Agri Articles
(e-Magazine for Agricultural Articles)
Volume: 03, Issue: 02 (MAR-APR, 2023)
Available online at http://www.agriarticles.com
©Agri Articles, ISSN: 2582-9882

DNA Marker, Molecular Markers and their Applications in Genetics & Plant Breeding

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Abstract
DNA markers are the utmost extensively used marker predominantly due to their abundance. They arise from various classes of DNA mutations like substitution mutations (point mutations), movements (insertions or deletions) or errors in replication of randomly repeated DNA (Paterson, 1996). These markers are selectively neutral because they are generally located in non-coding regions of DNA. Dissimilar to morphological and biochemical markers, DNA markers are practically unlimited in number and are not affected by environmental factors and the developmental stage of the plant (Winter & Kahl, 1995).

Introduction
DNA marker is a fragment of DNA revealing mutations/variations, which can be used to detect polymorphism between different genotypes or alleles of a gene for a particular sequence of DNA in a population or gene pool". Such fragments are associated with a certain locus within the genome and may be detected by some molecular technology. In simple words, DNA marker is a small region of DNA sequence showing polymorphism (base deletion, insertion and substitution) between different individuals. DNA markers that are tightly linked to candidate genes may be used as molecular tools for marker-assisted selection (MAS) in plant breeding (Ribaut and Hoisington, 1998).

What are Genetic Markers?
Genetic markers represent genetic differences between individual organisms or species. Generally, they do not represent the target genes themselves but act as 'signs' or 'flags. Genetic markers that are located in close proximity to genes (i.e., tightly linked) may be referred to as gene 'tags'. Such markers themselves do not affect the phenotype of the trait of interest because they are located only near or 'linked' to genes controlling the trait. All genetic markers occupy specific genomic positions within chromosomes (like genes) called 'loci' (singular 'locus'). There are three major types of genetic markers: (1) morphological (also 'classical' or 'visible') markers which themselves are phenotypic traits or characters; (2) biochemical markers, which include allelic variants of enzymes called isozymes; and (3) DNA (or molecular) markers, which reveal sites of variation in DNA (Jones et al., 1997; Winter & Kahl, 1995).

RFLP Markers: RFLP markers are the first generation of DNA markers and one of the important tools for plant genome mapping. They are a type of Southern-Bolting-based markers. In living organisms, mutation events (deletion and insertion) may occur at restriction sites or between adjacent restriction sites in the genome. Gain or loss of restriction

sites resulting from base pair changes and insertions or deletions at restriction sites within the restriction fragments may cause differences in size of restriction fragments. These variations may cause alternation or elimination of the recognition sites for restriction enzymes. They are powerful tools for comparative and synteny mapping. Most RFLP markers are co-dominant and locus specific.

RAPD Markers: RAPD is a PCR-based marker system. In this system, the total genomic DNA of an individual is amplified by PCR using a single, short (usually about 10 bases) and random primer. It is predominantly dominant marker which yields high levels of polymorphism and is simple and easy to be conducted. 1) neither DNA probes nor sequence information is required for the design of specific primers. 2) the procedure does not involve blotting or hybridization steps, and thus it is a quick, simple and efficient technique.

AFLP Markers: AFLPs are PCR-based markers. Technically, AFLP is based on the selective PCR amplification of restriction fragments from a total double-digest of genomic DNA under high stringency conditions, i.e. the combination of polymorphism at restriction sites and hybridization of arbitrary primers. The first step in AFLP involves restriction digestion of genomic DNA (about 500 ng) with two restriction enzymes, a rare cutter (6-bp recognition site, EcoRI, PtsI or HindIII) and a frequent cutter (4- bp recognition site, MseI or TaqI). The adaptors are then ligated to both ends of the fragments to provide known sequences for PCR amplification. The double-stranded oligonucleotide adaptors are designed in such a way that the initial restriction site is not restored after ligation. Therefore, only the fragments which have been cut by the frequent cutter and rare cutter will be amplified. This property of AFLP makes it very reliable, robust and immune to small variations in PCR amplification parameters (e.g., thermal cycles, template concentration), and it also can produce a high marker density.

SSR Markers: SSRs, also called microsatellites, short tandem repeats (STRs) or sequence-tagged microsatellite sites (STMS), are PCR-based markers. They are randomly tandem repeats of short nucleotide motifs (2-6 bp/nucleotides long). Di-, tri- and tetra-nucleotide repeats, e.g. (GT)n, (AAT)n and (GATA)n, are widely distributed throughout the genomes of plants and animals. The copy number of these repeats varies among individuals and is a source of polymorphism in plants. Because the DNA sequences flanking microsatellite regions are usually conserved, primers specific for these regions are designed for use in the PCR reaction.

SNP Markers: An SNP is a single nucleotide base difference between two DNA sequences or individuals. SNPs can be categorized according to nucleotide substitutions either as transitions or transversions. SNPs provide the simplest form of molecular markers as a single nucleotide base is the smallest unit of inheritance and thus, they can provide maximum markers. Typically, SNP frequencies are in a range of one SNP every 100-300 bp in plants SNPs may present within coding sequences of genes, non-coding regions of genes or in the intergenic regions. Advantages: SNPs are co-dominant markers, often linked to genes. High costs for marker development, high-quality DNA required and high technical/equipment demands limit, to some extent, the application of SNPs in some laboratories and practical breeding programs.

Conclusion

The choice and use of DNA markers in research and breeding is still a challenge for plant breeders. A number of factors need to be considered when a breeder chooses one or more molecular marker types. A breeder should make an appropriate choice that best meets the requirements according to the conditions and resources available for the breeding program.