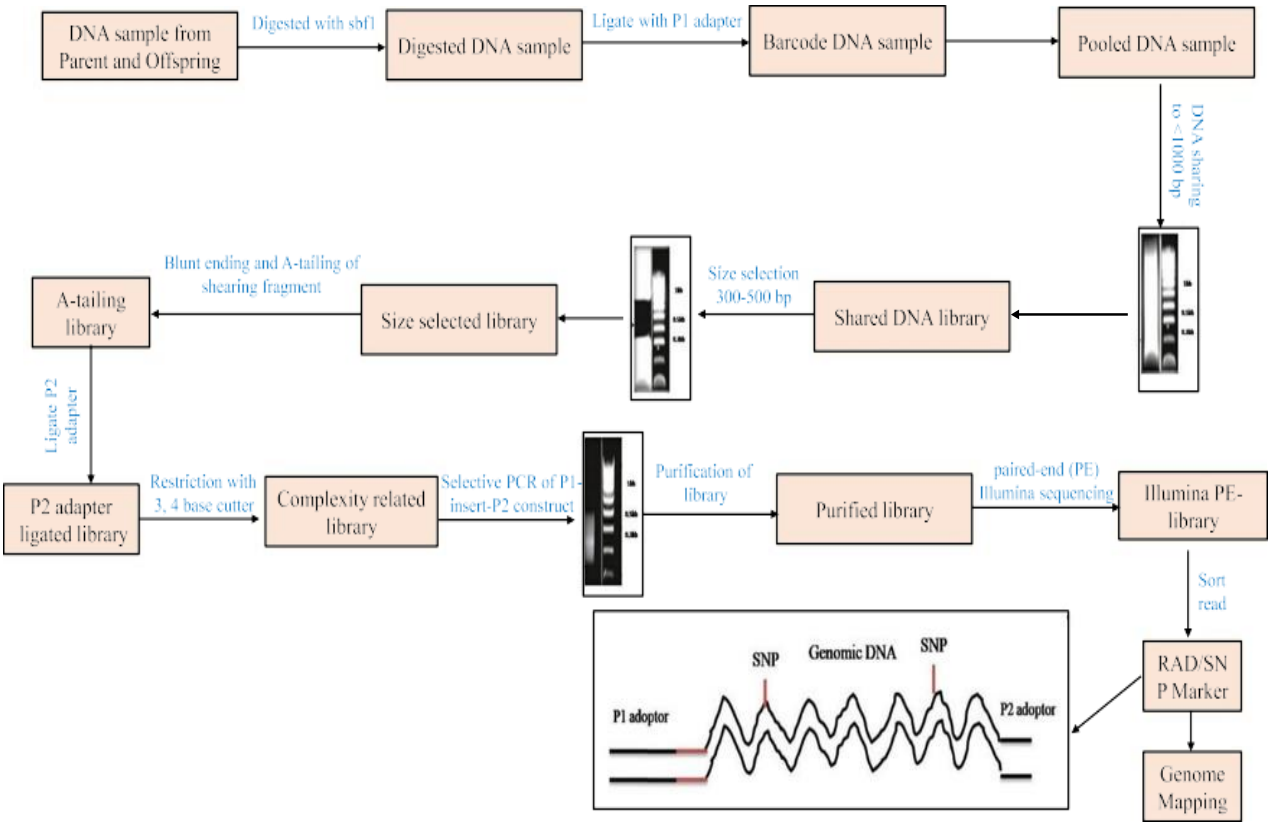

Figure 2 For genome mapping, RAD-sequence detects and genotypes SNPs using next-generation sequencing. The adapter's adapter_P1-EcoRI and adapter_P2-MspI ligate EcoRI and MspI to create RAD-tags (Silvia et al., 2017)

Markers of Transposability

TEs, which are "transposable elements," are DNA sequences that can move around and place themselves into coding regions of the genome. She first found them in maize in 1950 by Barbara McClintock. Since then, more studies have shown that they are more common in eukaryotic genomes (Feschotte, 2023). Before we can fully understand TE markers, we must look at what they are like and how they connect to other genes. Each method uses different TEs' unique properties and uses different primer annealing sites in transposable regions differently. There are two types of TEs: class I, also called retrotransposons, uses RNA molecules to move to new locations in the genome and copy itself; and class II, also called "cut and paste" transposable elements, doesn't need RNA intermediates and can remove itself from a donor site before moving to a specific location in the acceptor genome (Piao et al., 2017).

