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6. Molecular Breeding and Its Current Scenario in Crop Improvement

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Abstract:

Plant breeding is the important branch for the sustainability of agriculture. Crop improvement in broad way is not only to increase quantity and better quality but it also saves the agricultural resources. Production of better plant variety depends on the identification of plant characters. Environment is the major factor which interact with plant to produce phenotype. Selection of ideal plant is tedious job due to variable influence of environment. Genes present in genome is not affected by the external conditions.

So, validation of plant using molecular breeding concept is advantageous over conventional breeding techniques. Molecular breeding existing technologies can manipulate the DNA to achieve remarkable success to improve nutritional quality, biotic and abiotic stress management in agriculture. Molecular breeding fastens the way of plant selection with higher accuracy and significant result.

6.1 Introduction:

Food is the prime requirement for the survival of a living organism. Increasing population with urbanization leads to more food demands and reduce farming area, respectively. In addition, climate change effects, emerging insect pest of crop reduce the crop production as well as productivity. Traditional crop breeding approaches used different germplasm of crop either individual or combinations for crop improvement up to certain limit.

Molecular breeding add value to traditional breeding approaches and also create new varieties by its own using genome editing technologies. It helps to produce climate resilient, biotic stress resistance, better quality plant with higher productivity. Molecular breeding is faster, time saving and accurate technique to mitigate current challenges in food production.

Plant breeding with direct and indirect implementation of molecular biology is called molecular breeding. In broad sense, direct way means molecular biology provide assistance to breeding technique like Marker assisted backcrossing (MABC) - the transfer of gene of interest eliminating linkage drag and recovery of genetic background of recurrent parent. Indirect use means genetic manipulating by inserting transgene, double stranded break (DSB) followed by DNA repair mechanism and change expression level of gene using RNA interference (RNAi) technology.

6.2 Existing Molecular Breeding Technologies:

6.2.1 QTL Mapping:

Quantitative trait loci (QTL) mapping is one of the widely used method to identify the location of the group of gene or loci on chromosome governing the quantitative trait. It also clarity the number of genes influence the expression of a quantitative trait, level of influence of each gene/locus on the trait, gene action is responsible for the trait and which alleles are having favorable effect on the trait. Quantitative trait-related loci may be dispersed throughout the genome or grouped together.

Polygenes control quantitative features, which exhibit discontinuous variation and are strongly impacted by the environment. Both phenotypic and genotypic data require to identify the QTL. To find QTLs, regression and likelihood statistics are used. Having at least 200 plants is thought to be a desirable standard.

High-resolution mapping requires larger populations. The mapping population must first be phenotypically assessed. If the map will be used for QTL investigations, before further QTL mapping. Biparental populations with Recombinant Inbred Lines (RILs) and Double Haploid (DH) are thought to be superior. However, for joint linkage and association analysis, NAM and MAGIC populations are also used. Higher the genome size, more markers are required for analysis.

6.2.2 Marker Assisted Selection (MAS):

The process of identifying the gene of interest and specific loci associated with a trait using molecular markers, such as is known as Marker-Assisted Selection (MAS). This technique is utilized in both plant and animal breeding. Molecular markers exhibit qualitative inheritance patterns. MAS is an indirect selection method where the desired trait is chosen based on a marker that is linked to it, rather than directly selecting for the trait itself. Selecting plants based on their phenotypes can sometimes negatively affect the efficiency of the selection process.

Essential requirements of MAS: 1) The identified DNA marker(s) should co-segregate or be closely related to the trait (**preferably 1 cM or less**). 2) Emergence of effective DNA marker screening methods that can feasibly manage vast populations. 3) The screening method needs to be reasonably priced, simple to use, and highly reproducible.

MAS can be helpful for qualities that are challenging to quantify, have poor heredity, or express themselves later in development. However, because genotype does not exhibit the effects of the environment, molecular selection of plants allows for effective selection. Types of MAS are given below:

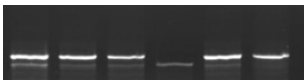
A. Marker assisted backcrossing
B. Marker assisted recurrent selection
C. Marker assisted gene pyramiding
D. Genomic selection

A. Marker Assisted Backcrossing:

The simplest kind of MAS is marker-assisted backcrossing, where the objective is to introduce a key gene into a superior cultivar or breeding line (the recurrent parent) from a source that is agronomically inferior.

