International Journal of Chemical and Molecular Engineering
Vol. 3: Issue 1
www.journalspub.com

Applications and Advantages of Molecular Markers in Plants

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ABSTRACT

Genetic improvement of crop plant through conventional plant breeding has made tremendous contribution to the breakthrough in the global agricultural production. Recently, arrays of tools and techniques in the fields of molecular biology have become available for supplementing the conventional genetic approaches.

Keywords: genetic approaches, genetic improvement, marker assisted selection, molecular marker, plant breeding

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INTRODUCTION

Genetic improvement of crop plant through conventional plant breeding has made tremendous contribution to the breakthrough in the global agricultural production. Recently, arrays of tools and techniques in the fields of molecular biology have become available for supplementing the conventional genetic approaches. Consequently, new interacted approaches are being designed. One of the approaches employs molecular markers for genome mapping, gene tagging and marker assisted selection (MAS) with the complete sequencing of whole plant genomes and a large number of random cDNAs in many different crops species, newer opportunities are emerging [1].

Molecular Marker

There are heritable differences in nucleotide of DNA at corresponding position on homologous chromosomes of two different individuals which follows a simple Mendelian pattern of inheritance. These differences are detected employing mainly two basic techniques.

(a) Southern blot hybridization
(b) Polymerase chain reaction

Briefly southern blot hybridization developed buy Prof. EM Southern in 2975 involves restriction of plant DNA electrophoretic separation of DNA fragment, denaturation and transfer of fragments from gel to nylon membrane. Hybridization of radiolabeled probe DNA and autoradiography to develop the X-ray films. The PCR reaction designed by Dr. Kerry Mullis in 1985. Involves denaturation to template DNA amplification, the fragments are separated on agarose or polyacrylamide gel obtain the DNA profiles. Using these two techniques and their variations different molecular markers or DNA markers have been developed [1].

METHODS

Restriction Fragment Length Polymorphism (RFLP)

Restriction Fragment Length Polymorphism (RFLP) is a difference in homologous DNA sequences that can be detected by the presence of fragments of different lengths after digestion of the DNA samples in question with specific restriction endonucleases.

Restriction fragment length polymorphism (RFLP) is a technique invented in 1984 by

the English scientist Alec Jeffreys during research into hereditary diseases (Figure 1). It is used for the analysis of unique patterns in DNA fragments in order to genetically differentiate between organisms – these patterns are called Variable Number of Tandem Repeats (VNTRs). Restriction endonucleases are enzymes that cut lengthy DNA into short pieces. Each restriction endonuclease targets different nucleotide sequences in a DNA strand and therefore cuts at different sites. The distance between the cleavage sites of a certain restriction endonuclease differs between individuals. Hence, the length of the DNA fragments produced by a restriction endonuclease will differ across both individual organisms and species. The RFLP technique exploits these differences in DNA sequences to recognize and study both intra species and interspecies variation [2].

