Marker Assisted Breeding Technique and its Consequences in Development of Biotic and Abiotic Stress Resistance in Crop Plants

Syed Mohd Quatadah[1], Nagmi Praween[2], Aneeta Yadav[1], Jitendra Kumar[1] and Vinay Joseph Silas[1].

1. Faculty of Agricultural Sciences and Allied Industries, Rama University, Kanpur-209217.
2. Department of Crop Improvement, ICAR-Indian Institute of Pulses Research, Kanpur-209217.
3.

Corresponding author mail: syedquatadah.fas@ramauniversity.ac.in

Abstract

Agriculture is continuously facing severe challenges due to increasing population, climate change, emerging pathogens, and degradation of natural resources. Conventional breeding methods have contributed significantly towards crop improvement, but they are often time consuming and less precise in transferring desirable traits. Marker assisted breeding (MAB) has emerged as one of the most important modern breeding approaches for improving crop plants against biotic and abiotic stresses. During the last two decades, enormous progress has been achieved in molecular marker technologies, genome mapping, quantitative trait loci (QTL) identification, and marker assisted selection (MAS). These advancements have accelerated crop improvement programs in several cereal, pulse, oilseed, fruit, and vegetable crops.

*Marker assisted breeding enables breeders to select plants carrying desirable genes without depending completely on phenotypic screening(**Collard and Mackill, 2008**). This approach has reduced breeding duration and improved selection efficiency. Various types of molecular markers such as RFLP, RAPD, AFLP, SSR, SNP and DArT markers have been widely utilized in crop breeding programs. In recent years, genomic selection and high throughput genotyping technologies have further strengthened marker assisted breeding.*

The present review article discusses the development of marker assisted breeding techniques and their applications in developing resistance against biotic stresses including diseases, insect pests and nematodes as well as abiotic stresses such as drought, salinity, heat, flooding and cold stress. The article also highlights recent advancements, limitations, challenges and future prospects of marker assisted breeding in sustainable crop improvement.

Key Words:*Molecular marker, Marker assisted selection, MAB, QTL, genotyping, PCR*

Introduction

Crop production throughout the world is seriously affected by several biotic and abiotic stresses. According to recent estimates, around 30 to 40 percent of global crop yield is lost every year due to plant diseases, insect pests and adverse environmental conditions. Conventional plant breeding has played an important role in development of improved crop varieties;however, the process is slow and largely dependent upon environmental interactions. Because of this reason, breeders started integrating molecular biology techniques with traditional breeding methods.

Marker assisted breeding is a technique in which molecular markers closely linked to target genes or quantitative trait loci are used for indirect selection of desired traits. It has become a very effective tool in modern agriculture because selection can be carried out even at seedling stage without waiting for trait expression. The rapid advancement in genomics and molecular marker systems during the last two decades has greatly transformed plant breeding(**Varshney *et al*., 2005**).

The concept of marker assisted selection was first developed after discovery of DNA markers during the 1980s, but large-scale utilization became possible after development of polymerase chain reaction (PCR) based markers and genome sequencing technologies. In present time, marker assisted breeding is widely employed in crops like rice, wheat, maize, barley, chickpea, soybean, cotton, tomato and many others.

Several successful examples of marker assisted breeding have been reported for resistance against bacterial blight in rice, rust diseases in wheat, drought tolerance in maize, salinity tolerance in rice and virus resistance in tomato. Despite tremendous progress, some limitations still exist regarding high cost, requirement of technical expertise and complex inheritance of quantitative traits.

Historical Development of Marker Assisted Breeding

The development of marker assisted breeding can be divided into several stages. Initially plant breeders relied mainly on morphological markers like plant height, seed colour and flower shape for identifying genetic variations. However, these markers were influenced by environmental conditions and were limited in number.

The introduction of biochemical markers such as isozyme markers during the 1970s represented the second stage of marker development. Isozymes provided more reliable genetic information but their polymorphism level was still low.

The real revolution started with development of DNA based molecular markers during the 1980s and 1990s. Restriction Fragment Length Polymorphism (RFLP) was among the earliest DNA marker systems used for genetic mapping. Later on, PCR based markers such as RAPD, AFLP and SSR became popular due to simplicity and efficiency.

During the last two decades, single nucleotide polymorphism (SNP) markers and next generation sequencing technologies have changed the scenario completely. High throughput genotyping platforms now allow analysis of thousands of markers simultaneously. Genome wide association studies (GWAS) and genomic selection approaches are increasingly being integrated with marker assisted breeding.

