

Article:

Article Title (3 to 12 words)	Molecular markers in crop improvement
Article Summary (In short - What is your article about – Just 2 or 3 lines)	Molecular markers have found large scale applications in crop improvement programs, because of several advantages over morphological and biochemical markers. Molecular markers are environmentally neutral, highly abundant, and independent of tissue and stage of the plants. Traditionally, breeders have relied on visible traits to select improved varieties however; MAS rely on identifying marker DNA sequences that are inherited alongside a desired trait during the first few generations. Molecular markers are also considered as useful tools for pyramiding of different resistance genes and developing multi-line cultivars targeting for durable resistance to the disease.
Category:	Genetics

Your full article (between 500 to 5000 words) - -	Do check for grammatical errors or spelling mistakes
Molecular markers in crop improvement Sundeep Kumar, Amit K. Singh, Ruchi Bansal, Sheel Yadav, Jyoti Kumari and Rakesh Singh National Bureau of Plant Genetic Resources, New Delhi Molecular markers are segment of DNA sequence of the genome that can differentiate two or more individuals/genotypes and follow the Mendelian pattern of inheritance. Molecular markers have been introduced over last two decades, which has revolutionized the entire scenario of biological sciences. Since the publication of restriction fragment length polymorphism (RFLP); first DNA marker techniques three decades ago, many DNA based marker techniques have been developed which includes RAPD, SSR, ISSR, CAPs etc. DNA based markers have found large scale applications in crop improvement programs, because of several advantages over morphological and biochemical markers. Molecular markers are environmentally neutral, highly abundant, and independent of tissue and stage of the plants. Traditionally, breeders have relied on visible traits to select improved varieties however; MAS rely on identifying marker DNA sequences that are inherited alongside a desired trait during the first few generations. Molecular markers are also considered as useful tools for pyramiding of different resistance genes and developing multi-line cultivars targeting for durable resistance to the disease. Although there is no marker technique which is without any limitation but an ideal molecular marker should have following features: 1) technically simple and easy to perform 2) follow co-dominant inheritance 3) require no prior sequence information about genome 4) reproducible and 5) generate more number markers per reaction However, most of the currently used molecular markers are random DNA markers (RDMs) derived from unknown regions of the genome, limiting their use in trait specific crop improvements. Therefore, the focus in the recent years has been to develop markers which are derived from the within the gene regions making them more useful for crop improvement per se. These markers are referred as gene targeted markers (GTMs). And those gene targeted markers	

which are derived from the polymorphic site within the genes having effect on phenotypes of the plants are referred as functional markers (FMs).

Some important terms: While dealing with markers, we must know following terms.

- **DNA Marker:** A fragment of known size used to calibrate an electrophoretic gel.
- **Genetic Marker:** is any allele of interest in an experiment.
- **Marker validation:** A process of examining behaviour of markers and associated polymorphism in different genetic backgrounds.

Eras in genetic marker evolution (Lin, 1997): There have been different eras of markers according to their uses. Different eras of marker evolution are as given here.

- **Until 1950's: Early Genetics Age** - Morphological & Cytological markers.
- **1960-Mid 1970's: Pre Recombinant DNA Age** - protein and allozyme
- **Mid 1970's - 1985: Pre PCR Age** - RFLP and minisatellites
- **1986-1995: Oligoscene Age** - RAPD, AFLP
- **1996 onwards: Computer robotic cyber genetics Age** - Complete DNA sequencing

Essential requirements for MAS: A marker is considered useful for MAS only if it fulfills the following three basic requirements.

- Markers should co-segregate or be closely linked (1 cM or less) with the trait
- Must have an efficient means of screening large population.
- Screening technique must be reproducible, economical and user friendly

Molecular Markers: Molecular markers can broadly be classified into three groups i.e., structural, gene targeted and functional markers.