Two types of selection are recognized by Hospital, 2003. Third type of selection is recombinant selection (Collard and Mackill 2008).

1st stage of selection – foreground selection

- Choice of target gene or QTL
- Valuable for assessing traits that are challenging to evaluate 
- Also advantageous for identifying recessive genes

2nd stage of selection – Recombinant selection

- Employing adjacent markers to select recombinants positioned between the target locus and flanking marker
- Reduces the presence of unwanted donor chromosome segments (referred to as linkage drag), which can adversely impact agronomic performance.
- Require large population sizes and it depends on distance of flanking markers from target locus. (Closer the distance, higher population size)

3rd stage of selection - Background selection

- Use unlinked (unlinked to the target locus) markers to select against donor. Markers should be polymorphic between donor and recurrent parents.
- Promotes the recovery of the recurrent parent genome more quickly.
- Savings of 2, 3 or even 4 backcross generations may be possible
- Minimum 5 markers per chromosomes are ideal for this.

B. Marker Assisted Recurrent Selection (MARS):

Conventional phenotypic recurrent selection focuses on choosing individuals based on offspring performance, followed by intercrossing between individuals demonstrating optimal progenies, to increase the frequency of desirable alleles in the population. Phenotypic selection has low efficiency due to environmental influence.

MARS could be helpful to reduce this environmental error in selection of multiple Quantitative Trait Loci (QTLs). MARS could increase recurrent selection's effectiveness and speed up the process, primarily by assisting in the integration of several advantageous genes or QTLs from various sources using recurrent selection based on multi-parent populations. (Asima Gazal *et al., 2015).*

C. Marker Assisted Gene Pyramiding:

The technique called "gene pyramiding" is employed to combine multiple desirable genes from different parents into a single genotype. This process aims to develop genotypes that possess all of the desired genes. Gene pyramiding serves two main purposes: 1) enhancing trait performance by combining two or more complementary genes, and 2) broadening the genetic diversity of released cultivars (Ye and Smith, 2008). Typically, gene pyramiding is used to improve qualitative traits such as disease and insect resistance. The process of gene pyramiding can be divided into two stages. The first stage involves creating a pedigree to accumulate all the target genes in a single genotype referred to as the root genotype. The second stage, known as the fixation phase, focuses on converting the target genes into a homozygous state (Joshi and Nayak, 2010). Various methods are employed during the fixation phase to accumulate the genes in a single parent. It includes three types of methods to cumulate the genes in a single parent as following:

a. Stepwise Transfer:

The donor parent (DP1) and recurrent parent (RP1) are crossed to create the F1 hybrid (Figure 6.1) and the third backcross generation (BC3) of backcrossing produces the improved recurrent parent (IRP1). Then, to pyramid multiple genes, this improved recurrent parent is crossed with another donor parent (DP2). Pyramiding is more accurate because it involves one gene at a time, but this technique is less desirable because it takes time.

b. Simultaneous Transfer:

The pyramiding is done in the pedigree step itself in the second strategy, where the recurrent parent (RP1) is crossed with the both donor parents simultaneously resulting in F1 hybrids that are then intercrossed to produce improved F1 (IF1), which is then backcrossed with the recurrent parent to produce the improved recurrent parent (IRP). However, when the donor parents are different, this method is less likely to be used because there is a chance.