The completion of genome sequencing projects in important crops has also accelerated identification of genes and QTLs responsible for stress resistance. Nowadays breeders can develop varieties carrying multiple resistance genes through marker assisted pyramiding.

Types of Molecular Markers Used in Plant Breeding

1. Restriction Fragment Length Polymorphism (RFLP)

RFLP markers were the first DNA markers widely used in plant genetics. They are based on variation in restriction enzyme cutting sites within DNA sequences. RFLP markers are co-dominant and highly reliable, but the procedure is laborious and expensive.

Advantages

- Highly reproducible
- Co dominant inheritance
- Useful for genetic mapping

Limitations

- Requires large amount of DNA
- Time consuming process
- Radioactive probes were often used

RFLP markers played an important role in early genome mapping studies in rice and maize.

2. Random Amplified Polymorphic DNA (RAPD)

RAPD markers utilize short arbitrary primers for amplification of random DNA segments through PCR. These markers became popular because they do not require prior sequence information.

Advantages

- Simple and rapid
- Low-cost technique
- Requires small amount of DNA

Limitations

- Dominant markers
- Poor reproducibility sometimes occur

RAPD markers were extensively used for diversity analysis and preliminary mapping studies.

3. Amplified Fragment Length Polymorphism (AFLP)

AFLP combines restriction digestion and PCR amplification. This technique generates large number of polymorphic markers and is highly reproducible.

Advantages

- High polymorphism
- Good reproducibility
- Suitable for genetic diversity studies

Limitations

- Technically complex
- Expensive compared to RAPD

4. Simple Sequence Repeats (SSR)

SSR or microsatellite markers are short tandem repeats distributed throughout the genome. SSR markers became highly popular because of their co dominant inheritance and high polymorphism(**Gupta *et al.,* 2008**).

Advantages

- Highly informative
- Co dominant nature
- Excellent reproducibility

Applications

- Gene mapping
- Marker assisted selection
- Variety identification
- QTL analysis

SSR markers have been extensively used in rice, wheat, barley and chickpea breeding programmes.

5. Single Nucleotide Polymorphism (SNP)

SNP markers are based on single base pair changes in DNA sequences. They are the most abundant markers in plant genomes.

Advantages

- High throughput genotyping
- Genome wide coverage
- Suitable for automation

SNP based platforms are now widely used in genomic selection and precision breeding.

Marker Assisted Selection (MAS)

Marker assisted selection refers to the indirect selection of target traits using linked molecular markers. MAS is especially useful for traits that are difficult to evaluate phenotypically or expressed at later growth stages(Hospital, 2009).

The process of MAS generally involves following steps:

1. Identification of markers linked with target trait

2. Validation of marker-trait association
3. Screening of breeding populations using markers
4. Selection of plants carrying desirable alleles
5. Advancement of selected lines

MAS has several advantages over conventional breeding. Selection can be carried out irrespective of environmental conditions and recessive genes can be identified easily. It also reduces population size and breeding duration.

However, efficiency of MAS depends upon tight linkage between marker and target gene. Recombination between marker and gene can reduce selection accuracy.

Marker Assisted Backcross Breeding

Marker assisted backcross breeding (MABB) is one of the most successful applications of molecular markers in crop improvement. In this approach, molecular markers are used to transfer one or few target genes from donor parent into elite cultivar while retaining maximum recurrent parent genome.

MABB involves three types of selection:

1. Foreground Selection

Selection for target gene using closely linked markers.

2. Recombinant Selection

Selection against linkage drag near target gene region.

3. Background Selection

Recovery of recurrent parent genome using genome wide markers.

This technique has been highly successful in rice improvement programmes for bacterial blight resistance(**Sundaram *et al.*, 2008**).

Marker Assisted Gene Pyramiding

Gene pyramiding refers to combining multiple genes controlling similar trait into a single genotype. Molecular markers have made gene pyramiding much easier and accurate.

Disease resistance breeding frequently uses pyramiding strategy because pathogens can easily overcome single resistance gene. By combining multiple resistance genes, durability of resistance can be enhanced.

Examples include:

- Pyramiding of bacterial blight resistance genes Xa21, xa13 and xa5 in rice

- Combining rust resistance genes in wheat
- Stacking of blast resistance genes in rice

Marker assisted pyramiding has significantly improved stability of resistance under field conditions(**Collard and Dwivedi, 2019**).

Role of Marker Assisted Breeding in Biotic Stress Resistance

Disease Resistance

Plant diseases caused by fungi, bacteria, viruses and nematodes result in huge yield losses worldwide. Marker assisted breeding has become a major strategy for developing resistant cultivars.

