Molecular Mapping and Molecular Characterization of Crops

Sristhi Yadav[1], Deep Nithun Senathipathi[2]*, Jyoti Barthawal[1], Vasudha Kak[3], Basista Rabina Sharma[3], Gururaj Moorthy[2], Mruganxi Sharma[2].

[1]Department of Biotechnology, School of Basic and Applied Sciences, Shri Guru Ram Rai University, Dehradun, Uttarakhand, 248001, India.
[2]International Centre of Excellence in Seafood Science and Innovation, Faculty of Agro-Industry, Prince of Songkla University, Hat Yai, Songkhla, 90110, Thailand.
[3]Sharda School of Allied Health Sciences, Sharda University, Greater Noida, Uttar Pradesh 201310, India.

Corresponding author: deepnithunsenathipathi@gmail.com

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.

1. 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 its known that it's about the genome of a plant. As most of the traits of the 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 the crop improvement such as its production, resistance to biotic and abiotic stresses (drought, pest, insects etc). Now the 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 plant simply defines the characteristics of the gene of interest that has been introduced 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 idea about the topic.

2. 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 done namely Genetic mapping and Physical mapping (Brown *et al.,* 2002). Both methods guide toward the location of a gene on 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 amount 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).

3. Molecular characterization

A method called "molecular characterization" attempt 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 in 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 plant's genome as a result of 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 characterisation comes in. Essentially, molecular characterisation 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 characterisation. 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 into 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 safety and gain a deeper understanding of plant behaviour. (OECD, 2010).

3.1 Approaches for understanding the function of a gene

Before catching up to the main subject of the discussion its necessary to understand the functioning of a gene. Two approaches are there that are used to understand the functions of gene i.e., Forward, and Reverse genetics. Forward genetics start with a well characterized

phenotype, example resistance of a gene in plant and the identifying the responsible gene for that phenotype (Wasim *et al*., 2018). While Reverse genetics first begins with 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. The Mapping is the base for the further characterization of the crops genome to study its expression and marker play important role in mapping that will be discussed in this chapter (Wasim *et al.,* 2018).

3.2 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 in 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 organised inside a genome.

Genetic Mapping: Genetic maps show the specific site of distinctive features that is achieved by generating the information about the presence or absence of molecular markers. Previously genes were used as the markers to identify specific site (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 at molecular and cellular areas, that has provided a powerful weapon with precise selection of desired genotype in a genome of a plant (Phillips and Vasil 2001; Gupta and Varshney 2004). Molecular markers and high-density mapping of genome is available in a variety that has broadened the methods in field of conventional breeding of plants with complex and desired traits. Molecular marker with a DNA sequence with known position on

chromosome or a map that may or may not be linked with the expression of a gene, can be easily distinguish between the two close related individuals (Schulmann 2007; Henry 2013). This is used in a variety of applications example in 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 has been discussed below (Jonah *et al*. 2011; Khan 2015):

a) The quantity of DNA sample or tissues required for analysis should be in less amount.
b) The marker should be selectively neutral to environment conditions.
c) Highly reproducible marker that can provide data exchange between laboratories.
d) Marker should be polymorphic.
e) Throughout the genome the inheritance pattern should be even.
f) Sequence based genome information is not prerequisite.
g) The markers should be easily assessed that shows strong heritability from one generation to another.
h) The marker should be in expensive, quick to assess and simple.

The properties are the basic requirement that should be present in the markers. Now the question rises 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).

