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Advancements in genomic selection and genome editing through molecular markers in modern era of plant breeding

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Abstract
Today's fluctuating climate change era, shows sevior effects on crop growth, so there is need to develop climate resilient crop variety. Recent advancements in the area of -omics of crops have facilitated effect on plant response to environmental stresses. As germplasm plateu occur in many crops so there is need to examine genes from wild variety, which are high yielding and resistant to climate change. As global warming occur temperature fluctuation rate is very high. To better adapt genetics and epigenetics transiently or lastingly to stimuli from the surrounding environment, the chromatin states in plant cells vary to allow the cells to fine tune their transcriptional profiles. Through conventional methods of crop growing and improving there is lot of time consuming process. So their is need to Specifically focuses on genes/DNA sequences. This has paved the way to exploitation of epigenetic variation in crop breeding. That helps to develop climate resilient crop varieties. Several marker techniques have been generated in the last decade starting from the first generation molecular marker, RFLP, that was based on DNA-DNA hybridization and later the invention of PCR gave rise to a second generation of faster and less expensive PCR based markers followed by the third generation sequence based makers.

Keywords: genomic selection, genome editing, molecular markers, modern era, plant breeding

Introduction
In Plant Breeding variations present within and between various plant populations play a beneficial role in the efficient utilization of plants. The process of gene flow, mating system, evolutionary background and population density are important factors used in the detection of structure and level of these variations. To investigate the Genetic Variation, diversity and other important characteristics, different types of agronomic and morphological parameters have been used successfully. Molecular marker investigation is helpful to find accurate gene of interest for improvement of any character which has revolutionized the molecular genetics and its efficiency in plant breeding programmes. By use of genetic markers there are several advantages in plant breeding programs as the potential value of genetic marker used in linkage maps and direct selection in plant breeding

Marker is a DNA sequence or gene with a known chromosome location controlling a particular gene or trait. So instead of search of gene breeder search for marker, ultimately the gene of interest is find attached to marker. Genetic markers are closely related with the target gene Genetic markers are broadly grouped into two categories: classical markers and molecular markers. Classical markers are three types Morphological, cytological and biochemical markers and some examples of DNA markers are restriction fragment length polymorphism (RFLP), amplified fragment length polymorphism (AFLP), simple sequence repeats (SSRs), single-nucleotide polymorphism (SNP) and diversity arrays technology (DArT) markers.

On the basis of phenotype, Morphological markers can visually distinguish qualities like seed structure, flower colour, growth habit and other important agronomic traits. Morphological markers are easy to use, with no requirement for specific instruments. They do not require any specialized biochemical and molecular technique. Breeders have used such type of markers successfully in the breeding programmes for various crops.

Cytological markers shows variations reveal differences in the distributions of euchromatin and heterochromatin. For example, G bands are produced by Giemsa stain, Q bands are produced by quinacrine hydrochloride and R bands are the reversed G bands. These chromosome landmarks can be used in the differentiation of normal and mutated chromosomes. Such markers can also be used in the identification of linkage groups and in

physical mapping. Cytological markers Markers that are related with variations present in the numbers, banding patterns, size, shape, order and position of chromosomes are known as cytological markers. Isozymes, are multi-molecular forms of enzymes which are coded by various genes, but have the same functions are known as biochemical markers. They are allelic variations of enzymes and thus gene and genotypic frequencies can be estimated with biochemical markers.

Biochemical markers are co-dominant, easy to use and cost effective. However, they are less in number; they usually detect less polymorphism and they are affected by various extraction methodologies, plant tissues and different plant growth stages. Molecular markers are nucleotide sequences and can be investigated through the polymorphism present between the nucleotide sequences of different individuals. Insertion, deletion, point mutations duplication and translocation are basis of polymorphisms; however, they do not necessarily affect the activity of genes. Co-dominant DNA marker are ideal if they should be, highly reproducible, evenly distributed throughout genome and having ability to detect higher level of polymorphism.

Molecular markers are classified into several groups on the basis of: (1) method of detection (hybridization-based molecular markers); (2) mode of gene action (co-dominant or dominant markers); (3) mode of transmission. Different types of DNA molecular markers have been developed and successfully applied in genetics and plant breeding activities in various agricultural crops. Due to use of molecular markers exact location of the genes are located.

There are different types of molecular markers:

RFLP

RFLP was the first molecular marker technique and Hybridization-based markers (RFLP), it is only marker system based on hybridization. In this molecular marker there is not any use of PCR, Individuals of same species exhibit polymorphism as a result of insertion/deletions, translocations, duplications, point mutations and inversions. DNA Isolation is the first step in the RFLP technique. DNA is mixed with restriction enzymes which are isolated from bacteria and these enzymes are used to cut DNA at particular loci (known as recognition sites). After that huge number of fragments with different length are obtained. Then preparation of Agarose or polyacrylamide gel electrophoresis (PAGE) is applied for the separation of these fragments by producing a series of bands. When bands are separated then through spectrophotometer gel photograph is taken and numbering in 0 and 1. If the bands are present then numbering is given as 1, and if the band is absent then numbering is given as 0. Each band represents a fragment having different lengths. Base-pair deletions, mutations, inversions, translocations and transpositions are the main causes for the variation resulting in the RFLP pattern. The restriction enzymes will not cut the fragment if a single base-pair variation occurs in the recognition site. However, if this point mutation occurs in one chromosome but not the other, it is called heterozygous for the marker, as both bands are present.