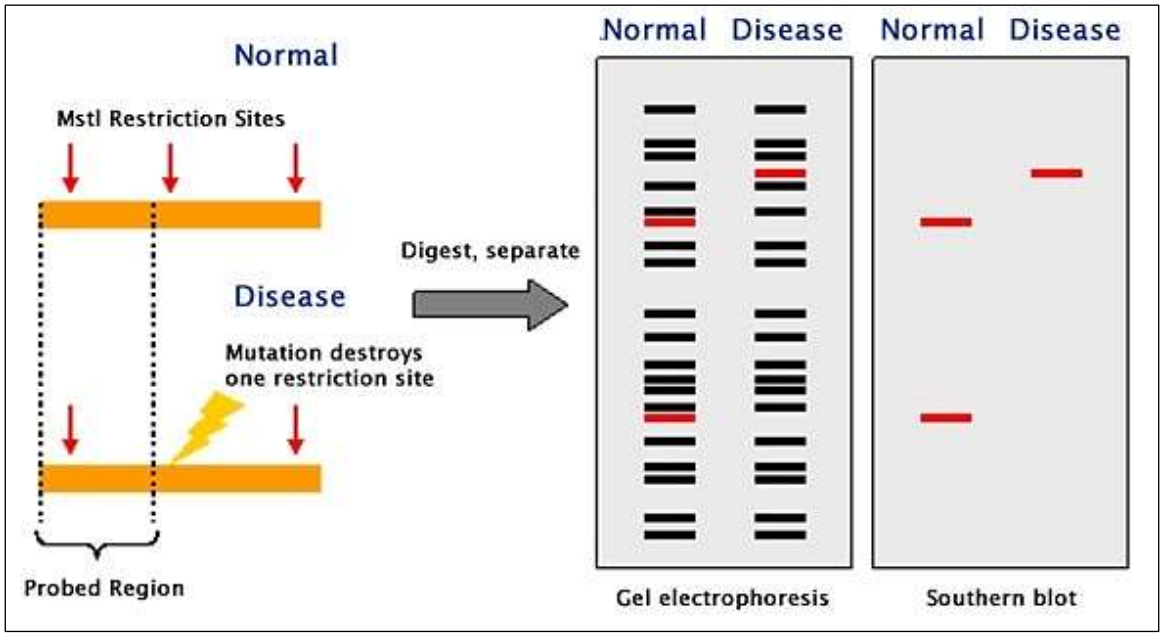

Fig. 1. *Restriction fragment length polymorphism.*

Applications of RFLP

RFLP has been used for several genetic analysis applications since its invention.

Some of these key applications of RFLP are listed below:

- To determine the status of genetic diseases such as Cystic Fibrosis in an individual.
- To determine or confirm the source of a DNA sample such as in paternity tests or criminal investigations.
- In genetic mapping to determine recombination rates that show the genetic distance between the loci.
- To identify a carrier of a disease-causing mutation in a family.

Random Amplified Polymorphic DNA (RAPD)

Random Amplified Polymorphic DNA (RAPD) markers are DNA fragments from PCR amplification of random segments of genomic DNA with single primer of arbitrary nucleotide sequence (Figure 2). Unlike traditional PCR analysis, RAPD does not require any specific knowledge of the DNA sequence of the target organism: the identical 10-mer primers will or will not amplify a segment of DNA, depending on positions that are complementary to the primers' sequence. For example, no fragment is produced if primers annealed too far apart or 3' ends of the primers are not facing each other. Therefore, if a mutation has occurred in the template DNA at the site

that was previously complementary to the primer, a PCR product will not be produced, resulting in a different pattern of amplified DNA segments on the gel [2].

