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DNA Molecular Markers

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Abstract

With the development of molecular marker technology in the 1980s, the fate of plant breeding has changed. Different types of molecular markers have been developed and advancement in sequencing technologies has geared crop improvement. To explore the knowledge about molecular markers, several reviews have been published in the last three decades; however, all these reviews were meant for researchers with advanced knowledge of molecular genetics. This review is intended to be a synopsis of recent developments in molecular markers and their applications in plant breeding and is devoted to early researchers with a little or no knowledge of molecular markers. 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 diversity available for crops and greatly complemented breeding stratagems. Genotyping-by-sequencing and association mapping based on next-generation sequencing technologies have facilitated the identification of novel genetic markers for complex and unstructured populations. Altogether, the history, the types of markers, their application in plant sciences and breeding, and some recent advancement in genomic selection and genome editing are discussed.

Keywords: Molecular markers • QTL mapping • MAS • Functional marker • Genomic selection • Genome editing

Introduction

Information about the genetic variations present within and between various plant populations and their structure and level can play a beneficial role in the efficient utilization of plants. The evolutionary background, process of gene flow, mating system and population density are important factors used in the detection of structure and level of these variations. To investigate the diversity and other important characteristics, different types of agronomic and morphological parameters have been used successfully [1]. During the last three decades, the world has witnessed a rapid increase in the knowledge about the plant genome sequences and the physiological and molecular role of various plant genes, which has revolutionized the molecular genetics and its efficiency in plant breeding programmes [2].

Genetic markers

Genetic markers are important developments in the field of plant breeding. The genetic marker is a gene or DNA sequence with a known chromosome location controlling a particular gene or trait. Genetic markers are closely related with the target gene and they act as sign or flags. Genetic markers are broadly grouped into two categories: classical markers and DNA/molecular markers. Morphological, cytological and biochemical markers are types of classical 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.

Classical markers

Morphological markers: 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. Main disadvantages of morphological markers are: they are limited in number, influenced by the plant growth stages and various environmental factors. Since ancient times, humans have successfully used various morphological markers to investigate the variation for utilization in plant breeding.

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. These 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.

Biochemical markers: Biochemical markers, or isozymes, are multimolecular forms of enzymes which are coded by various genes, but have the same functions. They are allelic variations of enzymes and thus gene and genotypic frequencies can be estimated with biochemical markers. Biochemical markers have been successfully applied in the detection of genetic diversity, population structure, gene flow and population subdivision. They are co-dominant, easy to use and cost effective. However, they are less in number; they detect less polymorphism and they are affected by various extraction methodologies, plant tissues and different plant growth stages.

Molecular markers/DNA markers: 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 these polymorphisms; however, they do not necessarily affect the activity of genes. An ideal DNA marker should be co-dominant, evenly distributed throughout genome, highly reproducible and having ability to detect higher level of polymorphism.

Classification of molecular markers

Molecular markers are classified into various groups on the basis of:

1. Mode of gene action (co-dominant or dominant markers);
2. Method of detection (hybridization-based molecular markers or polymerase chain reaction (PCR)-based markers);
3. Mode of transmission (paternal organelle inheritance, maternal organelle inheritance, bi-parental nuclear inheritance or maternal

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nuclear inheritance).

Different types of DNA molecular markers have been developed and successfully applied in genetics and breeding activities in various agricultural crops.

- Hybridization-based markers (RFLP)
- PCR-based markers
- PCR primers
- Randomly amplified polymorphic DNA (RAPD)
- AFLP
- SSRs or microsatellites
- Chloroplast microsatellites (cpSSRs)
- Mitochondrial microsatellites
- RAMP (Randomly amplified microsatellite polymorphisms)
- Sequence-related amplified polymorphism (SRAP)
- Inter simple sequence repeat (ISSR)
- Inter-retrotransposon amplified polymorphism (IRAP)
- Retrotransposon microsatellite amplification polymorphisms (REMAP)
- Retrotransposon-based insertion polymorphism (RBIP)
- Inter-primer binding site (iPBS)
- Cleaved amplified polymorphic sequences (CAPS)
- SCAR (Sequence-characterized amplified regions)

Conclusion

The last 30 years have witnessed a continuous development in the molecular markers technology from RFLP to SNPs and a diversity of array-technology-based markers. Advancements in the sequencing technologies have led to the development of NGS platforms that are low cost with high throughput. In spite of the presence of these highly advanced molecular genetic techniques, we are still not achieving our goals. The main reason behind this lies in inaccurate phenotyping. High-throughput phenotyping techniques solve these problems by using light, cameras, sensors, computers and highly modified devices for the collection of very precise phenotypic data, which is a core requirement to achieving our breeding goals successfully. CRISPR technology 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.

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