MOLECULAR MAPPING AND MOLECULAR CHARACTERIZATION OF CROPS

Abstract

Agriculture is the core of the functioning of the world. From ancient times many new techniques have been introduced to produce good quality crops. From the 18th century the improvement in biotechnology and the study of genes have paved the new paths for the development in agriculture. The current works in agriculture biotechnology has improved the agriculture in many ways that includes the production in abundance, production of good quality crop with abundance in nutrients, pest and insect resistance, and improvement in crops by using the Molecular mapping and then characterizing the required gene of interest for the development of the crop. This genetic engineering in plants has benefitted agriculture around the globe, the specific selection of the genome and study of it without affecting the plants have been used to work more easily and effectively. This chapter includes the first milestone in the current agriculture biotechnology that is the molecular mapping of the plant genome using Marker assisted techniques and Quality trait loci and the Molecular characterization of the genome that helps in the study of the genome of the crop is discussed in detailed that will provide a good understanding of the most important topics of the agriculture biotechnology.

Keywords: Agriculture engineering, Mapping and Molecular, breeding, biotechnology

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I. INTRODUCTION

The foundation of the global economy and a crucial element in maintaining life is agriculture. Agriculture has seen major changes over the years, starting with the green revolution and continuing with the current era of genetically modified crops. Challenges brought on by changes in the climate and human actions have caused these alterations. In response, scientists have worked to create a wide variety of plants that can endure such challenges. In order to improve plant characteristics and produce crops that can thrive in shifting conditions and suit the needs of modern agriculture, genetic engineering has emerged as a key technology. In this chapter we are going to discuss about a part of it i.e., Molecular Mapping and Molecular Characterization from the word Molecular it's known that it's about the genome of a plant. Most of the traits of evolutionary or agricultural importance are found to be controlled by a complex trait (multiple quantitative trait loci). Molecular or genetic mapping and characterization of the complex trait and functional traits facilitate the research in the genome-aided breeding that works for crop improvement such as its production, and resistance to biotic and abiotic stresses (drought, pest, insects,etc.). Now Mapping states that chromosome in a genome is studied to know about the specific locations and forms a map that will help in understanding the genome of a plant whereas characterization of a plant simply defines the characteristics where the gene is of our interestand is being incorporated to the plant genome by using methods of genetic engineering. This chapter gives a brief idea of molecular mapping and characterization and the technologies used in it such as Marker-assisted selection (MAS), Qualitative trait loci (QTL), etc. molecular markers and their applications. This chapter may set the first milestone that will give an idea about the topic.

II. MOLECULAR MAPPING

In genetics, mapping entails figuring out and comprehending the connections between genes located on chromosomes. A technique that assists in pinpointing the specific positions of molecular markers inside a genome is called molecular mapping. These markers are frequently linked to certain genes or traits of interest, allowing researchers to learn more about the genetic setting and structure of an organism's genome.There are two ways by which the mapping of the genetic material is donenamely Genetic mapping and Physical mapping (Brown et al., 2002). Both methods guide toward the location of a gene on a chromosome but are based on different information.In order to identify genetic differences between chromosomes or between various areas on the same chromosome during cell division, genetic mapping depends on molecular markers. These molecular markers serve as genetic loci that simplify monitoring and measurement within a plant genome or population since they are connected to particular genes or features of interest. Physical mapping, on the other hand, is based on differences between known DNA sequences, mostly based on the number of base pairs separating them. In this method, unique DNA sequences are physically measured in order to map out their relative positions on chromosomes or within genomes (Guo et al., 2017).

III. MOLECULAR CHARACTERIZATION

A method called "molecular characterization" attempts to identify an organism's genetic characteristics, especially in the context of plants. This process is used to understand the effects of genetic material injected into the plant and to determine how it expresses the

traits and behaviours of the plant. Characterization methods include mapping, which makes use of genetic markers like restriction fragment length polymorphism (RFLP), recombinant DNA, and random amplified polymorphic DNA (RAPD). These molecular markers act as indications of genetic variation occurring inside the genome of the plant due to which gene exchanges or other genetic alterations. The goal of molecular characterization is to evaluate plants created using contemporary biotechnologies. The objective of this procedure is to understand the molecular specifics of the inserted DNA within the plant genome. The exact place of insertion, the expressed material, and any intentional or unforeseen effects carried on by the insertion of the desired DNA are all detailed in this information. The plant's phenotype must be predicted in order to determine whether the recombinant DNA includes any potential risk factors. This is where molecular characterization comes in. Essentially, molecular characterization makes sure that genetically modified plants are thoroughly evaluated and assists in making decisions about their suitability for various purposes as well as their safety. (OECD, 2010).

The plant genome's particular regions are included in the scope of molecular characterization. This entails describing the inserted DNA, identifying the insertion site, and taking into account any potential genetic deletions or rearrangements brought on by the transformation procedure. The expression of proteins or RNA from the inserted DNA in plant tissues at various developmental stages is included in the analysis. Additionally, many propagation cycles are used to study the stability of the implanted DNA and evaluate its inheritance patterns. Outlining the specifics of the transformation and prospective DNA sequences that might be included in the plant genome are additional steps in the process. Overall, molecular characterization offers a thorough comprehension of the altered genetic landscape, making it easier to assess the safety and gain a deeper understanding of plant behaviour. (OECD, 2010).