Gene-Based Resistance Markers

Many animals and plants have defence mechanisms with resistance gene markers (Andersen et al., 2018). Knowing plant diseases is crucial before discussing these indicators. Many plants have effective biotic and abiotic disease defences. These systems have innate and adaptive immunity. R proteins (resistance proteins) and pathogen and pattern recognition receptors mediate innate immunity in plants and animals (Gouveia et al., 2017). In contrast, interference RNA controls plant adaptive immunity, notably during virus infections. Pathogen or pattern recognition receptors identify molecular patterns of microbes and pathogens. Similar-class bacteria have largely conserved receptors (Hennessy, 2017; Milc et al., 2019). Resistance proteins identify non-conserved Avr (avirulence) factors and initiate a signaling cascade that produces reactive oxygen species and programmed cell death in plant cells (Song et al., 2021). A new plant-pathogen interaction study reveals that cell death does not prevent pathogen transmission but inhibits its mobility in neighbouring live tissues through an unknown mechanism (Köhl et al., 2019). *Table 2* shows crop gene-marker relationships for disease resistance.

Polymorphism of Resistance Gene Analogs

Resistance gene analogue polymorphism (RGAP) can be used to find resistance genes in DNA. This method used degenerate primers and genomic DNA that haven't been cut to find R gene copies in conserved regions. Agar-gel electrophoresis, on the other hand, was not sensitive enough to find most PCR fragment length polymorphisms in early tests on crop species. The best way to separate PCR bands in plant profiling studies is to use polyacrylamide gel electrophoresis (PAGE), which has greatly improved the ability to separate fragment-length polymorphisms (Johnston-Monje and Lopez Mejia, 2020). They found that using R-like gene-specific primers with PAGE makes PCR markers correctly related to R genes (Hasan et al., 2021). According to research, RGAP has been used

successfully in many areas, such as making molecular markers for R genes that make cells resistant to pathogens and studying biodiversity to learn more about R gene regions like NBS and LRR (Sahu et al., 2022).

Table 2 Comparison of Crop Disease Resistance Gene-Marker Associations

Species varieties	Characteristics	Genes	Molecular markers	References
Triticum Aestivum L.	Classification and Characteristics of Leaf Rust on Wheat (Puccinia recondita f.sp. tritici)	Lr34 from Triticum aestivum	Repeat a simple sequence (3 times)	(Peng and Yang, 2017)
		Lr35 from Triticum speltoides	It has two parts: sequence-tagged site (STS) and cleaved amplified polymorphic sequences (CAPS).	(Gupta et al., 2017; Kthiri, 2017)
	Introduction to Wheat Stem Rust (Puccinia graminis f. sp. tritici)	Wheat Sr31 Discovery	STS	(Lapochkina et al., 2017)
	Puccinia striiformis strain causing Yellow Rust Tritici.	Wheat Yr15 Gene	Short form DNA (SSR) and Random Amplified Polymorphic DNA (RAPD)	(Khanfri et al., 2018)
Oryza Sativa L.	Pyricularia oryzae makes rice blast.	Wheat Pi5(t) Function Investigation	CAPS	(Paul et al., 2022)
	The Gall Midge is a species of insect.	Soybean Gm7 Identification	Marker for Sequence Characterized Amplified Region (SCAR) SA598	(Li et al., 2020)
Zea Mays	Understanding SCMV: Sugarcane Mosaic Virus	Corn Scm1 and Scm2 Characterization	The use of SCAR and CAPS to study DNA	(Kumawat et al., 2020)
Hordeum Vulgare	Malignant Barley Yellow Mosaic Virus	rym4/rym5 study	SSR to Gene Type	(Jasrotia et al., 2017)
	Puccinia Hordei: Leaf Rust Cause	Barley Rph7 Isolation	CAPS for building cells	(Dinh, 2020)