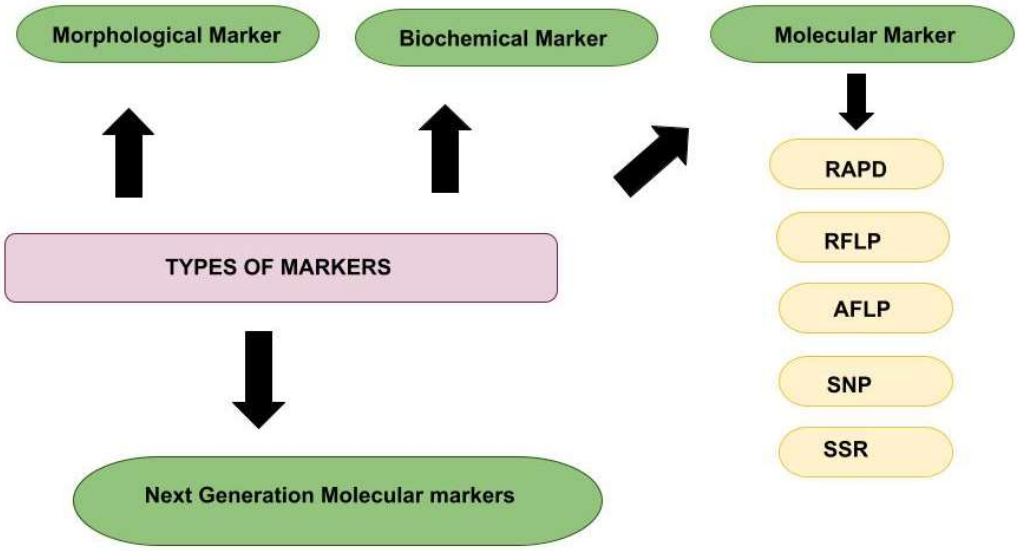

Figure 1: Types of markers

3.2.1 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). The use of biochemical markers as a genetic marker for genetic diversity 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 were the biochemical or isozyme markers. Isozyme markers are described as structurally distinct enzyme molecules with qualitatively identical catalytic activities. These are originated 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 for careful technology or application optimization (Kumar *et al*., 2009).

Disadvantages: There are number of advantages as well as the disadvantages for using isoenzyme markers. There are only 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).

3.2.2 Morphological Markers

Morphological markers have qualitative traits that are less in number. The morphological markers can be scored visually without 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 pioneer work in plants, these were based on complex qualitative traits that can be selected based on segregation of the traits with the simple mendelian inheritance. The morphological markers mostly appear on the late stage of their development (example colour of flower, seed coat colour), that makes it difficult to score it early (Palakurthy *et al.,* 2020) beside this the large areas of filed 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 programmes, 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).

3.2.3 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 forms a basis that design strategy to exploit for applied purposes. A marker must be a polymorphic i.e., it must be in different forms that will make it easy to distinguish it from the chromosome with normal gene as the marker will be carrying a mutant gene. The simultaneous occurrence of a trait in same population of two different variants or genotype is termed as 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 environment 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 amplifications 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 has been developed (Kumar *et al.,* 2009). Let's discuss them one by one:

3.2.3.1 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 on which the RFLP technique is used (Kumar *et al.,* 2009). The RFLP have their own origin in DNA fragments that has 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 membrane, the fragments are then identified by the hybridization with radioactive 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 genomic library or cDNA library (Kumar *et al.,* 2009). RFLPs have high genomic abundance due to the presence of ample amount of different restriction enzymes and random distribution throughout the genome that makes it suitable for the studies in gene mapping. RFLPs applied in phylogenetic and diversity studies that range from individuals within populations or species, to close related species (Kumar *et al.,* 2009).

Advantages: Restriction fragment length polymorphism were used for the first time in construction of genetic maps by Botstein *et al*. (1980). Restriction fragment length polymorphism are generally found to be polymorphic. Restriction fragment length polymorphism have high reproducibility and shows codominant alleles. Restriction fragment length polymorphism markers are reliable in breeding and linkage analysis. It can easily define the linkage trait is heterozygous or in homozygous state in individual, this information is useful for recessive traits (Kumar *et 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, that is highly inconvenient specially in the crosses for the species that are closely related. The inability of the Restriction fragment length polymorphism to detect single base changes restrict its use in different tests like detection of point mutations that occurs within the which are already detecting polymorphism (Kumar *et al.,* 2009).