1. Rice

Rice is one of the most important food crops globally. Several diseases including bacterial blight, blast and sheath blight reduce rice productivity.

Bacterial Blight Resistance

Genes such as Xa21, xa13 and xa5 have been successfully transferred into elite rice cultivars using MAS.

Sundaram et al. (2008) developed bacterial blight resistant rice varieties through marker assisted selection. Their work demonstrated high efficiency of MAS in rice breeding.

Blast Resistance

Blast disease caused by Magnaporthe oryzae is another serious threat. Genes like Pi1, Pi2 and Pi54 have been pyramided into rice cultivars using molecular markers.

2. Wheat

Wheat suffers from rust diseases including stem rust, leaf rust and stripe rust.

Marker assisted breeding has facilitated incorporation of resistance genes such as Sr2, Lr34 and Yr36 into commercial cultivars.

The emergence of Ug99 race of stem rust highlighted the importance of rapid resistance breeding. Several resistant wheat lines have been developed through marker assisted backcrossing(**Poland and Rutkoski, 2016**).

3. Maize

Maize diseases like downy mildew and maize streak virus significantly affect production.

MAS has been utilized for identification and transfer of resistance QTLs against these diseases. Marker based breeding has also improved resistance against ear rot and leaf blight diseases.

4. Tomato

Tomato breeding programmes have used markers for resistance against bacterial wilt, late blight and tomato yellow leaf curl virus.

Genes Ty1, Ty2 and Ty3 associated with virus resistance have been transferred through MAS(Foolad, 2007).

Insect Pest Resistance

Insect pests are major constraints in crop production. Marker assisted breeding has contributed towards development of resistant cultivars.

Rice Brown Planthopper Resistance

Genes Bph14 and Bph26 have been introgressed into rice varieties through marker assisted selection.

Maize Stem Borer Resistance

QTLs related to stem borer resistance have been identified and utilized in breeding programmes.

Cotton Bollworm Resistance

Molecular markers linked with resistance genes have assisted in development of improved cotton cultivars.

Nematode Resistance

Root knot nematodes and cyst nematodes cause severe damage in crops.

In soybean, the Rhg1 locus associated with soybean cyst nematode resistance has been effectively utilized through MAS.

Tomato Mi gene conferring nematode resistance has also been transferred into elite varieties.

Role of Marker Assisted Breeding in Abiotic Stress Resistance

Abiotic stresses are becoming more severe due to climate change. Marker assisted breeding has emerged as a valuable approach for improving tolerance against drought, salinity, heat and cold stress.

Drought Tolerance

Drought is considered one of the most destructive abiotic stresses affecting crop productivity.

Rice:QTLs such as qDTY1.1, qDTY2.2 and qDTY12.1 associated with drought tolerance have been identified and introgressed into rice cultivars.

Marker assisted breeding has led to development of drought tolerant rice varieties suitable for rainfed areas.

Maize: Several drought related QTLs have been mapped in maize. CIMMYT breeding programmes have utilized molecular markers for developing drought tolerant hybrids.

Wheat:MAS has been used to improve root traits, water use efficiency and stay green characteristics in wheat.

Although drought tolerance is complex quantitative trait, marker assisted breeding has shown considerable success during recent years.

Salinity Tolerance

Soil salinity severely limits crop production in arid and semi-arid regions.

Saltol QTL in Rice: The Saltol QTL located on chromosome 1 has been widely used for improving salinity tolerance in rice.

Marker assisted introgression of Saltol into elite varieties has produced salt tolerant cultivars with good yield performance(**Mackill, 2006**).

Wheat and Barley: QTLs related to sodium exclusion and ion homeostasis have been utilized for breeding salt tolerant genotypes.

Heat Stress Tolerance

Increasing global temperature is becoming a serious threat for agriculture.

Marker assisted breeding has identified several heat responsive QTLs in wheat, rice and maize.

Heat tolerant wheat varieties with improved grain filling ability have been developed using marker assisted selection.

Flooding and Submergence Tolerance

Flooding causes oxygen deficiency and severe yield reduction in many crops.

Sub1 Gene in Rice: The Sub1 gene conferring submergence tolerance has been successfully introgressed into popular rice varieties through marker assisted backcrossing.

The development of Sub1 rice varieties is considered one of the greatest achievements of marker assisted breeding(**Collard and Mackill, 2008**).

Farmers in flood prone regions greatly benefited from these varieties.

Cold and Frost Tolerance

Cold stress affects germination, flowering and reproductive development.