RNA Markers

During growth and development, gene expression regulates plant biological responses to biotic and abiotic stimuli. There are many ways to study these reactions, including using PCR-based markers. Fingerprinting markers amplify a specific subset of RNA or DNA fragments. These methods rely on transcribing a functional genomic region (Amiteye, 2021). Adhikari et al. (2017) examined cDNA/EST markers, which are molecular markers obtained from expressed or transcribed genomic areas. From the RNA pool or cDNA or ESTs, bioinformatics tools can construct primers selectively or randomly.

Studying tiny RNA polymorphism

In eukaryotic genomes, several 20–24-base endogenous noncoding short RNAs regulate genes (Deogharia and Gurha, 2022). These small RNAs are a useful source for making molecular labels. Flanking sequences and the ability to create primers for PCR reactions and fingerprinting are the same. This trait is used by a method called Inter small RNA polymorphism (iSNAP), created by (Amiteye, 2021). Starting a PCR reaction with primer pairs of small RNAs on either side of the gene is how this method finds length differences caused by InDels in the pool of small RNAs (Lev et al., 2017). This high-throughput, noncoding marker method is suited for repetitive application. It assists in genomic mapping and genotyping.

Analysis of EST-SSR markers for Assessing Genetic Diversity

EST data from cDNA conversion is in many open databases. ESTs, short transcribed sequences that read in one direction, are essential for gene expression and genetic diversity analysis. Researchers can access 5′ and 3′ gene sequences by turning cDNA into ESTs. The former are connected with protein coding, while the latter are more abundant in UTRs. Bioinformatics can generate EST-based molecular markers from these databases (Singh et al., 2019). New EST data has enabled the creation of plant species-specific microsatellites or SSR markers (Sun et al., 2021). EST-SSRs from transcribed genome areas amplify like genomic (gSSR) microsatellites but require different primers and positions. Sequencing data employing in silico approaches in plant group databases like Triticeae or NCBI-EST yields them.

Species Identification using Fingerprint Markers

Targeted fingerprinting markers (TFMs) were created using genetic elements. These multilocus markers target specific genomic areas and are developed purposefully. They are compared to gene or gene-related polymorphism sites regardless of functioning. The gene-targeted indicators produced may not be linked to phenotypic mutations TFM indicators help discover genetic variances and uniqueness in numerous ways. These markers randomly band certain, unnamed regions using plant genetic features (Huider et al., 2021). Through primer design or

changed PCR procedures, this distributes the complete genome and enhances reproducibility. TFM can adapt to diverse organisms and offer alternatives to AAD markers using genomic features.

Possible Plant Breeding Uses of MAS

The next sections show how MAS could transform crop plant breeding. This overview emphasizes marker-assisted selection and molecular markers' value in plant breeding (Cobb et al., 2019). This includes evolution and phylogeny, marker assessment of breeding materials, genetic diversity-based parental selection, promoting heterosis, genomic region identification, introgression, backcrossing, trait pyramiding, and early-stage selection. These categories overlap; however, they all help establish high-quality plant lines (Asif et al., 2020; Balqees et al., 2020; Farooq et al., 2021; Ghafoor et al., 2020; Iqbal et al., 2021). Integrating molecular markers into plant breeding programs or replacing phenotypic selection in line development is possible (Cobb et al., 2019).