3.2.3.2 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 method is based on random (Kumar *et al.,* 2009). 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 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 does amplification of several discrete loci in the Genome. Due to the stoichastic nature of DNA amplification with random sequence primers in the assay its necessary to maintain a consistent reaction conditions for the DNA amplification. Random amplified polymorphic DNA are 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 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 difference in the amplified sequence between the sites if the primer annealing (Kumar *et al*., 2009). The product obtained is derived from a region of 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 small amount of DNA and are 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 in it. Random amplified polymorphic DNA have high abundance of genomic material. They do not require blotting or hybridization. They do not require radioactive probes. Random amplified polymorphic DNA assay have been used as an efficient tool in agronomically important traits for the

identification of the markers that are introgressed during 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 and 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 and band profiles cannot be interrupted in terms of loci and alleles (Kumar *et al*., 2009).

3.2.3.3 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 analysed by using gel. Amplified fragment length polymorphism (AFLP) is a like DNA fingerprinting that detects DNA fragments of restriction by using PCR amplification. Amplified fragment length polymorphism (AFLP) involves the restriction of DNA, that 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 involve both PCR and RFLP. The banding profiles of AFLP are result of the variations in the intervening region or restriction sites (Kumar *et al*., 2009).

AFLPs can be applicable in studies that involves phylogenetic studies, parentage and identification of clones and cultivars, genetic identity of the closely related species as the fingerprinting profiles generally obtained in AFLPs are highly informative. The presence of high abundance of genome that are randomly distributed throughout the genome makes it

highly valued and widely used technology for the gene mapping studies. The AFLPs are widely used technique by the breeders to increase crop production, improvement in 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 are required (Kumar *et al*., 2009). Due to its capability to know a high number of polymorphic markers by just a single reaction expand the use of AFLP in the genetic marker technologies (Kumar *et al*., 2009).

Disadvantages: The disadvantages in AFLPs include the requirement for the high molecular weight DNA, dominance of alleles, and the non-homology of co migrating fragments that belongs 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 tag (EST- SSRs): these are PCR based genetic markers; Single nucleotide polymorphism (SNPs).

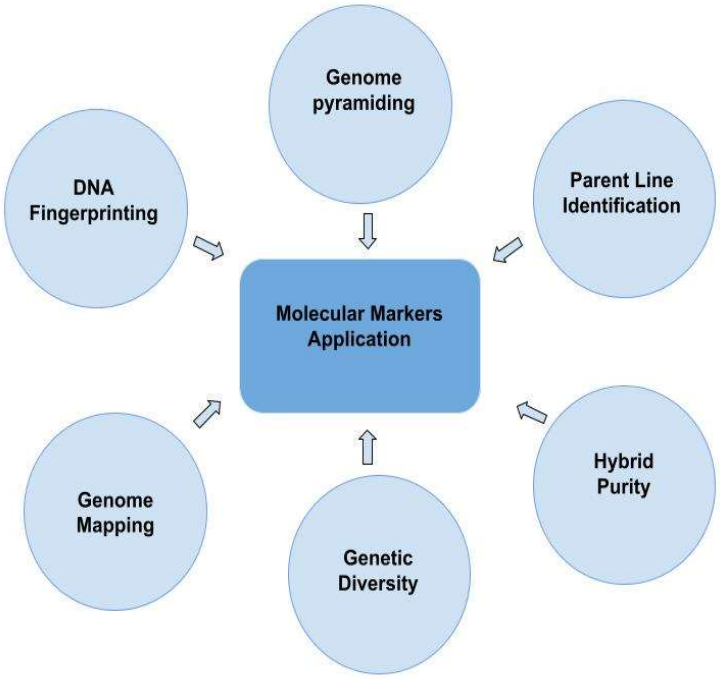

Figure 2: Applications of Molecular Markers

3.2.4 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 collectively known as next generation sequencing (NGS) technologies that have brought revolution in the conventional breeding strategies into genomics based genetic resources selection and the improvement of crops. The next-generation technologies have been used for ddRAD sequencing, de novo sequencing, genotyping by sequencing, singe cells that are based on transcriptome and metagenome analysis, 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 a way for conversion of non-automated version type marker 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 technology or method that are still to be find and researchers are working on it, this is an ongoing process that is improving day by day (Palakurthy *et al.,* 2020).