Some Markers are PCR-based. Cary Mullis in 1983 developed PCR technique, as a technique which could amplify a small quantity of DNA without the application of any living organisms. Denaturation, annealing and extension are the three major steps involved in PCR reactions. By using PCR technique we need PCR primers, the primer is a small part of DNA or RNA from which synthesis of DNA starts.

The primer efficiency is essential and depends on the following main factors: (1) association and dissociation of primer–template duplex during the annealing step and the extension temperature; (2) stability of the duplex to mismatched nucleotides; (3) efficiency of polymerase in the identification and extension of mismatched duplex. Designing of a primer is a most crucial parameter in successful reaction mixture. If all things are balanced except a primer, it will lead to no/false working of the PCR protocol. Primer length is also critical for a successful PCR.

RAPD

Another molecular marker is randomly amplified polymorphic DNA (RAPD) this technique was developed by Williams *et al*. and Welsh and Mcclelland independently and all the three steps denaturation, annealing and extension are worked in RAPD. Amplification of genomic DNA is achieved by PCR using single, short (10 nucleotide) and random primer. During PCR, process amplified fragments are totally dependent on the length and size of both the target genome and the primer. Amplification takes place when two hybridization sites are similar to each other and in opposite direction. For the visualization of the genes, the PCR product is separated in agarose gel stained with ethidium bromide. By confirming the presence or absence of specific bands polymorphism detected in any agrose gel. PCR buffer, magnesium chloride concentration, annealing temperature, DNA of gene of interest and Taq DNA (type of DNA polymerase) are some important factors affecting the reproducibility of randomly amplified polymorphic DNA (RAPD) markers. RAPD are dominant markers.

AFLP

Another useful molecular marker is AFLP. Some limitations present in the RAPD and RFLP technique were overcome through the development of AFLP markers. AFLP markers combine the RFLP and PCR technology, in which first digestion of DNA is done and then PCR is performed. In AFLP markers there is no need of prior sequence information. In AFLP, both good-quality and partly degraded DNA can be used; however, this DNA should not contain any restriction enzymes or PCR inhibitors. In AFLP, two restriction enzymes (a frequent cutter and a rare cutter) are used for the cutting of DNA. Each end of the resulting fragments is ligated with the oligonucleotides. Oligonucleotides are short nucleic acid fragments used for the ligation in PCR.

SSR

SSRs are also called as Microsatellites, short tandem repeats and simple sequence length polymorphisms. SSRs are tandem repeat motifs of 1–6 nucleotides that are present abundantly in the genome of various taxa. Microsatellites can be mononucleotide, dinucleotide, trinucleotide, tetranucleotide, pentanucleotide and hexanucleotide. Various Studies have confirmed the presence of SSRs in protein-coding genes and expressed sequence tags (ESTs). In SSRs their are lesser repetition per locus with higher polymorphism level. This high polymorphism level can be detected with ease by PCR and it is due to the occurrence of various numbers of repeats in microsatellite regions. Occurrence of SSRs may be due to recombination of double-strand DNA, slippage of single-strand DNA, transfer of mobile elements (retrotransposons) and mismatches. The sequences which are flanking the SSRs are conserved and are used in the development of primers.

SSR markers involves detection of specific microsatellites. After this, the detection of favorable regions for primer designing is done and after that PCR is performed. On the basis of banding patterns, Interpretation and evaluation of PCR gel are performed for investigation of polymorphism.

SSR markers are co-dominant markers, with high reproducibility and greater genome abundance, and they can be used efficiently in plant mapping studies. Each markers have their benefits as well as drawback on the basis of our study and gene of interest, we select our molecular marker. If we consider Microsatellite markers they exhibit greater level of polymorphism with the drawback of being labour intensive. While RAPD markers are cost effective as compared to microsatellites, their level of polymorphism detection is low as compared to that of microsatellite markers as they are dominant markers and they didn't show polymorphism on codominant gene. To overcome the imperfection of these two molecular markers, randomly amplified microsatellite polymorphisms (RAMP) markers were developed. This marker system involves an SSR primer which is utilized for the amplification of genomic DNA in the absence or presence of RAPD primers. RAMP markers are cost effective, reflect higher polymorphism and have wide distribution in the genome. They have been successfully applied in various plants for molecular characterization