1. **Approaches for understanding the function of a gene:** Before catching up to the main subject of the discussion it is necessary to understand the functioning of a gene. Two approaches are there that are used to understand the functions of genes i.e., Forward, and Reverse genetics. Forward genetics start with a well-characterized phenotype, for example, resistance of a gene in a plant and identifying the responsible gene for that phenotype (Wasim et al., 2018). While Reverse genetics first begins with a gene, and then works on its phenotype to know what it determines. To make it simple forward genetics focus on finding the genetic basis of a phenotype while reverse focus on finding possible phenotype that may have a particular DNA sequence that is present in detail in DNA sequencing. Genetic mapping or molecular mapping works on the forward genetics methods such as QTLs (Quantitative trait Loci) mapping and association mapping. Mapping is the base for the further characterization of the crop genome to study its expression and markers play an important role in mapping that will be discussed in this chapter (Wasimet al., 2018).

2. **Methods of Molecular Mapping & Molecular Characterization:** In the past, conventional plant breeding techniques were the standard, relying on desirable features found in parental lines. This strategy, nevertheless, was time-consuming and had a low success rate. A breakthrough in science that began in the 1990s made it possible to explore genetic variants at the DNA level. The start of genetic research for plant improvement was signalled by this crucial event. The scientific and industrial

applicability of research has significantly increased with the study of genetic markers. As a result, agricultural production and quality standards have increased. The methods used for molecular mapping are categorized into two: - Physical Maps and Genetic Maps

- **Physical Maps:** Physical maps offer a nucleotide-based depiction of genomic distances. These maps provide information on the actual distances between particular genomic regions, allowing for a more precise knowledge of how DNA sequences are arranged and organized inside a genome.

- **Genetic Mapping:** Genetic maps show the specific site of distinctive features that are achieved by generating information about the presence or absence of molecular markers. Previously genes were used as the markers to identify specific sites(Brown et al., 2002)

Molecular mapping and characterization are based on the markers in the genome. Molecular markers have changed a lot in the field of life sciences because of their broad applications in molecular and cellular areas, that has provided a powerful weapon with a precise selection of desired genotype in the genome of a plant (Phillips and Vasil 2001; Gupta and Varshney 2004). Molecular markers and high-density mapping of the genome are available in a variety that has expanded the methods in the field of standard methods of breeding plants with complex and desired traits. This is used in anarray of purposes, for example in the diagnosis of a disease, conservation biology, forensic sciences, paternity tests, etc (Varshney et al., 2005).

The ideal marker that is required for the study has some specific properties which have been discussed below (Jonah et al. 2011; Khan 2015):

- The amount of DNA samples or tissues required for analysis should be in less amount.
- The marker should be selectively neutral to environmental conditions.
- Highly reproducible marker that can provide data exchange between laboratories.
- The marker should be polymorphic.
- Throughout the genome the inheritance pattern should be even.
- Information regarding the sequence-based genome is not a requirement.
- The markers should be easily assessed that show strong heritability from one generation to another.
- The marker has to be inexpensive, quickly assessable, and simple.

The properties are the basic requirements that should be present in the markers. Now the question arises How many types of markers are there and they work? So first let's look at the types of markers that are used and present in the system. A marker is polymorphic, and that polymorphism can be detected at four levels namely biochemical markers, Morphological markers, molecular markers, and next-generation molecular marker technologies (Palakurthy et al., 2020) (Figure 1).

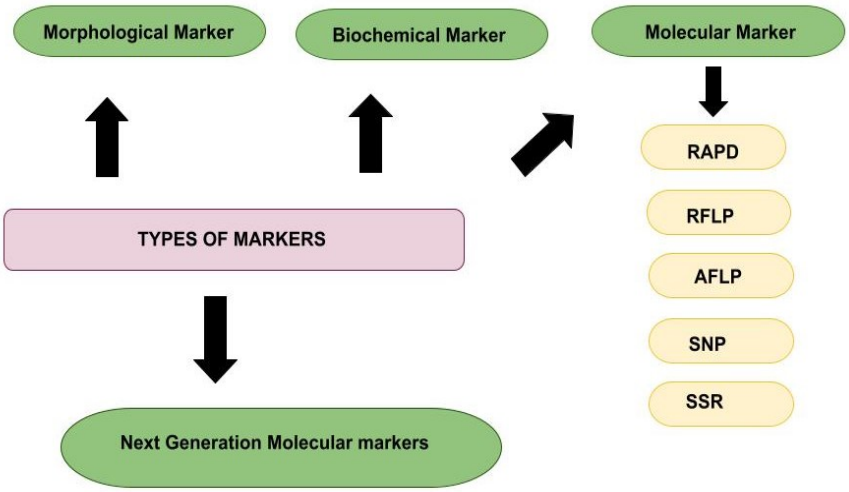

Figure 1: Types of markers

- **Biochemical markers:** Isozyme markers, another name for biochemical markers, rely on variations that can be identified using SDS PAGE. These markers have a high degree of genetic neutrality, which enables their use in linkage studies and genetic variability research. (Tanksley and Orton 1983). Using biochemical markers as a genetic marker for gene assortment or variation in plant breeding is constrained by the insufficient availability of genetic markers in the assay and the development stage-dependent expression of isozyme loci. One of the first groups to be investigated for genetic linkage maps and genetic diversity was the biochemical or isozyme markers. Isozyme markers are described as structurally distinct enzyme molecules with qualitatively identical catalytic activities. These originate through the alterations in the amino acids that cause changes in net charge or spatial conformation of the enzyme molecules and their electrophoretic mobility. The isozyme profile of sample individuals is observed after specific staining. Iso-loci is the loci that has usually two or more loci that can be distinguished for an enzyme. Because of this, the allozyme variations are also referred to as isozyme. In the field of plant genetics and breeding studies, isoenzymes have proven to be reliable genetic markers. Their consistency in expression, which is unaffected by environmental factors, accounts for their dependability. (Palakurthy et al., 2020).