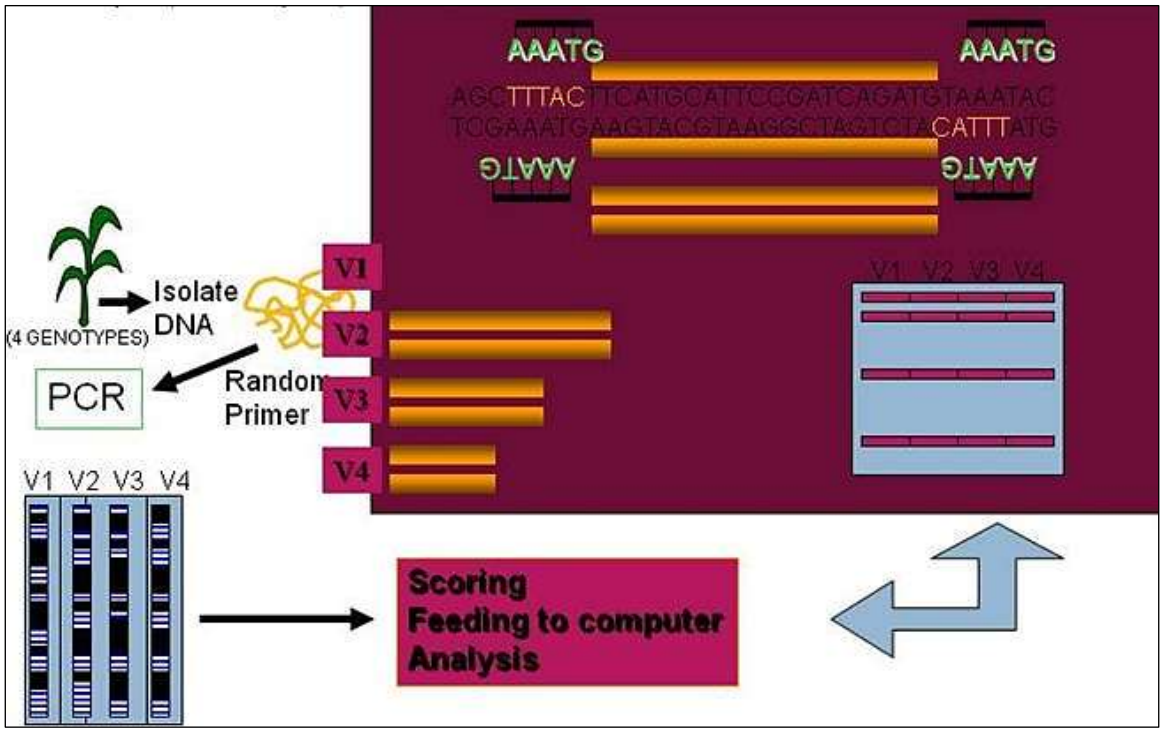

Fig. 2. *Principal of random amplified polymorphic DNA.*

Application

- The polymorphic RAPD marker band is isolated from the gel.
- It is amplified in the PCR reaction.
- The PCR product is cloned and sequenced.
- New longer and specific primers are designed for the DNA sequence, which is called the Sequenced Characterized Amplified Region Marker (SCAR).

Sequences Tagged Sites (STS)

A sequence-tagged site (STS) is a short region along the genome (200 to 300 bases long) whose exact sequence is found nowhere else in the genome. The uniqueness of the sequence is established by demonstrating that it can be uniquely amplified by the PCR. The DNA sequence of an STS may contain repetitive elements, sequences that appear elsewhere in the genome, but as long as the sequences at both ends of the site are unique, unique DNA primers complementary to those ends can be synthesized, the region amplified using PCR, and the specificity of the reaction demonstrated by gel electrophoresis of the amplified product.

Applications of STS

STSs are very helpful for detecting microdeletions in some genes. For example, some STSs can be used in screening by PCR to detect microdeletions in Azoospermia (AZF) genes in infertile men. Identification of genes in elephants could provide additional information for evolutionary studies and for evaluating genetic diversity in existing elephant populations. Sequence tagged sites (STSs) were identified in the Asian and the African elephant for the following genes: melatonin receptor 1a (MTNR1A), retinoic acid receptor beta (RARB), and leptin receptor (LEPR) [2].

Sequences Tagged Microsatellite Sites (STMS)

Polymorphic loci present in nuclear DNA and organellar DNA that consist of repeating units of 1-10 base pairs, most typically, 2–3 bp in length, also called Simple Sequence Repeats (SSR), Sequence-Tagged Microsatellite Sites (STMS) or Simple Sequence Repeats Polymorphisms (SSRP). SSRs are highly variable and evenly distributed throughout the genome. This type of repeated DNA is common in eukaryotes. These polymorphisms are identified by constructing PCR primers for the DNA flanking the microsatellite region. The flanking regions tend to be conserved within the species, although sometimes they may also be conserved in higher taxonomic levels (Figure 3) [3].

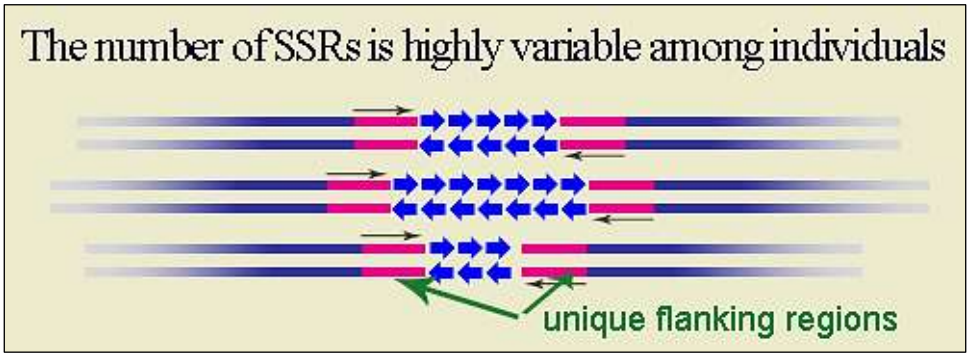

Fig. 3. *Sequenced target site microsatellite.*

Amplified Fragment Length Polymorphism (AFLP)