Transforming Cultivar Identity and Assessing "Purity"

Mixing seeds from different types is common because it can be hard to keep track of all the seed samples needed for inter- and intra-plant breeding programs. It is possible to identify each plant by its marker. For grain hybrids to produce the most heterosis, it is important to keep the genetic purity high. SSR and STS markers in hybrid rice have made it easier to confirm purity. This contrasts the old "grow-out tests" method, which involved waiting for the plant to fully develop and evaluating its physical and reproductive traits (Kumar et al., 2021).

Investigating plant heterosis

Research indicates a maize-sorghum genetic mix. DNA markers revealed a promising heterosis group that could lead to hybrid vigour (Ali et al., 2013; Ali et al., 2016; Ali et al., 2014; Iqbal et al., 2021; Iqra et al., 2020; Mulima, 2017). Developing this hybrid line to develop hybrids takes time and money. DNA markers alone cannot properly predict heterosis, despite efforts.

Criteria for selecting appropriate genomic regions

Breeders need to be able to spot changes in the frequency of alleles in the genome to look for particular alleles or haplotypes and plan their breeding processes ahead of time (Ahmar et al., 2020). This information can also be used for other things, like QTL mapping, where the studied areas can confirm or expand on links between markers and traits that have already been found (Balsalobre et al., 2017). This information can also help make new types with the right mix of alleles, using marker-assisted methods like backcrossing or early generation selection.

Assessing Genetic Variation and Parental Selection

Plant breeding programs depend on having a lot of different genetic material to choose from. Finding and using different strains to hybridize with elite cultivars is important for increasing the genetic base of core breeding materials (Aaliya et al., 2016; Ali and Malik, 2021; Juma et al., 2021; Mazhar et al., 2020; Mustafa et al., 2018; Naseem et al., 2020). Much research has been done on the genetic diversity of breeding materials for different crops. Molecular markers have improved this approach by providing valuable data.

Use of markers to help with introgression

Through hybridization and repeated backcrossing, introgression moves a good trait from one type of plant to another. Through crossing two populations and then repeatedly backcrossing to "B," the receiver or recurrent parent, Zhang et al. (2021b) explained introgression as a way to get a desired trait or QTL from a plant in population "A" into a plant in population "B." Markers on DNA can help with this process because they show when the desired quality or QTL is present and help the recipient's background genome blend in better. Using molecular markers in introgression is a great way to add genes or QTLs from landraces because it speeds up the growth of better varieties and eliminates the problem of linkage drag (Hernandez et al., 2020).

Regeneration and lineage

Historically, geographical factors and physical differences within groups were used extensively to study how species or traits changed. But thanks to progress in molecular biology, we now have a better idea of how an organism's genes are put together. To learn about a person's development and make a genetic map, phylogenetic studies need to use a lot of molecular markers (Ramesh et al., 2020). Chloroplasts have been very helpful because their genetic make-up is simple and constant.

Precision Gene Modification Using CRISPR Genome Editing

Using the CRISPR gene editing method by Zhang et al. (2021a) greatly improved the growth and development of many crop plants. Cas9 has become the most popular genome-altering method in recent years. This approach offers numerous advantages, including its user-friendly nature, ability to target methylated loci, and versatility (Pajares et al., 2021; Singer, 2019). Core to CRISPR are CRISPR RNAs and Cas proteins. TracrRNA and crRNA are essential for cutting a target site. They work with the most-studied Cas protein, Cas9 endonuclease. Combining these two RNA molecules creates sgRNA (Zhou et al., 2018). When sgRNA and Cas proteins join together, they make RNA-guided endonuclease. This enzyme precisely cuts target regions in the genome (Manghwar et al., 2020). The three types of the CRISPR-Cas system are named after the type of Cas protein they contain. Some bacteria and archaea share Cas1 and Cas2, but only bacteria have type I, archaea only have type II, and some bacteria also have type III (Hidalgo-Cantabrana et al., 2019). Many Genome editing has helped Nicotiana tabacum (Vats et al., 2019), Arabidopsis (Vats et al., 2019), maize (Li et al.,

2021b), and wheat (Wang et al., 2018) succeed (*Figure 3*).