Markers linked with cold tolerance genes have been used in crops like rice, barley and chickpea.

Several cold tolerant barley lines have been developed through MAS.

Genomic Selection and Advanced Breeding Approaches

During the last decade, genomic selection has emerged as advanced form of marker assisted breeding. Unlike conventional MAS which uses few markers, genomic selection uses genome wide marker information for predicting breeding values.

This approach is highly useful for complex quantitative traits controlled by many genes.

High throughput genotyping technologies such as genotyping by sequencing (GBS), SNP arrays and next generation sequencing have accelerated genomic selection(**Cobb *et al.*, 2013**).

Machine learning and bioinformatics tools are also being integrated with breeding programmes. These technologies can analyse massive genomic datasets and improve prediction accuracy.

Some researchers believe that integration of genomic selection with gene editing technologies may revolutionize crop improvement in future.

Consequences and Impact of Marker Assisted Breeding

Marker assisted breeding has produced both positive and challenging consequences in agricultural systems.

Positive Consequences

1. Faster Development of Improved Varieties

Breeding duration has reduced significantly due to early generation selection.

2. Improved Precision

Selection accuracy has increased because markers are less influenced by environment.

3. Enhanced Stress Resistance

Many resistant cultivars against diseases and environmental stresses have been released.

4. Conservation of Genetic Resources

Wild relatives carrying useful genes can be effectively utilized.

5. Reduction in Chemical Usage

Disease resistant cultivars reduce dependence on pesticides and fungicides.

Negative Consequences and Limitations

1. High Cost

Molecular laboratories and genotyping platforms require substantial investment.

2. Requirement of Skilled Manpower

Technical expertise in molecular biology and bioinformatics is necessary.

3. Limited Success in Quantitative Traits

Complex traits controlled by many genes remain difficult to improve.

4. Possibility of Linkage Drag

Undesirable genes linked with target genes may also get transferred.

5. Dependence on Marker Validation

Markers identified in one population may not work effectively in another genetic background.

Despite these limitations, marker assisted breeding remains one of the most promising technologies in modern agriculture.

Challenges in Marker Assisted Breeding

Although marker assisted breeding has achieved remarkable success, several challenges still persists.

1. Complex Nature of Stress Tolerance

Abiotic stress tolerance involves interaction of multiple genes and environmental factors.

2. Genotype × Environment Interaction

Performance of markers may vary under different environmental conditions.

3. Limited Phenotyping Facilities

Accurate phenotyping is essential for successful QTL mapping.

4. High Throughput Data Management

Modern genomic technologies generate massive datasets which require advanced computational tools.

5. Small Effect QTLs

Many stress related QTLs have minor effects and are difficult to utilize effectively.

Researchers are continuously working to overcome these limitations through integration of genomics, phenomics and computational biology.

Future Prospects

Future of marker assisted breeding appears very bright because genomic technologies are advancing rapidly. Development of cheaper sequencing platforms and automated phenotyping systems will further increase breeding efficiency.

Genome wide association studies and genomic selection are expected to become routine tools in crop breeding program.

Integration of CRISPR based genome editing with marker assisted selection may produce climate resilient crops in coming years.

Artificial intelligence and machine learning are also expected to improve prediction of complex traits. Breeders may soon be able to design crop varieties with precise combinations of stress resistance genes.

Participatory breeding and precision agriculture approaches can also strengthen utilization of marker assisted breeding technologies at farmer level.

Still, public investment and international collaboration are necessary for ensuring equal access to these technologies, especially in developing countries.

Conclusion

Marker assisted breeding has revolutionized crop improvement during the last two decades(**Varshney *et al.*, 2021**). The technology has greatly enhanced efficiency, speed and precision of plant breeding programmes. Molecular markers have facilitated identification and transfer of genes associated with disease resistance, insect resistance, drought tolerance, salinity tolerance, heat stress and flooding tolerance.

Several successful examples including Sub1 rice, bacterial blight resistant rice and rust resistant wheat demonstrate the immense potential of marker assisted breeding. The integration of genomic selection, next generation sequencing and bioinformatics has further strengthened modern breeding strategies.

Although some limitations and challenges still exist, continuous advancement in molecular technologies is expected to overcome many of these problems. Marker assisted breeding will continue to play a vital role in development of climate resilient and high yielding crop varieties required for future food security.

Overall, the last two decades have witnessed remarkable progress in marker assisted breeding and its application in stress resistance breeding. The coming years may bring even more innovative approaches which could transform global agriculture completely.

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