    - ❖ **Advantages:** The main benefit of isoenzymes is their simplicity because they don't require the availability of sequence information or procedures like DNA extraction, probing, or primer construction. It takes little time or effort to use them. Depending on the materials used for enzyme staining, various allozymes can be applied with the least amount of expense by using straightforward analytical procedures. However, in other circumstances, particular enzymes may need careful technology or application optimization (Kumar et al., 2009).

- ❖ **Disadvantages:** There are a number of advantages as well as disadvantages for using isoenzyme markers. There are only a few biochemical assays per species with a few genetic markers. Post-translational modifications are the main cause of the lower levels of isozyme polymorphism compared to DNA-level polymorphism. It is difficult to monitor different enzymes in one area since different biochemical techniques are required to see allele differences. Because a sizable section of the genome does not encode proteins or isozymes, there is complexity involved. Because of this, the variations that exist at the protein level may be hidden by this factor (Kumar et al., 2009).

- **Morphological Markers:** Morphological markers have qualitative traits that are less in number. The morphological markers can be scored visually without the use of any specialized techniques and follow dominant inheritance. This can be seen in the work of geneticist Alfred H. Sturtevant who constructed the genetic linkage map of drosophila melanogaster with six morphological traits (Sturtevant 1913). Further, Sax (1923) there has been reported some pioneering work in plants, these were based on complex qualitative traits that can be selected based on the segregation of the traits with the simple Mendelian inheritance. The morphological markers mostly appear in the late stage of their development (for example colour of the flower, and seed coat colour), which makes it difficult to score it early (Palakurthy et al., 2020) beside this the large areas of field or environmental conditions are required to tag morphological markers, that makes it more expensive to use. Significant environmental sensitivity exists for morphological markers. In the context of plant breeding programs, they typically have an influence on other particular markers or traits, frequently as a result of pleiotropic gene effects. Less often used morphological markers can be seen in wheat breeding, which serves as a typical example of this. of this situation, male sterility and the dwarf character of plants are related. (Liu 1991). The assessment of genetic diversity using these morphological markers is limited because of the Phenotypic plasticity and environmental effects (Mondini et al. 2009).

- **Molecular Markers:** A distinguishable DNA sequence with straightforward inheritance tracking is referred to as a molecular marker. These DNA-based molecular markers are used in genetic variation research because they provide a useful way to link genotypic and phenotypic variants. (Kumar et al., 2009). Based on the naturally occurring DNA polymorphism the molecular markers work that form a basis that design strategy to exploit for applied purposes. A marker must be polymorphic i.e., it must be in different forms that will make it easy to distinguish it from the chromosome with a normal gene as the marker will be carrying a mutant gene. The simultaneous occurrence of a trait in the same population of two different variants or genotypes is termed genetic polymorphism. DNA markers are the best method for the efficient and effective selection and evaluation of the plant genetic material, unlike the morphological marker and biochemical (isozyme) markers, as the DNA markers segregate as a single gene that make it unaffected by the environmental conditions. As DNA can be easily extracted from plant material with a cost-effective analysis and labour this makes it advantageous to use. Most of the molecular markers are developed utilizing arbitrary amplification of genomic regions that are done by using PCR (e.g., Rapid amplified polymorphic DNA) or by using genomic libraries (e.g.,

Restriction fragment length polymorphism or Expressed sequence tags (SSRs)) or a hybrid that uses both methods using enzymes following selective amplification using PCR (e.g., Amplified fragment length polymorphism) (Kumar et al., 2009).The first DNA markers used were fragments that were produced by restriction digestion – the restriction fragment length polymorphism (RFLP) based DNA marker. After that several markers were developed (Kumar et al., 2009).Let's discuss them one by one:

- **Restriction Fragment Length Polymorphism (RFLP):** The RFLP (Restriction Fragment Length Polymorphism) method uses DNA cleavage to examine patterns and distinguish between different organisms. When DNA is digested by a certain restriction endonuclease in two organisms, the lengths of the resultant fragments will change depending on how far apart the cleavage sites are in each organism (Kumar et al., 2009). To differentiate species or even strains from one another the similarity of the patterns can be used. Restriction endonuclease is a special class in which the RFLP technique is used (Kumar et al., 2009). The RFLP have their own origin in DNA fragments that have occurred due to point mutations within restriction enzyme recognition site sequences, evolutionary processes, deletion, or insertion in the fragments or crossing over unequally. The size of fragments or size fractionation is accomplished by gel electrophorese and after that southern blotting by transferring it to other membranes, the fragments are then identified by the hybridization with radioactively labelled probes. The different sizes of fragments that are produced are mainly due to the testing of different individuals. In the RFLP analysis method the genomic DNA that is restriction enzyme digested is firstly resolved by using gel electrophoresis and then by the southern blotting method, it is blotted on the nitrocellulose membrane. The specific band patterns then produced are visualized using the radioactively labelled probes or with alternative non-radioactive stains such as fluorescein or digoxigenin. The probes used are mostly species-specific single locus probes, that are obtained from a genomic library or cDNA library (Kumar et al., 2009).RFLPs have high genomic abundance due to the presence of ample amounts of different restriction enzymes and random distribution throughout the genome that makes it suitable for studies in gene mapping. RFLPs are applied in phylogenetic and diversity studies that range from individuals within populations or species to closely related species (Kumar et al., 2009).