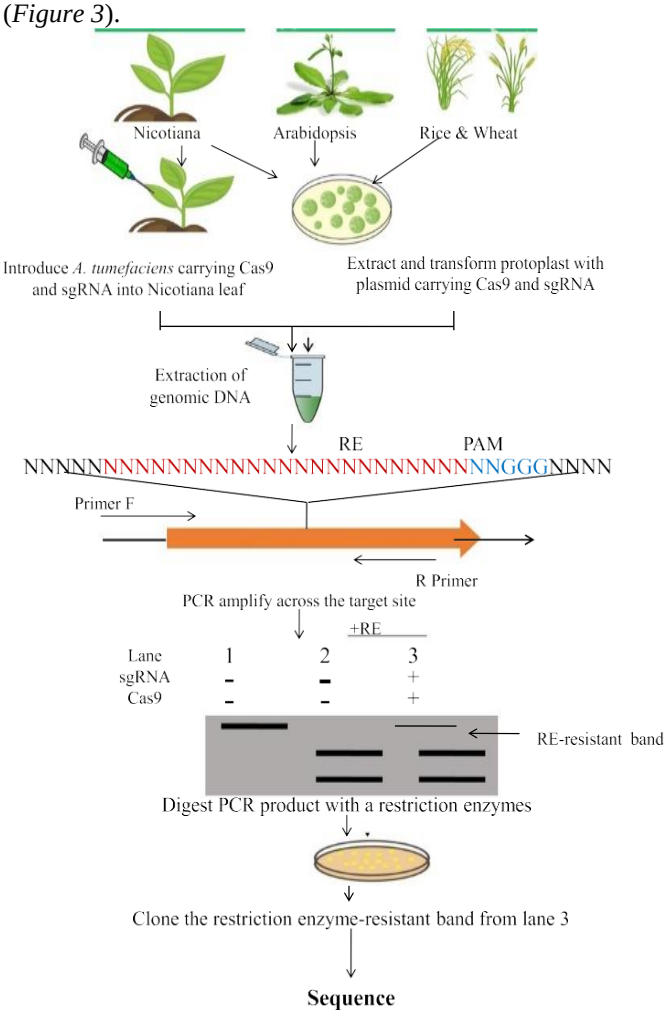

Figure 3 shows a schematic of CRISPR/Cas9 genome editing for crop enhancement (Wang et al., 2018)

Improving Crops Using MAS

With the rise of biotechnology and conventional breeding, genetic mapping has become an important method for finding key genes and QTLs connected to agricultural traits. This mapping information and molecular markers enable marker-assisted selection (MAS) to improve breeding results. The genetic basis of phenotypes, population size, and targeted gene genetic background determines MAS's efficacy. MAS has improved qualities impacted by a single gene, but yield and environmental stress tolerance have been difficult to improve. MAS improved quantitative features despite these challenges (Das et al., 2017). Improved genotyping and comparative and functional genomics have made it easier to identify genotypes with enhanced agronomic performance.

Maximizing Yield with MAS

Numerous studies have shown that MAS can boost agricultural output. However, identifying the best crops for diverse locations requires a comprehensive strategy integrating classic agricultural approaches [174] with crop modeling and QTL mapping (Mores et al., 2021). We'll examine how MAS has increased crop yields like maize and rice.

Oryza Sativa l. (rice)

Recent research have examined yield component QTL genes using advanced BC populations and Oryza rufipogon wild rice. These findings suggest that 53% (Yadavalli et al., 2022) and 33% (Duan et al., 2022) of O. rufipogon's QTL alleles increase yield and yield components in top rice cultivars despite their poor performance. The second investigation found a lower percentage, possibly due to higher yield QTL allele genetic similarities between the elite line and O. rufipogon. Most of the identified loci in this crossover may have superior alleles in the elite cultivar. Several productive QTLs from O. rufipogon were not connected to any adverse QTLs, making them suitable for generating new parent lines. That these QTLs have stable effects has been shown by the fact that they have the same effects in people with different genetic backgrounds and different situations(Quero-García et al., 2021).