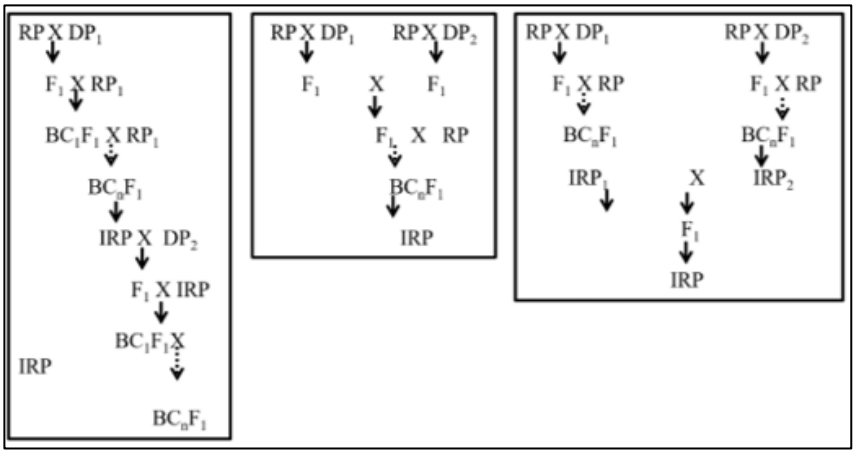


Figure 6.1: Strategies of gene pyramiding: Simultaneous transfer, stepwise transfer and Simultaneous and stepwise transfer

c. Simultaneous and Stepwise Transfer:

The third technique combines the first two and involves backcrossing numerous donor parents with a recurrent parent (RP1) at the same time, up to the BC3 generation. Pyramided lines are created by intercrossing the backcross populations with the specific gene. This is the most preferable strategy because it not only saves time but also completely ensures that the genes will be fixed.

D. Genomic Selection:

The genomic selection (GS) scheme was proposed by Meuwissen et al. (2001). Linkage mapping is limited by poor resolution, few alleles and need for mapping populations. QTL mapping cannot detect minor QTLs with low heritability. Association mapping is prone to false positives and unable to explain all the variance of a trait. MABC improves only for introgressed QTL. MARS is based on significant major and minor QTLs.

6.2.3 RNA Interference (RNAI) and Genome Editing Tools:

RNAi is double stranded RNA (dsRNA) inducing phenomenon for gene silencing or knockdown of gene. micro-RNA (miRNA) and small short interfering RNA (siRNA). in case of siRNA, dsRNA cleaved by dicer protein and form 21bp dsRNA with 2bp overhang at 3 ends. Its further binding with argonaute protein to form RNA induced silencing complex (RISC). Passenger strand remove from complex and guide strand binds with complementary mRNA and reduce the translation process by cleave mRNA of interest. In miRNA inducing silencing process, hairpin loop primiRNA cleaved by DROSHA and DGCR8 protein to nearly 70 nucleotide made RISC complex and transport to cytoplasm and bind to mRNA. This finally results in reduce gene expression.

One of the latest molecular breeding aspects is manipulation of DNA sequences using genome editing tools like CRISPER-Cas9 (Clustered Regulatory Interspersed Short Palindromic Sequences), TALENs (Transcription Activator-Like Effector Nucleases) and ZFNs (Zinc Finger Nucleases). When a virus attacks a bacterium, it destroys the bacterial genome while leaving portions of the viral genome at specific locations. This is known as Crisper mechanism. Bacterial systems create Cri RNA, which is complementary to viral RNA, when the same virus attacks again. The Cas protein recognizes a specific nucleotide sequence on Cri RNA called PAM (Protospacer Adjacent Motif), resulting in an interaction of RNA and proteins. DNA double strand breaks are caused by Cas 9, which is complementary to g RNA (guide RNA). Simply said, tracer RNA is a gene in the CRISPR system that activates cri RNA by maturing it and producing it along with it. CriRNA in the RNA fragment comes from the spacer sequences previously snipped by the bacteria.

The zinc-finger nucleases TALENs and ZFNs, which have distinct DNA-binding and DNA-cleavage domains, have emerged as the class of targeting agents that has demonstrated to be the most adaptable and successful in recent years. TALENs are fusion proteins made of the Fok1 endonuclease and the bacterial TALE protein, and their specificity is based on protein-DNA interaction. It typically consists of 33 to 35 modules of amino acids, each of which targets a specific nucleotide. This means that scientists can identify any particular

DNA sequence they choose by assembling various TALEN moieties. The crystal structure of a trio of fingers linked to DNA revealed that each finger makes remarkable modular contact with 3 bp of DNA on average (Pavletich and Pabo 1991). This implied that by creating innovative assemblies, numerous distinct sequences could be tackled. Cleavage is done by heterodimer of type II restriction enzyme *Fok1*.