3.2.5 Marker Assisted Selection (MAS)

The molecular markers have provided the breeders the selection of desired traits and this is known as Marker assisted Selection (MAS). These tools enhance the breeding programs of crops. The 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 introgressing 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 utilised 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 years (Beckmann and Soller, 1986a) the molecular markers were introduced that were restriction fragment length polymorphism, Random amplified polymorphic DNA, amplified fragment length polymorphism etc, that has brought crop improvement that include theoretical issues that are related to the marker assisted selection and back crossing for the qualitative traits' improvement. Lande and Thompson (1990) pioneered the studies of the marker assisted selection for the traits that has 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 optimisation. 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 arise why the Marker assisted selection is used? The answer lies in the four broad areas of that are useful to all the target

crops that justifies the development and use of the MAS (Young and Tanksley, 1989; Ribaut and Hoisington, 1998; Xu, 2002, 2003; Koebner, 2004; Xu et al., 2005):

(a) Traits whose selection is dependent on the specific development and environment stages that influence the expression of the target phenotype.
(b) The speeding up of backcross breeding or the maintenance of the recessive alleles while backcrossing.
(c) Traits that are difficult to manage because they are expensive or time consuming through conventional phenotypic selections.
(d) Several QTL 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 utilising 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.

4. 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 twenty-first century, population is increasing which is leading to construction new buildings, roads, schools, hospitals, etc. to provide facilities to humans. But what the most important thing is food and that we get from plants, crops, and trees. The land what left is mostly unfertile because of that the production of grains, vegetables, fruits is getting less and 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 the molecular mapping and characterization is helping to enhance the agriculture sector more.

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

The process of molecular characterisation 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 localise the gene of interest within the genome. Molecular markers for trait selection have numerous advantages. In this DNA markers play important role in crop improvement in terms of productivity, nutrition, and quality. The main advantages of molecular markers that makes it best method for the crop are (Collard and Mackill, 2007; Mackill, 2007):

(a) **Time saving**: the proper genetic crosses by the DNA marker can be done on time before pollination

(b) **Consistency**: the DNA marker is neutral to environment variations

(c) **Biosafety**: molecular markers facilitate the introgression of traits in the crops in advance of disease.

(d) **Efficiency**: DNA markers allow early breeders to select the progenies to improve the genetic quality

(e) **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 a 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 marker, and quantitative trait loci analysis tools, though. These methods are generally focused on enhancing the staple food like Grains (Rice, wheat, Maize etc.), Vegetables & fruits, along with cash (commercial) crops (jute, cotton etc.). The focus area is their good production, better quality, growth even in harsh environmental conditions (high or low temperatures etc.).

Here we will discuss about three main staple food as they 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 world. Out of these three staple crops rice is most important that is consumed and grown in low-income countries (Pandey *et al.,* 2010). Let's discuss in detail about them.

4.1 Rice

Rice (Oryza sativa) is second most consumed food and in a great demand. Most of the production of the rice comes from Asia with great consumption rate. Rice is a pack of nutrient, vitamins, and minerals such as iron, potassium, zinc, starch that makes it versatile, but its losses 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 done and now with the help of molecular and cellular level advances in the recent years in agriculture biotechnology the production and quality both has been increased with good results (Jena *et al.,* 2008).

These advances i.e., (i)cultivation through anther and pollen culture, (ii) success in genetic transformation, (iii) Mapping the genome of rice and map-based gene cloning, (iv) Chloroplast and mitochondrial genetic maps, (v) Complete genome sequencing, (vi) development of BAC and YAC libraries etc. Several other advances in biotic and abiotic stress played 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 the plant molecular science as a modal system for the development of its own and for the other crops (Zhang *et al*., 2008; Han *et al*., 2008; Jung *et al*., 2008).

The reason why rice is chosen as modal are:

(i) among the other crops it has smallest genome.
(ii) genetic stocks and germplasm are abundant.
(iii) it shares substantial collinearity with grass family such as other cereals.
(iv) it has high precision sequence of genome.
(v) saturated molecular markers maps that enable mapping easier.
(vi) large scale breeding programmes.