  - **Advantages:** Restriction fragment length polymorphism was used for the first time in the construction of genetic maps by Botstein et al. (1980). Restriction fragment length polymorphism is generally found to be polymorphic. Restriction fragment length polymorphism has high reproducibility and shows codominant alleles. Restriction fragment length polymorphism markers are reliable in breeding and linkage analysis. It can easily define whether the linkage trait is heterozygous or in a homozygous state in an individual, this information is useful for recessive traits (Kumaret al., 2009).

  - **Disadvantages:** Restriction fragment length polymorphism has been hindered by the large quantities of high molecular weight DNA that are required for DNA digestion and southern blotting. The requirements of radioactive labelled probes make the analysis in Restriction fragment length polymorphism expensive and hazardous. The Restriction fragment length polymorphism assay is labour

intensive and time-consuming. In the Restriction fragment length polymorphism, one of the makers from many is polymorphic, which is highly inconvenient especially in the crosses for the species that are closely related. The inability of the Restriction fragment length polymorphism to detect single base changes restricts its use in different tests like the detection of point mutations that occurs within the which are already detecting polymorphism (Kumar et al., 2009).

- **Random Amplified Polymorphic DNA (RAPD):** Random amplified polymorphic DNA is a PCR (Polymerase chain reaction) bases technology. In 1991 Welsh and McClelland developed a PCR-based genetic assay Known as Random amplified polymorphic DNA (RAPD). In Random amplified polymorphic DNA by using a single primer of arbitrary nucleotide sequence it detects nucleotide sequence polymorphisms in DNA. In this reaction at two different sites of the complementary strands of the DNA template, a single piece of primer anneals to the genomic DNA (Kumar et al., 2009). If the primer sites are present at a site that can be amplified a discrete DNA product is formed through the thermos cyclic amplification process. Random amplified polymorphic DNA assay is useful for efficient screening of nucleotide sequence polymorphism between individuals as each primer amplification of several discrete loci in the Genome. Due to the stochastic nature of DNA amplification with random sequence primers in the assay its necessary to maintain consistent reaction conditionsfor the DNA amplification. Random amplified polymorphic DNA is the DNA fragments that are amplified by using short synthetic primers of random sequences by the Polymerase chain reaction. The reverse and forward primers in Polymerase chain reaction are oligonucleotides that can amplify 1 to 10 genomic sites one after another. The amplified products after the Polymerase chain reaction are separated on agarose gels by electrophoresis method in the presence of ethidium bromide and then observed under the ultraviolet light where the bands are observed if they are present or not. The polymorphism in Random amplified polymorphic DNA is considered due to the variation in the sites of primer annealing, these can also be generated by length differences in the amplified sequence between the sites of the primer annealing (Kumar et al., 2009). The product obtained is derived from a region of the genome that has two short segments in inverted orientation, that are present on opposite strands and are complimentary to the primer.RAPD has been used for many purposes from studies of individuals to studies of different closely related species (Kumar et al., 2009).

  - **Advantages:** RAPD requires a small amount of DNA and is less expensive and much easier to work with, Because the PCR is involved low amount of DNA is sufficient. In Random amplified polymorphic DNA, the primers that are used are commercially available, so the sequencing of the primer construction is not needed. Random amplified polymorphic DNA hasa high abundance of genomic material. They do not require blotting or hybridization. They do not require radioactive probes. Random amplified polymorphic DNA assays have been used as an efficient tool in agronomically important traits for the identification of the markers that are introduced during the development of near-isogenic lines (Kumar et al., 2009).

- **Disadvantages:** The main disadvantage of the Random amplified polymorphic DNA is that they have low reproducibility and because of these high standards procedures are needed because of their sensitivity. Random amplified polymorphic DNA analysis requires a purified and high molecular weight DNA with precautions to avoid any contamination of DNA as short random primers are used that are to amplify the DNA fragments in a variety of organisms. Random amplified polymorphic DNA is not specific to locus,similar-sized fragments may not be homologous andband profiles cannot be interrupted in terms of loci and alleles (Kumar et al., 2009).

- **Amplified Fragment Length Polymorphism (AFLP):** Amplified fragment length polymorphism (AFLP) is essentially an intermediate between restriction fragment length polymorphism and Polymerase chain reaction. Amplified fragment length polymorphism (AFLP) is basically based on a selective amplifying a restriction fragment from a complex mixture of DNA fragments that are obtained after the digestion of the DNA by the restriction endonucleases (Kumar et al., 2009). The polymorphism is detected by using the polyacrylamide gel electrophoresis or capillary electrophoresis. Amplified fragment length polymorphism (AFLP) technique involves main four steps: (a) restriction of DNA and ligation of oligonucleotides, (b) amplification before selection. (c) Amplification after selection, (d) amplified fragments analyzed by using gel. Amplified fragment length polymorphism (AFLP) is a DNA fingerprinting that detects DNA fragments of restriction by using PCR amplification. Amplified fragment length polymorphism (AFLP) involves the restriction of DNA, which is followed by the ligation of the adapters that are complementary to the restriction sites and followed by the selection amplification by PCR of a subset of the adapted restriction fragments. Then the fragments are viewed on the polyacrylamide gel electrophoresis by fluorescence methodologies or by autoradiographic. The AFLP involves both PCR and RFLP. The banding profiles of AFLP are the result of variations in the intervening region or restriction sites (Kumar et al., 2009).

  AFLPs can be applicable in studies that involve phylogenetic studies, parentage and identification of clones and cultivars, and genetic identity of closely related species as the fingerprinting profiles generally obtained in AFLPs are highly informative. The presence of a high abundance of genomes that are randomly distributed throughout the genome makes it a highly valued and widely used technology for gene mapping studies. The AFLPs are a widely used technique by breeders to increase crop production, and improve in a variety of criteria, by using the molecular maps to undertake the MAS (marker-assisted selection) for the special characters that are desired (Kumar et al., 2009).