6.3 Current Scenario of Molecular Breeding in Crop Improvement:

6.3.1 Molecular Breeding for Biotic and Abiotic Stress Resistance:

As said before field screening of a plant for disease and abiotic stress have environmental interference but molecular aspect gives better way to select the desirable plant. In recent times, areas affected by drought have experienced a significant decline in rice yields. This decline is primarily attributed to the increased frequency and severity of drought stress. Researchers (Kumar et al., 2014) conducted a study using grain yield as a selection criterion and identified 14 quantitative trait loci (QTLs) that have a significant impact on high-yielding rice cultivars in drought-prone regions. Among these QTLs, some exhibited positive effects on rice grain production under drought conditions in both lowland and upland ecosystems. These QTLs proved valuable in enhancing rice productivity in drought-affected areas across different genetic backgrounds.

By employing a well-planned marker-assisted backcross breeding strategy, the yield of prominent rice varieties like IR64 and Vandana has been successfully increased, and ongoing efforts are focused on introgressing QTLs into several other well-known varieties. Marker-assisted breeding has also played a significant role in the development of high-yielding rice cultivars resistant to bacterial blight (BB). For instance, Samba Mahsuri, a BB-susceptible variety, has been improved through the stacking of *Sub1* and *Sub4* BB-resistant genes, while other combinations involving the BB resistance gene, *Sub1* QTL, and yield QTL have been developed (Mohapatra *et al.,* 2021).

6.3.2 Molecular Breeding in Quality Improvement:

A. Modifying Carotenoid Levels in Vegetable Crops:

Vitamin-A deficiency is a common problem in many developing regions, leading to childhood blindness and other health issues. To combat this, Ingo Potrykus, Peter Beyer, and their colleagues developed "golden rice" by genetically modifying rice genotypes with carotenoid biosynthetic genes to increase the availability of vitamin-A precursors in the diet (Ye *et al*, 2000). Golden rice has shown potential for manipulating carotenoid biosynthesis in other crops (DellaPenna & Pogson, 2006). One advantage of increasing carotenoid levels is the potential for more vibrantly colored vegetables, which can be more appealing to consumers. Therefore, enhancing carotenoid levels is beneficial not only for nutrition but also for aesthetics. Breeding programs have successfully improved the nutritional value of sweet potatoes, specifically the orange-fleshed variety, which has the potential to enhance the health of millions of people in developing countries. This type of sweet potato contains carotenoids that can be transformed into vitamin A when consumed, providing an important source of this essential nutrient for the body.

Phil Simon and Gold man (2007) at the University of Wisconsin, USA have identified molecular markers associated with genes related to carotenoid content in carrots and used them to identify carrot lines with higher levels of carotenoids, which are now being utilized by commercial seed companies. Through traditional breeding, the overall levels of carotenoids in carrots have significantly increased in the past 40 years, reaching up to 1,000 ppm carotenoids on a fresh weight basis (Simon and Gold man, 2007).

Genetically modified potato, which normally accumulates lutein and violaxanthin, to accumulate zeaxanthin instead (Römer *et al*, 2002). This modification also resulted in increased levels of α-tocopherol (vitamin E). The carotenoid and tocopherol pathways are linked, so modifications aimed at one pathway may have effects on the other.