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 several abiotic factors such as salinity, cold and drought. This

has paved way for the Marker Assisted selecti0on and Marker assisted backcross in rice genome for its improvement in breeding. The 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 reduce 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 Bph7 gene on the long arm of the 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 indicates the importance of the chromosome 4 of rice as it consists of QTLs mapped in large number for the BPH resistance.

In other 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 for the use of genetic technology in rice development is the production of golden rice that has been known as the genetic modified crop in which specific genes were selected and modified to make the rice grain rich in vitamin A. this golden rice is in market but not in much areas, but it's the first modification for the rice that has enhanced the quality of the rice by enriching it with vitamin. There are many advances have been done till now that concerns 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.

4.2 Wheat

Wheat (*Triticum aestivum*) is the second largest crop grown worldwide, with the increasing production and land allocation. the durum species of the wheat is the one that is grown and consumed in great quantity in most parts of the world (Arriagada *et al*., 2020). Wheat is the important part of the culture worldwide, as bread is an important part of diet. To meet its future demand and consumption an increase in grain yield is necessary. The strategies that will allow this objective to achieve and to have a production that is sustainable over time is through the development and release of new variety of wheat with improved yield under different environmental conditions. Therefore, the development of the wheat crop with a high yield has become the major objective for the 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 the 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), that 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 1930s and is then become 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 controlled by the genes on chromosomes 4A, 5A, 7A, 7B, 4D and 2D that is based on the monosomic analysis this was reported in Liao *et al*. study. It has also been reported that Sumai 3, a Chinese FHB resistance, has a QTL on the 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 have the same genomic region i.e., on 3BS. The FHB resistance in the chromosome 3BS has been identified that can be used to improve wheat resistance by gene pyramiding. Two other small QTLs in chromosome 1R and 3B are new that has been identified. These are the data from the studies that have been done for the FHB disease. The Marker assisted selection (MAS) for the FHB resistance can also be performed using the closed linked markers, SSR markers that is breeder friendly. AFLP-SSR integrated map can be used to identify the marker associated traits of the crops such as heading date and lant 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 infect 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 the chromosome 4B that was between the markers, IWB66138 and BS00022785 (Emebiri *et al.*, 2016). These was

mapped by using the molecular marker technologies that made it possible to identify the specific genes. These two examples were for the biotic stresses that occur in wheat crop. The time for the swing of wheat till the time of its harvest faces difference in the temperatures and in some cases, drought can also be a cause for the decrease 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).

4.3 Maize

As a versatile resource for numerous industrial goods as well as a 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 designing strategies for the improvement of maize. Recent studies (Messing *et al.,* 2004) shows that maize contains about 59000genes, that accounts for 7.5% of the genome. About 58% of the genome that is composed of all the types of the repeat elements, DNA transposons and retroelement. 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 have been done, that 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 the only SNP alleles in 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 the recent years the genome sequencing of B73 (US corn belt inbred) and Palomero, a popcorn landrace in Mexico have set landmark in the research of maize, that has a significant

application in understanding of the maize genome evolution and organization (Prasanna, 2012).

A pest *Rhopalosiphum maidis* (maize leaf aphid) is a destructive pest that is affecting the production of maize globally. A RIL mapping population 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 the 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 cause for maize reducing production in Mediterranean countries. Six QTLs have been mapped for the resistance against the MCB on the chromosomes 5, 8, 9, and 10 in A637 xA509 based RILs. For the western corn rootworm, double haploid (DH) population developed by the crossing of UR2 with Mo47 that was used for mapping QTLs for the rootworm (Jiménez-Galindo *et al.,* (2017).