  - **Advantages:** AFLPs are high in genomic abundance, high reproducibility, generation of many informative bands per reaction, a wide range of applications, and no sequence of data for primer construction is required (Kumar et al., 2009). Its capability to know a high number of polymorphic markers by just a single reaction expands the use of AFLP in genetic marker technologies (Kumar et al., 2009).

- **Disadvantages:** The disadvantages of AFLPs include the requirement for high molecular weight DNA, dominance of alleles, and the non-homology of co-migrating fragments that belong to different loci. AFLPs fragments are not independent always, as this is important for the analysis of genetic relatedness. The major disadvantage of the AFLP markers is that they are dominant markers (Kumar et al., 2009).

There are some more molecular markers that are being used (Palakurthy et al., 2020)namely Single sequence repeats (SSRs): also known as variable tandem repeats; Expressed sequence tags (EST- SSRs): these are PCR-based genetic markers; Single nucleotide polymorphism (SNPs).

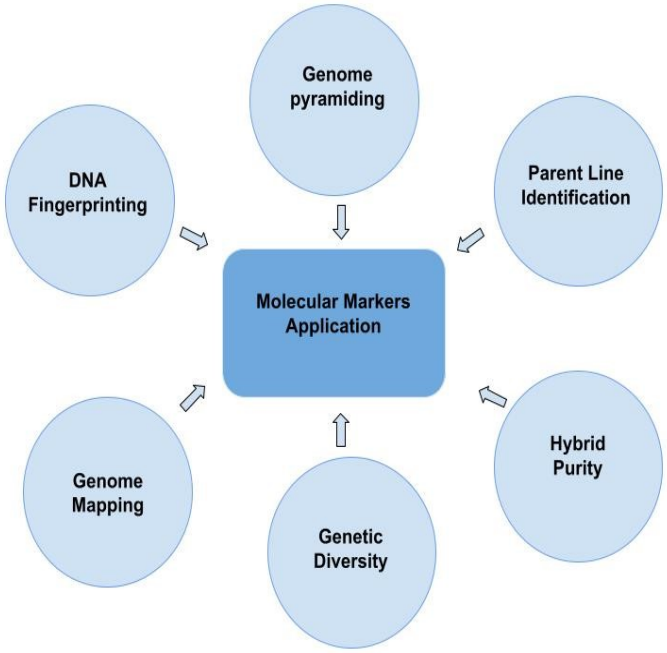

Figure 2: Applications of Molecular Markers

- **Next-generation molecular markers:** The area of plant breeding has undergone a dramatic change because of the development of next-generation molecular marker technologies. With marker-assisted approaches, genotype selection has been incorporated, ushering in the widespread use of molecular markers. Since then, the tactics for plant breeding and the parental selection process have undergone significant changes as a result of the advances in sequencing (Palakurthy et al., 2020). These technologies with high throughput technologies are collectively known as next-generation sequencing (NGS) technologies that have brought a revolution in the conventional breeding strategies into genomics-based genetic resource selection and the improvement of crops. The next-generation technologies have been used for

ddRAD sequencing, de novo sequencing, genotyping by sequencing, singlecells that are based on transcriptome and metagenome analysis, and whole genome sequencing (WGRS). The NGS technologies mainly fall under three main categories namely Single-molecule sequencing, Sequencing by synthesis, and Sequence by ligation (Palakurthy et al., 2020).

Next-generation technologies have paved the way for the conversion of non-automated version type markers like STS, RFLP, or other markers that are linked to a phenotype into markers' automatic systems.These markers are based on the presence of insertion/deletion in restriction enzymes at the recognition sites or substitution within the restriction sites. There are several new technologies or methods that are still to be found and researchers are working on it, this is an ongoing process that is improving day by day (Palakurthy et al., 2020).

- **Marker-Assisted Selection (MAS):** The molecular markers have provided the breeders with the selection of desired traits and this is known as Marker-assisted Selection (MAS). These tools enhance the breeding programs of crops. Plant breeding will be more benefited by the genomics tools through more effective quantifying, identifying, and characterizing the genetic variations from the germplasm resources, cloning, tagging, and introgression genes or QTL (quantitative trait loci) that use genetic transformation and molecular technologies to enhance the target trait, manipulating the variations of genes in the breeding process. When isozyme markers were utilized for the cultivation background to hasten the introduction of monogenic traits from foreign germplasm in the early 1980s, the creation of molecular markers in plant breeding first came to light (Tanksley and Rick 1980; Tanksley 1983). Later in the years (Beckmann and Soller, 1986a) molecular markers were introduced that were restriction fragment length polymorphism, Random amplified polymorphic DNA, amplified fragment length polymorphism, etc., which has brought crop improvement that includes theoretical issues that are related to the marker-assisted selection and backcrossing for the qualitative traits' improvement. Lande and Thompson (1990) pioneered the studies of marker-assisted selection for the traits that have triggered the studies and publications for MAS. As innovative theoretical insights have been investigated in their application within crop breeding, the Marker Assisted Back Cross (MABC) system and Marker Assisted Selection (MAS) have recently undergone optimization. Through the use of allele pyramiding techniques, these initiatives have provided paths for the combining of advantageous alleles. These investigations within MAS and MABC have provided information on a number of basic genetic features important to the improvement and development of MAS systems. The size of the genome, the size of the population, the number of markers, and the sample size have all been taken into account. The design of ideal methods for plant breeding procedures is the result of these theoretical investigations.Now the question arisesas to why Marker-assisted selection is used. The answer lies in the four broad areas that are useful to all the target crops that justify the development and use of the MAS (Young and Tanksley, 1989; Ribaut and Hoisington, 1998; Xu, 2002, 2003; Koebner, 2004; Xu et al., 2005):

  - Traits whose selection is dependent on the specific development and environment stages that influence the expression of the target phenotype.