AFLP is a trademark of KeyGene (Wageningen, The Netherlands). AFLPs are DNA fragments (80–500 bp) obtained from digestion with restriction enzymes, followed by ligation of oligonucleotide adapters to the digestion products and selective amplification by the PCR (Figure 4). Thus, AFLPs involve both RFLP and PCR. The PCR primers consist of a core sequence (part of the adapter), and a restriction enzyme specific sequence and 1–5 selective nucleotides (the higher the number of selective nucleotides, the lower the number of bands obtained per profile). The AFLP banding profiles are the result of variations in the restriction sites or in the intervening region. The AFLP technique simultaneously generates fragments from many genomic sites (usually 50–100 fragments per reaction) that are separated by polyacrylamide gel electrophoresis and that are generally scored as dominant markers. Selective Fragment Length Amplification (SFLA) and Selective Restriction Fragment Amplification (SRFA) are synonyms sometimes used to refer to AFLPs. A variation of the AFLP technique is known as Selectively Amplified Microsatellite Polymorphic Locus (SAMPL). This technology amplifies microsatellite loci by using a single AFLP primer in combination with a primer complementary to compound microsatellite sequences, which do not require prior cloning and characterization [4].

Applications

Because of the highly informative fingerprinting profiles generally obtained, AFLPs can be applied in studies involving genetic identity, parentage and identification of clones and cultivars, and phylogenetic studies of closely related species. Their high genomic abundance and generally random distribution throughout the genome make AFLPs a widely valued technology for gene mapping studies. SAMPL is considered more applicable to intraspecific than to interspecific studies due to frequent null alleles [4].