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

5. 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 the plant biotechnology and genetics provide an exciting way to control the pest population in the environment in an environment friendly manner. The presence of the multiple gene stacking has increased protection against the organisms that are harmful and has reduced the risk of emergence of new resistance for herbivore and added durability advantages. The use of the genetic breeding technologies has offered a high potential to design a crop that are pest resistance. The genetic modification technologies are coevolving with the institutions, accumulating knowledge, and policy

regimes around the world. The 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 the genetic modification technology on the land use changes, farm structure, agriculture productivity, commodity prices, and the need of the environment should be done in more accurate form. The future of the genetically modified crops in the agricultural production system in developing and developed economies with an effective integration will be dependent on the effective and transparent communications about the strategies that inform the public and their understanding the role of technologies or more specifically the genetic technologies in the agricultural productions (Bennett *et al.*, 2013).

6. 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 has been made possible in numerous ways thanks to molecular characterisation, mapping, and marker assisted selection. The genotype that has resulted from the transformation can be characterised at a molecular level using the inserted DNA analysis. The technology of molecular markers has played the 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 has 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 crop in ways such as quality, quantity. The resistance of the crops toward the 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 has made it possible for the breeders to grow crops even in unfavourable conditions with a good result. The crops that are genetically modified does 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 for 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 researchers view and the safety measures that has been taken while preparing for the crop's molecular changes the molecular mapping and characterization plays a great role during this process. As the molecular maps provides a map of the genome and the molecular

characterization work on that simultaneously by characterizing the gene that is inserted in the plant that how it is expressed or if there any changes that occurs 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 assessments.

REFERENCES

Arriagada, O., Marcotuli, I., Gadaleta, A., & Schwember, A. R. (2020). Molecular Mapping and Genomics of Grain Yield in Durum Wheat: A Review. *International journal of molecular sciences*, *21*(19), 7021.

Beckmann, J.S., and M. Soller. 1986a. Restriction fragment length polymorphisms in plant genetic improvement. Oxford Surv. Plant Mol. Cell Biol. 3:196–250.

Bennett, A. B., Chi-Ham, C., Barrows, G., Sexton, S., & Zilberman, D. (2013). Agricultural biotechnology: economics, environment, ethics, and the future. *Annual Review of Environment and Resources*, *38*, 249-279

Brkić A, Šimić D, Jambrović A, Zdunić Z, Ledenčan T, Raspudić E, Brmeţ M, Brkić J, Mazur M, Galić V (2020) QTL analysis of Western corn rootworm resistance traits in maize IBM population grown in continuous maize. Genetika 52:137–148

Brown TA. Genomes. 2nd edition. Oxford: Wiley-Liss; 2002. Chapter 5, Mapping Genomes.

Castro-Álvarez FF, William M, Bergvinson DJ, García-Lara S (2015) Genetic mapping of QTL for maize weevil resistance in a RIL population of tropical maize. Theor Appl Genet 128:411–419

Collard, B.C.Y., and D.J. Mackill. 2007. Marker-assisted selection: An approach for precision plant breeding in the twenty-& rst century. Philos. Trans. R. Soc. Lond. B Biol. Sci. 17:1–16.

Emebiri LC, Tan MK, El-Bouhssini M, Wildman O, Jighly A, Tadesse W, Ogbonnaya FC (2016) QTL mapping identifies a major locus for resistance in wheat to Sunn pest (Eurygaster integriceps) feeding at the vegetative growth stage. Theor Appl Genet 130:1–10

Fraley, R. 2006. Presentation at Monsanto European Investor Day, 10 November 2006.

Gupta PK and Varshney RK 2004 Cereal genomics: an overview (Kluwer Gupta Academic Press, Dordrecht, The Netherlands)

Han B, Zhang Q. 2008. Rice genome research: status and future perspectives. Plant Genome 1:71–76

Henry RJ 2013 Molecular markers in plants (WileyBlackwell, Ames, IA) pp 200

International Rice Genome Sequencing Project. 2005. The mapbased sequence of the rice genome. Nature 4.