(a) Structural markers:

- **RFLP (Botstein et al., 1980)**
- **RAPD (William et al., 1990)**
- **SCAR (Paran and Michelmore, 1993)**
- **STS (Olson et al., 1989)**
- **SSR (Tuntz et al., 1989)**
- **AFLP (Vos et al., 1995)**

(b) Gene targeted markers:

- **Sequence related amplified polymorphism (SRAP)**
- **Target region amplification polymorphism (TRAP)**
- **Start Codon Targeted (SCoT) markers**
- **Conserved Domain Derived Polymorphism (CDDP)**

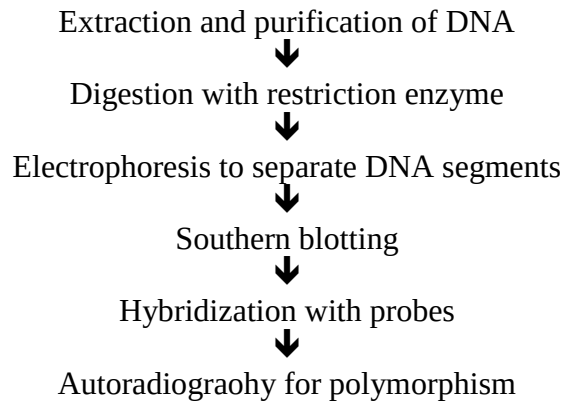
(c) Functional markers

(a) Structural Markers:

(i) RFLP (Botstein et al., 1980):

- It is "Restriction Fragment Length Polymorphism arising due to differences in the restriction sites"
- When genomic DNAs from several individuals belonging to one of more species are digested separately with REs, electrophoresed, blotted on a membrane and probed with a labeled DNA clone, polymorphism in the hybridization pattern is termed as RFLP

Methodology:



Advantages

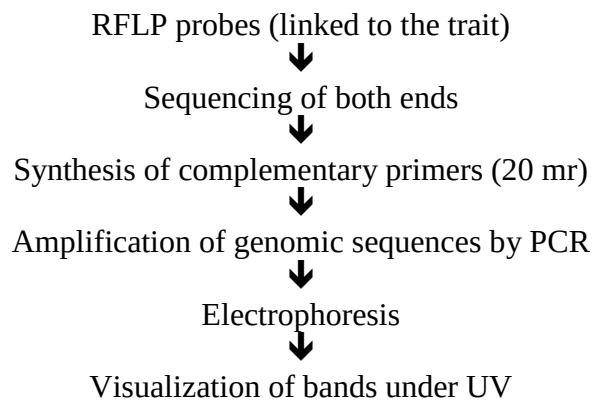
- Reliable polymorphism
- Co-dominant and can identify a unique locus

Disadvantage

- Labour intensive and time consuming
- Requires large amount of DNA

(ii) Sequence tagged site (STS): Invented by Olson *et al.* (1989). STS is a short unique sequence that identifies a specific locus and can be amplified by PCR.

Methodology:



Advantages

- Identifies specific locus and a single critical band is observed

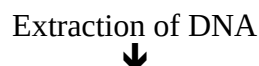
Disadvantages

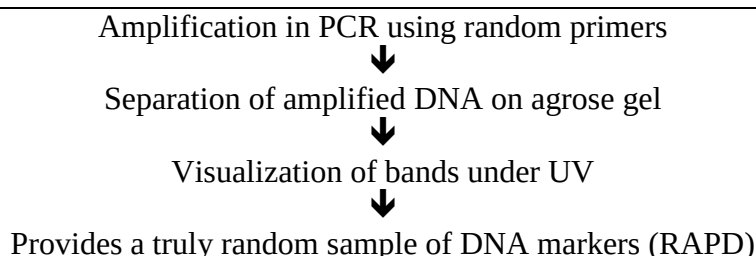
- Reduced polymorphism than the corresponding RFLP markers

(iii) RAPD:

- In case of RAPD, we set the PCR reaction with primers having arbitrary sequences (unbounded or random primers) for amplification.