B. Anthocyanins in Barley:

Barley grain can have a range of colors, such as yellow, purple, red, and blue, which can also appear in other parts of the seed, Pigments are important for protecting the plant under various biotic and abiotic stress conditions, as well as providing valuable health benefits (Koes *et al*., 2005). Pigments also have a physiological function in attracting pollinators and seed dispersers (Chaves-Silva *et al*., 2018). Additionally, anthocyanins play a role in preventing different chronic diseases in humans (Li *et al*., 2021). Only dominant alleles of Ant1 and Ant2 loci (Gordeeva *et al*., 2019), and both genes lead to anthocyanin accumulation in the pericarp. Classical and molecular techniques have been utilized to create new barley varieties with improved anthocyanin accumulation and diverse colors. However, genetically-modified organisms (GMOs) may face market challenges due to consumer attitudes and perception. To address this, CRISPR/Cas9 has emerged as a promising non-transgenic method for generating mutations. Gasparis *et al*. (2018) demonstrated that an optimized RNA-guided Cas9 system can create homozygous knockout mutants in the offspring of transgenic barley plants. Transcriptional repressors such as AtMYBL2 and FaMYB1 have been identified and shown to negatively regulate anthocyanin biosynthesis in Arabidopsis and strawberry, respectively (Matsui *et al*., 2008). Inhibiting these repressors could enhance anthocyanin content. A better understanding of the molecular mechanisms governing anthocyanin repression may improve breeding efforts in barley using non-transgenic methods.

C. Molecular Approaches for Calcium Biofortification in Finger Millet:

Calcium is a crucial nutrient for both plants and animals, and it is essential for structural integrity and signaling processes. In humans, insufficient calcium intake has been linked to several diseases, which can have severe long-term health consequences. Unfortunately, major food crops do not contain much calcium. However, finger millet, which is an underutilized crop, has exceptionally high levels of calcium and is a promising nutritional security crop. To develop crops with higher calcium content, it is crucial to understand the genetic variation and molecular mechanisms involved in calcium uptake, transport, and accumulation in grains. The goal of this research is to provide a comprehensive overview of the molecular mechanisms involved in regulating calcium nutrition and highlight the importance of biofortification. By identifying potential candidate genes and regulatory elements in finger millet, it may be possible to alleviate calcium malnutrition and develop

nutraceuticals or designer crops. Finger millet can serve as a model for understanding the mechanisms of calcium accumulation in grains and may pave the way for developing crops with elevated calcium levels.

Researchers used association mapping studies to find QTLs for calcium content and discovered two minor QTLs connected to grain calcium content on linkage groups 3 and 8, respectively (Yadav *et al.,* 2014). Despite having a few small QTL, chromosome 8 may also contain genes or areas that contribute to mineral accretion (Srinivasachary *et al.,* 2007). Rice chromosome 3 and finger millet LG 3 share co-linearity, and rice chromosome 3 also contains Ca2+ QTLs. These results show that to uncover all the genes that regulate this complicated feature and its variance in a population, a comprehensive, genome-wide search is required.

Two calcium-binding proteins, Calcineurin-B and Calreticulin, were found in finger millet seeds using peptide mass fingerprinting (Singh *et al.,* 2016). Their expression was examined throughout seed development and grain filling (Singh *et al.,* 2014). Finger millet transcriptome sequence data showed that Ca2+ transporter genes were significantly expressed in genotypes of the plant with high seed Ca2+, offering a viable explanation for the high calcium buildup in finger millet. Through genetic engineering or marker-assisted selection techniques, calcium biofortification programmes can make use of this genetic information. In order to speed up breeding for high grain Ca2+ in finger millet, closely related markers to the found genes (EcCIPKs) can be used.

Genetic modification is an alternate strategy for raising the calcium (Ca2+) content of important food crops. To increase the concentration of calcium (Ca2+) in the edible portions of plants, three transgenic approaches can be used. The first technique involves expressing calcium (Ca2+) transporter proteins such Ca2+ ATPase and Ca2+/H+ antiporters in order to boost the calcium (Ca2+) storage capacity. The second strategy involves overexpressing calcium (Ca2+) channel proteins to increase calcium (Ca2+) buildup. The third technique entails increasing calcium (Ca2+) levels by overexpressing calcium (Ca2+) binding proteins.