- The speeding up of backcross breeding or the maintenance of the recessive alleles while backcrossing.
- Traits that are difficult to manage because they are expensive or time-consuming through conventional phenotypic selections.
- (Several QTLs for a single trait or multiple monogenic traits pyramiding with complex inheritance (e.g., drought tolerance or other adaptive traits).

Monsanto created the first farming method utilizing marker-assisted selection (MAS), which had its market debut in the United States in 2006. According to Fraley (2006), this was a key turning point and predictions were made that in the years to come there would be a noticeable movement towards the development of commercial crops in the United States through molecular breeding. Numerous substantial breeding programs have been established during the past 20 years in several different nations. For instance, initiatives have been started in the USA and Australia for the growing of wheat, respectively. Many additional public sector programs have adopted MAS breeding as a result of these efforts, which have been replicated throughout the world. Breeders have thereby benefited from these activities, demonstrating their fruitful results.

IV. IMPORTANCE OF MOLECULAR MAPPING AND CHARACTERIZATION OF CROPS

What is the most important thing we need at this time of our lifetime? Have you ever thought about that? if not let us discuss it now. At this point in the twenty-first century, the population is increasing which is leading to the construction of new buildings, roads, schools, hospitals, etc. to provide facilities to humans. But the most important thing is food that we get from plants, crops, and trees. The land that left is mostly unfertile because of that the production of grains, vegetables, and fruits is getting less to solve that biotechnology is what helped and now there are several techniques and tools are being used to increase the growth as well as the nutrient of the food we consume. Now molecular mapping and characterization is helping to enhance the agriculture sector more.

As stated above molecular mapping helps in the genome by using genetic mapping methods that further help in identifying the specific location of the desired gene that is needed for agriculture biotechnology applications.

The process of molecular characterization entails assessing the distinctive characteristics of the genetic makeup of a particular plant. Understanding the molecular details of the inserted DNA within the plant's genome is aided by this approach. It includes looking into where the genetic material was inserted and how it was expressed afterward in the plant's genome. For successful gene insertion at the appropriate position in the plant's genetic structure, it is essential to understand the molecular-level interactions and precisely localize the gene of interest within the genome.Molecular markers for trait selection have numerous advantages. In this DNA markers play an important role in crop improvement in terms of productivity, nutrition, and quality. The main advantages of molecular markers that make it the best method for the crop are (Collard and Mackill, 2007; Mackill, 2007):

- **Time-saving:** The proper genetic crosses by the DNA marker can be done on time before pollination
- **Consistency:** The DNA marker is neutral to environmental variations
- **Biosafety:** molecular markers facilitate the introgression of traits in the crops in advance of disease.
- **Efficiency:** DNA markers allow early breeders to select the progenies to improve the genetic quality
- More accurate selection of complex traits: polygenic traits are difficult to select do DNA markers linked to QTLs allow them to be a single factor.

Each gene that contributes to the quantitative traits has little impact on the environment. It is challenging to study quantitative qualities because it is commonly accepted that their heritability is low. The genetic foundation of quantitative traits has been substantially facilitated by the development of genome mapping, molecular markers, and quantitative trait loci analysis tools, though.These methods are generally focused on enhancing staple foods like Grains (Rice, wheat, Maize, etc.), Vegetables &and fruits, along with cash (commercial) crops (jute, cotton, etc.). The focus area is their good production, better quality, and growth even in harsh environmental conditions (high or low temperatures, etc.).

Here we will discuss three main staple foods asthey are a necessity for the world. We will discuss about three main crops.Maize, rice, and wheat are the most widely grown in all parts of the world. Out of these three staple crops rice is the most important that is consumed and grown in low-income countries (Pandey et al., 2010). Let’s discuss in detail about them.

1. **Rice:** Rice (Oryza sativa)is the second most consumed food and is in great demand. Most of the production of the rice comes from Asia with a great consumption rate. Rice is a pack of nutrients, vitamins, and minerals such as iron, potassium, zinc, and starch that make it versatile, but itloses its nutrients and minerals during the milling process. With the increase in the demand to make rice sufficient to feed the growing population from past decades, many advances have been made and now with the help of molecular and cellular level advances in recent years in agriculture biotechnology, the production and quality both have been increased with good results (Jenaet al., 2008).

   These advances i.e.,
   - Cultivation through anther and pollen culture,
   - Success in genetic transformation,
   - Mapping the genome of rice and map-based gene cloning,
   - Chloroplast and mitochondrial genetic maps,
   - Complete genome sequencing,
   - Development of BAC and YAC libraries etc. Several other advances in biotic and abiotic stress played a major role in the increase of the productivity of rice in modern cultivation (International Rice Genome Sequencing Project, 2005).Rice is also adopted to study plant molecular science as a modal system for the development of its own and for other crops (Zhang et al., 2008; Han et al., 2008; Jung et al., 2008).

The reasons why rice is chosen as modal are:

- Among the other crops it has the smallest genome.
- Genetic stocks and germ plasm are abundant.
- It shares substantial collinearity with grass families such as other cereals.
- It has a high-precision sequence of genome.
- saturated molecular markers maps that enable mapping easier.
- Large-scale breeding programs.

Molecular mapping of the rice genome has identified several traits of the gene that are economically important as well as for the enrichment of the rice. Several QTLs (Quantitative trait loci) have been identified with several abiotic factors such as salinity, cold, and drought. This has paved the way for marker-assisted selection and Marker-assisted backcross in the rice genome for its improvement in breeding. Molecular biotechnology with the mapping and characterization methods provides scientists to further improve the crop.