ISSR

Zietkiewicz *et al*. [16] developed Inter simple sequence repeat (ISSR) technique are it is simple, easy to understand as compared to RAPD and there is no need of prior knowledge of DNA sequences. However, they are dominant markers and did not differentiate between co dominant genes and they have less reproducibility with homology of co-migrating amplification products. It is based on amplification of DNA segments located in between two identical but oppositely oriented microsatellite repeat regions, at a distance which allows amplification. Normally long primers having a size of 15–30 bases are used in this technique. The primers used in Inter simple sequence repeat (ISSR) may be unanchored or more typically they are anchored at the 30 or 50 end having 1 to 4 degenerate bases, which are extended into the flanking sequences. Segregating by simple Mendelian laws of inheritance, they are characterized as dominant markers; however, they can also be used in the development of co-dominant markers.

Genome Editing

With the advancements in the field of genetic engineering, many techniques have been evolved to modify a single locus of a target organism. This dream comes true with the development of CRISPR (clustered regularly interspaced short palindromic repeat), a gene-editing technology. Genome editing has revolutionized plant breeding and has been applied successfully in different economically important crops. This technique facilitates the direct improvement of less favourable alleles into more favourable alleles. For the production of improved crop varieties, it is necessary to utilize genome selection and genome editing collectively. Genome editing shortens the time when backcrossing is done between elite varieties and exotic germplasm. This exotic germplasm serves as the encyclopedia for the ancient alleles that are referenced for the development of modern varieties having resistance against biotic and abiotic stress.

For the recombination of alleles that are already adapted, GS is then applied. Genome editing (CRISPR). CRISPR is a genome-editing technique applied successfully in various plants. Cas9 is a recent advancement in the genome-editing technology and is becoming the technique of choice due to its many advantages, like its being easy to use, genome-editing versatility and ability to cleave methylated loci. CRISPR RNAs and Cas protein are the two most important parts in the CRISPR technique. CRISPR RNA (crRNA) and trans-encoded CRISPR RNA (tracrRNA) are two short RNAs that can cleave a particular target site with the help of Cas9 endonuclease (the most explored Cas protein). sgRNA, known as single guide RNA, results when crRNA and tracrRNA are fused artificially. When sgRNA is combined with Cas protein, this leads to the formation of RNA-guided endonuclease that mediates the cleavage at a particular sequence in the genome.

Types of CRISPR

On the basis of this Cas protein, the CRISPR–Cas system is grouped into three types; I, II and III. Cas1 and Cas2 are two different proteins which are commonly present in all three types. Type I is present in both archaea and bacteria, while type II is only present in bacteria; however, type III is most commonly present in archaea but also in some bacteria. Genome editing has been performed successfully in model plants like Nicotiana tabacum, Arabidopsis and some economically important crops like maize and wheat.

Use of CRISPR

Mechanism Acquisition, expression and interference are the three steps which are used by the CRISPR-Cas system to identify and target the pathogen genetic material. Identification and consolidation of foreign DNA is performed within the CASPR locus as a spacer during acquisition. During the acquisition of DNA fragments, a Protospacer having a short stretch (2–5) of conserved nucleotides (PAMs) is used as the identification motif. The AT (adenine–thymine) leader side of the CRISPR array, a 30-bp single copy of spacer is inserted and duplicated.

During the expression step, a long pre-crRNA is transcribed from the CRISPR locus, while tracrRNA and Cas proteins (Cas1, Cas2, Cas9 and Cas4/Casn2) are applied for its processing into crRNAs. The Cas protein complex is guided towards the particular target area of foreign DNA by crRNA for cleavage during the interference step, thus facilitating the immunity against the attack of pathogens.

Conclusion

For genome analysis Molecular markers are considered as valuable tools. With the development of molecular marker technology the scenario of plant breeding has changed. Different types of molecular markers have been developed and advancement in sequencing technologies has geared crop improvement. The progress made in molecular plant breeding, genetics, genomic selection and genome editing has contributed to a more comprehensive understanding of molecular markers and provided deeper insights into the variation and diversity available for crops and greatly complemented breeding studies and if we talk about CRISPR technology it has revolutionized the plant breeding and genetics and researchers are focusing on editing the genomes of all economically important plants. The coming years are likely to see continued innovations in molecular marker technology to make it more precise, productive and cost

effective in order to investigate the underlying biology of various traits of interest. The global population continues to rise, as does the likelihood of reduced yields of major food crops due to the changing climate, thus making the development of genetically improved, crops a research priority. The convergence of low-cost genome sequencing with improved computational power and high-throughput molecular phenotyping technologies has accelerated the identification of genes underlying important agronomic traits relevant to food production and quality. Simple or more advanced molecular markers possess inherent strengths and weakness and none of them are perfect with shortcomings. With rapid progress in molecular biology, more effective and superior markers may appear in near future which can significantly accelerate plant breeding research. Deployment of these products from the laboratory to the field remains hindered by biological and regulatory bottlenecks that require further development.

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