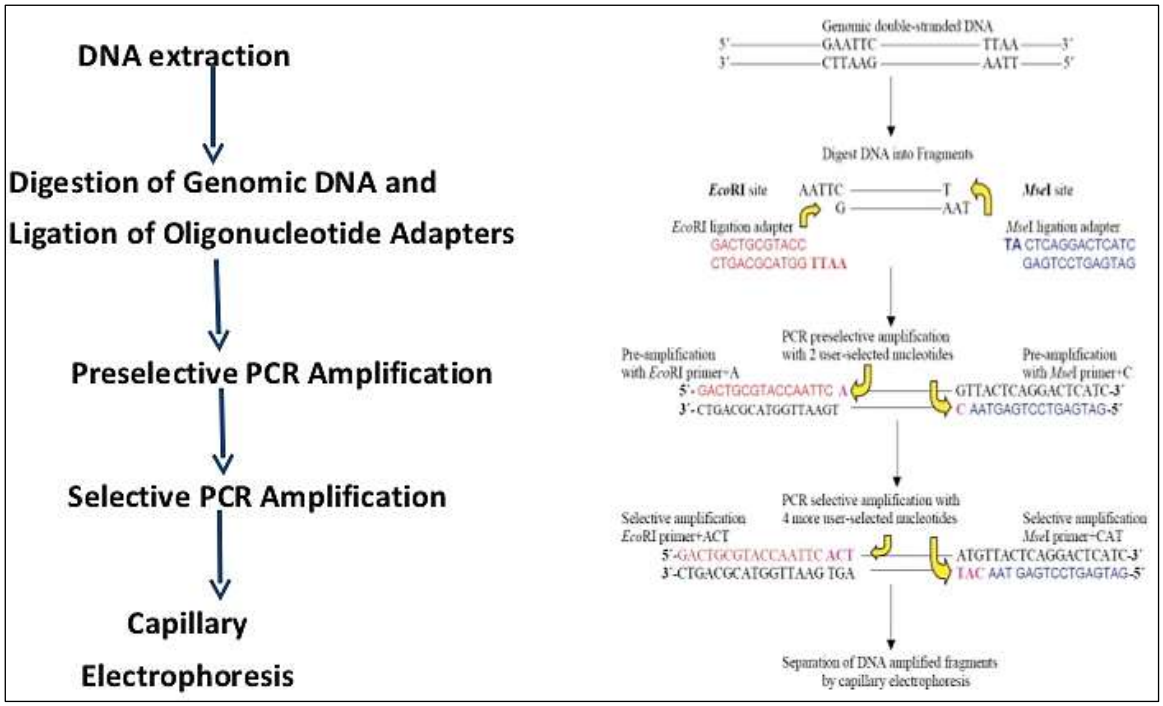

Fig. 4. *Principal of amplified fragment length polymorphism.*

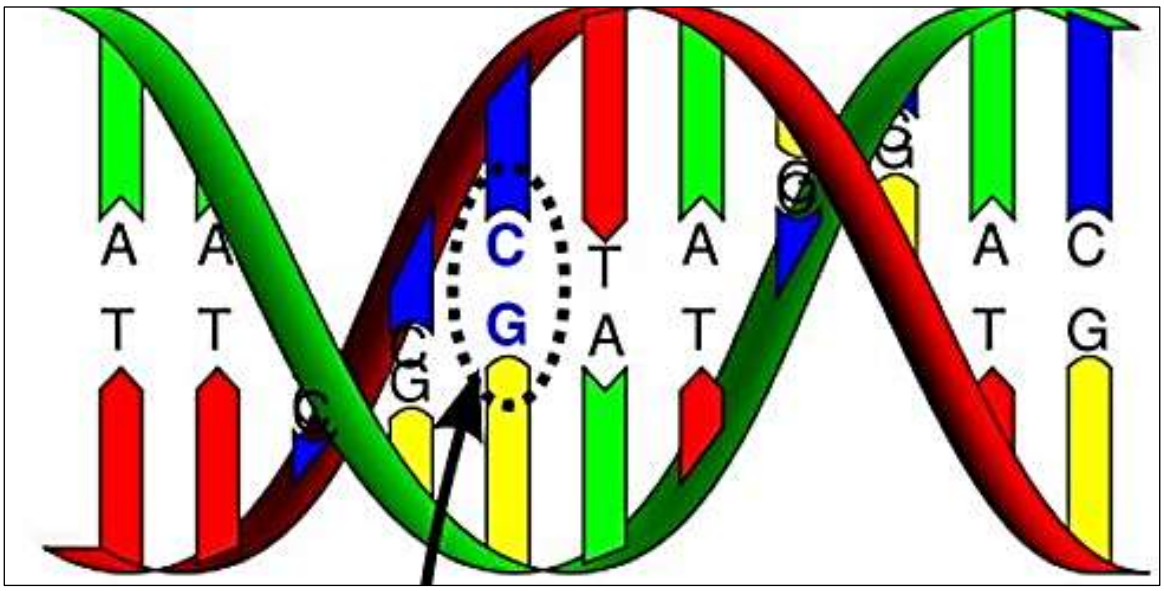

Fig. 5. *Single nucleotide polymorphism.*

Single Nucleotide Polymorphism (SNP)

Single nucleotide polymorphisms, frequently called SNPs (pronounced "snips"), are the most common type of genetic variation among people (Figure 5). Each SNP represents a difference in a single DNA building block, called a nucleotide. For example, a SNP may replace the nucleotide cytosine (C) with the nucleotide thymine (T) in a certain stretch of DNA. SNPs occur normally throughout a person's DNA. They occur once in every 300 nucleotides on average, which means there are roughly 10 million SNPs in the human genome. Most commonly, these variations are found in the DNA between genes. They can act as biological markers, helping scientists locate genes that are associated with disease. When SNPs occur within a gene

or in a regulatory region near a gene, they may play a more direct role in disease by affecting the gene's function. Most SNPs have no effect on health or development. Some of these genetic differences, however, have proven to be very important in the study of human health. Researchers have found SNPs that may help predict an individual's response to certain drugs, susceptibility to environmental factors such as toxins, and risk of developing particular diseases. SNPs can also be used to track the inheritance of disease genes within families. Future studies will work to identify SNPs associated with complex diseases such as heart disease, diabetes, and cancer [4].

ADVANTAGES OF MOLECULAR MARKERS

The molecular markers offer numerous advantages over the other genetic markers [5]. These include:

- Abundance in growth of plants
- Co-dominance characters
- Phenotypic characters in plants
- Epistatsis absent
- Developmental stages, tissue and environment expression

Several number of molecular markers can be acquired either by using DNA probes from a variation of sources in grouping with any of commercially obtainable restriction enzymes or by using a variety of DNA primers. This allows study of a number of markers in a particular population by construction of high density linkage maps of plant genomes. The exceeding features make molecular markers idea for categorization of quantitative traits.

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