Some marker-assisted selection for Biotic and abiotic stresses are discussed below:

In rice brown planthopper (BPH) is a destructive pest that reduces its production in Asia. Qui et al., (2014)carried out genetic mapping for the resistance in 93-11/T12 F2 population for brown plant hopper and then located the Bph7 gene on the long arm of chromosome 12, between the markers of SSR i.e., RM28295 and RM313. Yuexiong et al., (2020) and Yang et al., (2020) recently mapped the Bph38 and Bph35 on chromosome 4 of rice. The studies done by them have indicated the importance of chromosome 4 of rice as it consists of QTLs mapped in large numbers for BPH resistance.

In another study for the Small brown planthopper (SBPH) resistance QTL mapping is done using the RIL (recombinant inbreed line) populations that led to the identification of three QTLs namely Qsbph3, qSBPH2, QSBPH7.1 that are located on the chromosomes 3, 2 and 7 respectively (Wang et al., 2013).A very good example of the use of genetic technology in rice development is the production of golden rice which has been known as the genetically modified crop in which specific genes were selected and modified to make the rice grain rich in vitamin A. This golden rice is in the market but not in many areas, but it’s the first modification for the rice that has enhanced the quality of the rice by enriching it with vitamins. There are many advances that have been done till now that concern the production of rice in extra submergence of the rice seedling, in temperature that is not that favourable for the growth, and high yielding of the rice so that it can be available to the world.

2. **Wheat:** Wheat (Triticum aestivum) is the second largest crop grown worldwide, with increasing production and land allocation.the durum species of wheat is the one that is grown and consumed in great quantity in most parts of the world (Arriagada et al., 2020). Wheat is an important part of the culture worldwide, as bread is an important part of the

diet. To meet its future demand and consumption an increase in grain yield is necessary. The strategies that will allow this objective to be achievedand to have a production that is sustainable over time is through the development and release of a new variety of wheat with improved yieldunder different environmental conditions. Therefore, the development of a wheat crop with a high yield has become the major objective for plant breeders worldwide (Mengistu et al., 2016). For the genetic improvement the simultaneous selection based on grain yield-related trait approach is adopted including quantitative trait loci (QTL) are being applied to increase the wheat yield through marker-assisted selection (MAS). There are multiple QTLs with small and large effects that have been mapped on all wheat chromosomes (Arriagada et al., 2020). Now let's discuss about few applications of the mapping and characterizations on the molecular level of wheat:

There are many diseases that occur in the growing stage and even after the complete life cycle of the grain. One of them is Fusarium head blight (FHB), which is caused by Fusarium gramineraum, it is a destructive disease of wheat and barley that happens in humid and warm regions worldwide. It was first reported in China in the 1930s and is then became severe and spread worldwide. Fusarium head blight (FHB) reduces the yield of the grain greatly and significantly it lowers the quality of grain. The FHB resistance is controlled by the genes on chromosomes 4A, 5A, 7A, 7B, 4D, and 2D which is based on the monosomic analysis that was reported in the Liao et al. study. It has also been reported that Sumai 3, a Chinese FHB resistance, has a QTL on chromosome 3BS. That QTL was also placed on the map in the map interval Xgwm533.1 to Xgwm493 and has explained phenotypic variation for the FHB resistance of about 42% in Sumai 3, and then it was designated as Qfhs.ndsu-3BS. Also, it was reported by Shen et al. (2003) that QTL in Ning 894037 also has the same genomic region i.e., on 3BS. The FHB resistance in chromosome 3BS has been identified and can be used to improve wheat resistance by gene pyramiding. Two other small QTLs in chromosomes 1R and 3B are new andhave been identified. These are the data from the studies that have been done on the FHB disease. The Marker-assisted selection (MAS) for the FHB resistance can also be performed using the closed linked markers, SSR markers that are breeder friendly. AFLP-SSR integrated map can be used to identify the marker-associated traits of the crops such as heading date and plant height and can be used for the marker-assisted selection that can break the undesired associations between other agronomic traits and FHB resistance (Zang et al., 2004).

There is a pest Sunn Pest (Eurygaster integriceps) that infects wheat and is a serious concern worldwide. The mapping studies with 90K SNP iSelect assay and gene-based KASP markers in two different or separate populations from Cham6 x IG139431 and Cham6 x IG1 39883, respectively that has led to the identification of major QTL that has resistance to Sunn pest, they are Ei1 on chromosome 4BS. This QTL has been mapped on chromosome 4B that was between the markers, IWB66138 and BS00022785 (Emebiri et al., 2016). These weremapped by using molecular marker technologies that made it possible to identify the specific genes. These two examples were for the biotic stresses that occur in wheat crops. The time for the swing of wheat till the time of its harvest faces differences in the temperatures and in some cases, drought can also be a cause for the decreased yield of wheat so there are studies that have worked on the drought resistance gene of the wheat to overcome the disaster, including other agronomical or environmental unfavourable conditions (Prasanna, 2012).