A marker for thousand-grain weight (TGW) was found on chromosome 6 in a recent study. The researchers used a BC inbred line from the high-yielding japonica Nipponbare and the low-yielding indica cultivar Kasalath (Jena and Kim, 2020). The TGW-boosting gene was in the indica cultivar. When this allele was introduced into a Nipponbare near-isogenic line (NIL) using marker-assisted selection (MAS), it led to a 10% rise in TGW and a 15% rise in yield per plant, regardless of plant type (Gouda et al., 2020). Molecular markers can easily identify the part of the genome where this yield QTL is found. This makes it possible to introduce this QTL into other high-yielding rice cultivars and increase yield further.

Zea *mays* (maize)

The use of marker-mediated backcrossing to select maize breeding plants is becoming common. This method monitors the transmission of favorable alleles at QTLs by foreground selection and accelerates the genetic background in the remaining genome through background selection (Tourrette et al., 2021). This method creates Near Isogenic Lines (NILs) in maize by crossing a top-performing parental line with an exotic donor line. Selecting for target features and creating the right genetic background usually requires markers and repeated selfing (Wilde and Miedaner, 2021). It is possible to make different NILs with different introgressed genetic regions by only doing two backcrosses and one selfing generation. Field tests have shown that these NILs have gotten donor segments that make them produce more or less. This way of breeding not only makes top lines, but it also gives us useful information for finding and mapping yield QTLs. There is a possible problem with this method, though: it might not find good epistatic effects between QTLs. To solve this problem, many studies have tried to find a good way to guess how well a hybrid corn crop will do by using marker and phenotyping data together. Li et al. (2021a) examined heterosis and G×E interaction in maize by crossing two popular inbred lines, B73 and Mo17. Identifying

and mapping a QTL allele predicted higher hybrid yield. QTL-enhanced hybrids yielded more than those without QTLs (Dwivedi et al., 2018).

MAS breeding advantages above traditional breeding

Food plant breeding programs use molecular or DNA markers.

1. Plant growers are more interested in them because they have several benefits.
2. You can get genomic DNA markers from any part of a food plant and use them to check plants early on for certain traits.
3. This lets early selection happen and gives farmers control over blooming.
4. They also let you choose from many figures, which would be hard to keep up with and fix otherwise.
5. Molecular markers are especially helpful for complicated traits with many genetic parts.
6. They can also help choose the right alleles, especially for traits sensitive to the surroundings.
7. Molecular markers let you choose a single plant, no matter how heritable it is.
8. They can tell the difference between heterozygous food plants and homozygous, and they can also help reduce linkage drag during backcrossing.
9. DNA markers also make it easier and faster to determine the genetic background of a parent who appears more than once.
10. Using them can help reduce DNA diversity while choosing parents for crossing.
11. This helps heterosis exploitation and can make the gene makeup of top germplasm less complicated.

Limitations on the Application of MAS

1. MAS methods cost more than the old ways of choosing.
2. There needs to be a well-stocked lab with expensive drugs and tools.
3. Finding different DNA markers, like RFLP, RAPD, AFLP, SNP, SRP, etc., takes much time, effort, and work.
4. Highly advanced tools, DNA isolation, and the study of DNA markers all need skilled workers.
5. It's hard to do a QTL study with MAS because environmental factors and genetic background can have cumulative effects.
6. MAS that uses radioisotopes to name DNA can be very bad for your health, especially regarding RFLP markers.
7. In this case, PCR-based markers are better.
8. In the long run, MAS might not work as well as choosing traits directly.

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