Jena, K. K., & Mackill, D. J. (2008). Molecular markers and their use in marker-assisted selection in rice. *Crop science*, *48*(4), 1266-1276

Jiménez-Galindo JC, Ordás B, Butrón A, Samayoa LF, Malvar RA (2017) QTL mapping for yield and resistance against Mediterranean corn borer in maize. Front Plant Sci 8:698

Jonah PM, Bello, LL, Lucky O, Midau A and Moruppa, SM 2011 The importance of molecular markers in plant breeding programmes. Global J. Sci. Fro. Res. 11 1–9

Jung KH, And G, Ronald PC. 2008. Towards a better bowl of rice: assigning function to tens of thousands of rice genes. Nat. Rev. Genet. 9:91–10

Khan F 2015 Molecular markers: an excellent tool for genetic analysis. Mol. Biomark. Diag. 6 3

Koebner, R.M. 2004. Marker assisted selection in the cereals: The dream and the reality. p. 317–329. In P.K. Gupta and R.K. Varshney (ed.) Cereal genomics. Kluwer Academic, Dordrecht, the Netherlands.

Kumar, P., Gupta, V. K., Misra, A. K., Modi, D. R., & Pandey, B. K. (2009). Potential of molecular markers in plant biotechnology. *Plant omics*, *2*(4), 141-162.

Lande, R., and R. Thompson. 1990. Efficiency of marker-assisted selection in the improvement of quantitative traits. Genetics 124:743–756.

Liu B-H 1991 Development and prospects of dwarf male sterile wheat. Chin. Sci. Bull. 36 306

Mackill, D.J. 2007. Molecular markers and marker-assisted selection in rice. p. 147–168. In R.K. Varshney and R. Tuberosa (ed.) Genomics assisted crop improvement. Vol. 2. Genomics applications in crops. Springer, New York.

Meihls LN, Handrick V, Glauser G, Barbier H, Kaur H, Haribal MM, Lipka AE, Gershenzon J, Buckler ES, Erb M, Köllner TG (2013) Natural variation in maize aphid resistance is associated with 2, 4-dihydroxy-7-methoxy-1, 4-benzoxazin-3-one glucoside methyltransferase activity. Plant Cell 25:2341–2355

Mengistu, Dejene Kassahun, *et al*. "High-density molecular characterization and association mapping in Ethiopian durum wheat landraces reveals high diversity and potential for wheat breeding." *Plant Biotechnology Journal* 14.9 (2016): 1800-1812.

Messing J, Bharti AK, Karlowski WM, Gundlach H, Kim HR, Yu Y, Wei F, Fuks G, Soderlund CA, Mayer KF *et al*.: Sequence composition and genome organization of maize. Proc Natl Acad Sci USA 2004, 101:14349-14354.

Mondini L, Noorani A and Pagnotta MA 2009 Assessing plant genetic diversity by molecular tools. Diversity 1 19–35

OECD (2010), "Section 2 - Molecular characterisation of plants derived from modern biotechnology", in Safety Assessment of Transgenic Organisms, Volume 3: OECD Consensus Documents, OECD Publishing, Paris.

Palakurthi ramesh1, gunti mallikarjuna1, shaik sameena1, anand kumar1, kola gurulakshmi1, bvigneswara reddy1, p chandra obul reddy2 and akila chandra sekhar. Advancements in molecular marker technologies and their applications in diversity studies. J biosci (2020) 45:123

Pandey S, Byerlee D, Dawe D, et al. (Eds) (2010) Rice in the global economy: strategic research and policy issues for food security. Los Baños (Philippines): International Rice Research Institute – IRRI

Phillips RL and Vasil IK 2001 DNA-based markers in plants (Kluwer Academic Publishers, Dordrecht. The Netherlands)

Plant biotechnology. I. Siddiqui, Mohammed Wasim, editor II. Prasad, Bishun Deo, editor III. Sahni, Sangita, editor IV. Kumar, Prasant, editor V. Title. ISBN 978-1-77188-580-5 (v. 1: hardcover). ISBN 978-1-315-21374-3 (v. 1: PDF) 1.

Prasanna, B. M. (2012). Diversity in global maize germplasm: characterization and utilization. *Journal of biosciences*, *37*(5), 843-855.