3. **Maize:** As a versatile resource for numerous industrial goods as well as basic food, maize, scientifically known as Zea mays, is significant on a global scale. In addition to its usefulness, maize is unique as a genetic model organism famous for its extensive genetic diversity. Histories show that Mexico was where it first became domesticated. Numerous studies carried out in Mexico and other countries highlight the wide genetic variety found in maize germplasms. The use of molecular markers in maize research has created new opportunities for understanding and appreciating the genetic variety of the crop on a worldwide scale, including the numerous varieties that flourish all over the world. The high throughput genotyping technologies and next-generation sequencing can be used to understand the genetic diversity and design strategies for the improvement of maize. Recent studies (Messing et al., 2004)show that maize contains about 59,000 genes, that account for 7.5% of the genome. About 58% of the genome is composed of all the types of repeat elements, DNA transposons, and retroelements. The remaining 34.5% is occupied by the unknown sequences that are present in the space of known identifiable coding regions and repeat elements. Molecular characterization of 770 maize inbred lines with the 1034 SNP markers has been done, which has led to the identification of 446 high-quality markers, which have no germplasm and repeatability of specific biasing effects. The use of SNP haplotypes combinedly may be more beneficial to use rather than using only SNP alleles in the analysis of diversity (Prasanna, 2012).

   The new in silico tools and sequencing technologies has now empowered the extensive opportunities for maize to speed up its research in large-scale, high-resolution QTL mapping, linkage map construction, and genome studies in a wide range of association. In recent years the genome sequencing of B73 (US corn belt inbred) and Palomero, a popcorn landrace in Mexico have set a landmark in the research of maize, that has a significant application in understanding of the maize genome evolution and organization (Prasanna, 2012).

   The pest Rhopalosiphum maidis (maize leaf aphid) is a destructive pest that is affecting the production of maize globally. An RIL mapping population was developed by the crossing of CML322 and B73, for the leaf aphid resistance to map QTLs. This study was also useful for the identification of HDMBOAGIc on chromosome 1 between the markers, PZA03189.4 and PMH5098.25 (Meihls et al., 2013). Along with this on 6 different chromosomes a total of 15 QTLs for the maize have been mapped that exhibit a range between 14% and 51% of phenotypic variation, and these QTLs can be utilized for the improvement of the maize through marker-assisted selection technology (Castro-Álvarez et al., 2015).Another pest Mediterranean corn borer (MCB), Sesamianon agrioides, is a cause for maize-reducing production in Mediterranean countries. Six QTLs have been mapped for the resistance against the MCB on chromosomes 5, 8, 9, and 10 in A637 xA509-based RILs. For the western corn rootworm, a double haploid (DH) population developed by the crossing of UR2 with Mo47 was used for mapping QTLs for the rootworm (Jiménez-Galindo et al., (2017).

   Recently,(Brkićet al., 2020) mapped major four QTLs for root regrowth, root size, and root damage on chromosomes 1 and 6 using maize IBM intermated RILs. These QTLs co-locate with the genomic regions that govern plant defense against herbivores. Like this, there is research that is still in process that is strictly focusing on improving the quality and production fighting against all the biotic and abiotic stresses.

V. FUTURE ASPECTS

The current focus should be on creating crop varieties with the best genotypic combinations and a wide spectrum of disease and insect pest resistance. More research should be done on the impact of microbiomes: fungi, bacteria, foraminifera, endophytes, etc. on crop resistance. The ongoing developments in plant biotechnology and genetics provide an exciting way to control the pest population in the environment in an environment-friendly manner. The presence of multiple gene stacking has increased protection against the organisms that are harmful and has reduced the risk of the emergence of new resistance for herbivores and added durability advantages. The use of genetic breeding technologies has offered a high potential to design a crop that ispest-resistant. Genetic modification technologies are coevolving with institutions, accumulating knowledge, and policy regimes around the world. Future research should be focused on the evolution in a continuous manner of the genetic modification technologies including the Marker-assisted selection and Marker-assisted backcross technologies. The understanding of the impact of genetic modification technology on land use changes, farm structure, agriculture productivity, commodity prices, and the needs of the environment should be done in a more accurate form. The future of genetically modified crops in the agricultural production system in developing and developed economies with an effective integration will be dependent on effective and transparent communications about the strategies that inform the public and their understanding of the role of technologies or more specifically the genetic technologies in the agricultural productions (Bennett et al., 2013).

VI. CONCLUSION

Recent improvements in the breeding of the three major crops: rice, maize, and wheat have improved crop quality, resilience to biotic and abiotic stressors, and yield. The improvement and enhancement of crops have been made possible in numerous ways thanks to molecular characterization, mapping, and marker-assisted selection. The genotype that has resulted from the transformation can be characterized at a molecular level using the inserted DNA analysis. The technology of molecular markers has played an important role in understanding the genome of the plant that has to be manipulated in the form of insertion, deletion, duplication, etc. and the maps that have been produced by these methods have made this possible to study in detail about the plant genome and to select the desired DNA and to use it in the breeding of the plants. the recent studies have provided many applications of the molecular markers in the improvement of crops in ways such as quality, and quantity. The resistance of the crops toward biotic stress such as pests, insects, nematodes, fungi, bacteria, and viroid, and the abiotic stresses such as drought, submersion, high or cold temperatures, pH of the soil, etc. have made it possible for the breeders to grow crops even in unfavourable conditions with a good result. The crops that are genetically modified do have restrictions from a group of society that are against it that claims that the changes in the plants at the molecular level may be a threat towards the species of plant and may be harmful to the environment as well as for the human beings and animals who consume the crops (Bennett et al., 2013). The debate on this is still going on it whether it’s good or bad. But from the researcher’s view and the safety measures that have been taken while preparing for the crop’s molecular changes, molecular mapping and characterization play a great role during this process. As molecular maps provide a map of the genome and molecular characterization works on that simultaneously by characterizing the gene that is inserted in the plant how it is

expressed or if there are any changes that occur other than the desired one during the process. Gives us a better idea and helps to plan and work in a better way without interrupting the other parts of the genome and seeking every risk and safety assessment.

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