6.3.3 Molecular Breeding in Yield Improvement:

GWAS and GS are valuable tools for understanding the genetic basis of complex traits in soybean. Previous studies have utilized GWAS to identify molecular markers associated with various agronomic traits in soybean (Yao *et al*., 2015). For example, multiple markers related to maturity have been discovered (Zuo *et al*., 2013), with a concentration on chromosome 16. Candidate genes regulating maturity and plant height have also been identified (Contreras-Soto *et al*., 2017). Additionally, numerous molecular markers associated with seed weight have been found in soybean. Genome editing, particularly using CRISPR technology, plays a crucial role in developing environmentally-friendly and resilient agriculture. Researchers have successfully used CRISPR to edit genes such as OsGS3 and OsGL3.1 in rice, resulting in increased grain size and overall yield per plant. Multiplex gene editing has also led to higher grain yield in rice by targeting genes GS3, GW2, and Gn1a (Yuyu *et al*., 2020). Furthermore, CRISPR-based gene editing has been employed to improve photosynthesis by editing genes like NRP1, resulting in enhanced photosynthetic efficiency, grain yield, and biomass production in rice (Flexas *et al*., 2020).

Scientists are also focusing on enhancing crop photosynthesis by targeting Rubisco, a crucial enzyme in CO2 fixation. Recent research has used CRISPR to disrupt the RbcS multigene family in rice and replace it with RbcS from sorghum, leading to improved photosynthetic rates and increased crop productivity (Matsumura *et al.*, 2020). These advancements demonstrate the potential of genome editing in improving crop traits and overall agricultural productivity. Researchers have employed the CRISPR-Cas system to enhance the photosynthetic system of diploid and polyploid crops. For instance, in one study, multiple rbcS homologues (rbcS_S1a, rbcS_S1b, and rbcS_T1) were targeted and eliminated in tobacco, which is a tetraploid crop. As a result, the mutant plants exhibited a higher photosynthetic rate compared to the wild-type plants (Donovan *et al.*, 2020). This research highlights the potential of CRISPR-based gene editing to improve photosynthesis in both diploid and polyploid plant species.

6.4 Future Challenges and Prospective:

Plant transformation is a crucial technique for gene editing, but it faces challenges in certain plant species with complex genomes like wheat, cotton, and Brassica. Moreover, CRISPR-Cas gene editing often requires time-consuming and labor-intensive tissue culture. To overcome these limitations, scientists are exploring tissue culture-free genome editing methods, such as RNA virus-based systems for delivering gRNA. Despite the popularity of CRISPR-Cas technology, concerns remain regarding off-target effects and regulatory issues. To address these concerns, researchers are developing more precise and well-structured strategies, including biased and unbiased off-target detection methods, modification and engineering of gRNA, improved Cas variants, efficient delivery methods for the CRISPR system, development of antiCRISPR proteins, as well as effective base-editing and prime editing systems. The newly developed base-editing and prime editing tools have demonstrated reduced risks with minimal off-target effects in both plants and animals, presenting opportunities for the safe development of genetically engineered food crops and contributing to the global zero hunger goal.

The use of rapid cycling genomic selection in plant breeding programs has the potential to increase genetic gains, but adoption of these methods has been limited due to the high cost of genotyping, which is still a barrier for smaller breeding programs or public-sector programs with limited resources. To overcome this limitation, it is important to evaluate the feasibility of rapid cycling genomic selection in different crop species and design new breeding programs that integrate this technology more efficiently. Efforts are already underway, such as those of the International Maize and Wheat Improvement Center (CIMMYT) in Africa and Asia, which serve as examples of how public-sector breeding programs can increase genetic gains in important crops.

6.5 Conclusion:

The preceding literature underscores that although there have been recent advancements and successful instances of molecular plant breeding, a significant challenge in plant biology is still the identification of gene combinations that result in substantial enhancements in crops.

The suggestion put forth is that the advancement of molecular plant breeding can be accelerated by enhancing the integration of different research disciplines and activities that are essential to the field. It is also recommended that the corporate sector continues to invest in initiatives that promote this integration and create a favorable training environment for aspiring scientists in molecular breeding. In addition to financial support for graduate training and sponsored research, companies can provide non-monetary assistance to bridge the technological gap between public and private sector research in molecular plant breeding. By uniting the collective efforts of the diverse community of scientists dedicated to plant biology and crop improvement, molecular plant breeding will be able to make even greater contributions to meeting global demands for sustainable increases in agricultural productivity.

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