Qiu Y, Guo J, Jing S, Zhu L, He G (2014) Fine mapping of the rice brown planthopper resistance gene BPH7 and characterization of its resistance in the 93–11 background. Euphytica 198:369–379

Ribaut and Hoisington, 1998; Xu, 2002, 2003; Koebner, 2004; Xu et al., 2005)

Sax K 1923 The association of size differences with seedcoat pattern and pigmentation in Phaseolus vulgaris. Genetics 8 552–560

Schulmann AH 2007 Molecular markers to assess genetic diversity Euphytica 158 313–321

Section 2. Molecular characterisation of plants – 305.safety assessment of transgenic organisms: oecd consensus documents: volume 3 © oecd 2010.

Sturtevant AH 1913 The linear arrangement of six sex-linked factors in Drosophila, as shown by their mode of association. J. Exp. Zool. 14 43–59

Tanksley, S.D. 1983. Molecular markers in plant breeding. Plant Mol. Biol. Rep. 1:1–3.

Tanksley, S.D., and C.M. Rick. 1980. Isozyme gene linkage map of the tomato: Applications in genetics and breeding. Theor. Appl. Genet. 57:161–170

Varshney RK, Graner A and Sorrells ME 2005 Genic microsatellite markers in plants: features and applications. Trends Biotechnology. 23 48–55

Wang Q, Liu Y, Hu J, Zhang Y, Xie K, Wang B, Tuyen LQ, Song Z, Wu H, Liu Y, Jiang L (2013) Detection of quantitative trait loci (QTLs) for resistances to small brown planthopper and rice stripe virus in rice using recombinant inbred lines. Intl J Mol Sci 14:8406–8421

Wani, S. H., Choudhary, M., Barmukh, R., Bagaria, P. K., Samantara, K., Razzaq, A., Jaba, J., Ba, M. N., & Varshney, R. K. (2022). Molecular mechanisms, genetic mapping, and genome editing for insect pest resistance in field crops. *TAG. Theoretical and applied genetics. Theoretische und angewandte Genetik*, *135*(11), 3875–3895.

Xu, Y. 2002. Global view of QTL: Rice as a model. p. 109–134. In M.S. Kang (ed.) Quantitative genetics, genomics, and plant breeding. CABI Publishing, Wallingford, UK.

Xu, Y. 2003. Developing marker-assisted selection strategies for breeding hybrid rice. Plant Breed. Rev. 23:73–174.

Xu, Y., & Crouch, J. H. (2008). Marker-assisted selection in plant breeding: From publications to practice. *Crop science*, *48*(2), 391-407.

Xu, Y., S.R. McCouch, and Q. Zhang. 2005. How can we use genomics to improve cereals with rice as a reference genome? Plant Mol. Biol. 59:7–26

Yan J, Yang X, Shah T, Héctor Sánchez H, Li J, Warburton M, Zhou Y, Crouch JH, and Xu Y 2010 High-throughput SNP genotyping with the Golden Gate assay in maize. Mol. Breeding 25 441–451.

Yang M, Lin J, Cheng L, Zhou H, Chen S, Liu F, Li R, Qiu Y (2020) Identification of a novel planthopper resistance gene from wild rice (Oryza rufpogon Grif.). Crop J 8:1057–1070

Young, N.D., and S.D. Tanksley. 1989. Restriction fragment length polymorphism maps and the concept of graphical genotypes. Theor. Appl. Genet. 77:95–101.

Zhang Q, Li J, Xue Y, Han B, Deng XW. 2008. Rice 2020: A call for an international coordinated effort in rice functional genomics. Mol. Plant 1:715–19

Zhang, X., Zhou, M., Ren, L., Bai, G., Ma, H., Scholten, O. E., ... & Lu, W. (2004). Molecular characterization of Fusarium head blight resistance from wheat variety Wangshuibai. *Euphytica*, *139*, 59-64.

Guo, J., Zhao, Z., Song, J., Liu, C., Zhai, S., Li, H., Liu, A., Cheng, D., Han, R., Liu, J. and Kong, L., 2017. Molecular and physical mapping of powdery mildew resistance genes and QTLs in wheat: a review. *Agricultural Science & Technology*, *18*(6), p.965.