Methodology:





Advantages:

- Easy to use.

Disadvantages:

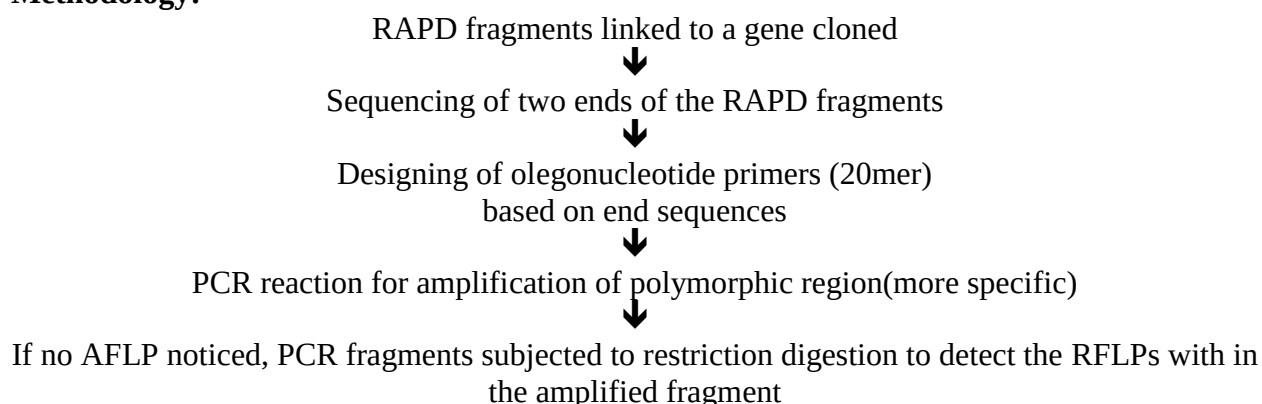
- Dominant in nature cannot distinguish homozygous dominant to heterozygous.
- Reproducibility is a big issue in RAPD markers.

(iv) SCAR (Sequence characterized amplified regions - Paran and Michelmore, 1993):

Advantage

Inherited in a co-dominant fashion in contrast to RAPDs which are inherited in an dominant manner.

Methodology:

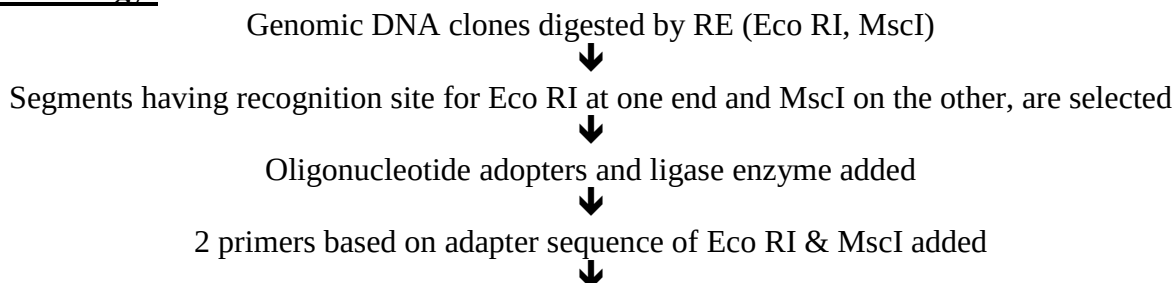


(v) AFLP (Amplified fragment length polymorphism, Vos et al., 1995):

Advantage

- Most suitable for fingerprinting
- Reproducible and versatile

Methodology:



PCR + Electrophoresis



Amplified product seen under UV

(vi) Microsatellites or SSR loci:

- Repeat units of di, tri and tetra nucleotides (CA)_n, (GT)_n, (AAT)_n or (AGAT)_n etc
- Ubiquitous in eukaryotic genomes
- Locus specific & high level of polymorphism make SSRs the marker of choice in future
- Reflect polymorphism based on the number of repeat units in a defined region of the genome (Litt et al., 1989)
- Number and composition of repeats differ in plants and animals. First microsatellite in plant by Candit & Hubbel, (1991)

	Plants	Animals
<i>Repeat units</i>	Every 33kb	Every 6kb
<i>Common repeat</i>	AT	AC or TC

Advantages:

- Polymorphism is highly reproducible
- Rapid and accurate detection of polymorphic loci

Disadvantages

- More expensive
- Unavailability of homologous sequence in the genome restrict their use in interspecific & interspecific genomic analysis
-

(vii) VNTR (Variable number of tandem repeats):

- These are the repeat units of 11-60 bp long nucleotides present in human genome

Methodology:

VNTR loci present in humans



Development of primers using conserved flanking regions of VNTR loci



Amplification of all available VNTR loci



PCR products differ on the basis of number of repeat units present in different VNTR loci



Variation exhibited in the form of different bands

Molecular markers: Some examples

<u>Gene & Source</u>	<u>Marker type & location</u>
<i>Lr47 (A. speltiodes)</i>	CAPS (7A) Helguera et al., 2000

<i>Pm26 (T. turgidum)</i>	RFLP (2BS) Rong et al., 2000
<i>Cold tolerance</i>	RFLP (5A) Vagujfalci et al., 2000
<i>High grain protein (T. turgidum)</i>	RFLP (6B) Meslin et al., 1999

(viii) Single Nucleotide Polymorphism (SNPs):

- ◆ SNPs do not require gel assay & less time consuming
- ◆ Subject to automation
- ◆ Earlier developed in human but recently started to use in plants
- ◆ Sufficient to detect variation even at a single nucleotide
- ◆ Biallelic in nature is an disadvantage

Matrix-assisted laser desorption/ionization –time of light (MALDI-TOF) mass spectrometry allow to detect biallelic loci

(b) Gene targeted markers:

A few of the gene targeted marker techniques used in plant genome analysis are described here.

(i) Sequence related amplified polymorphism (SRAP)

SRAP targets gene region and primers amplify ORFs dispersed throughout the genomes. This technique uses a pair of primers with AT- or GC rich cores to amplify intragenic fragments for polymorphism detection. It combines simplicity, reliability, moderate throughput ratio and facilitates sequencing of selected bands. SRAP marker system has found application in construction of linkage map, mapping of QTLs and assessment of genetic diversity.

(ii) Target region amplification polymorphism (TRAP)

TRAP is a PCR based technique which uses bioinformatics and EST database information to generate markers around the candidate gene sequences. It is a modification of SRAP and uses a pair of primers to generate markers. One of the primers, the fixed primer is designed from the EST database and other primer is arbitrary primer either with AT or GC core to anneal with intron or exon. As markers are targeted to specific genes, they can be used for generating markers associated for agronomically important traits

(iii) Start Codon Targeted (SCoT) markers

SCoT markers are PCR based marker generated using a single 18 nucleotide long primer designed from consensus region around start codon of plant genes. Since the start codon is a universal feature of genes, a set of SCoT primers should be able to generate markers across diverse group of plants. The SCoT markers are generally dominant and show higher reproducibility compared to arbitrary primer based PCR markers.

(iv) Conserved Domain Derived Polymorphism (CDDP)

CDDP marker technique exploits short conserved amino acid sequences in proteins for designing polymerase chain reaction (PCR) from the corresponding DNA sequence. Since primers are designed using highly conserved regions of genes, they are expected to generate markers in diverse plant species. This method can be used in conjunction with or as a substitute to other technically simple dominant marker methods for applications such as targeted quantitative trait loci mapping, especially in laboratories with a preference for agarose gel electrophoresis.

Development of functional markers

Development of functional markers requires allele sequences of functionally characterized genes from which polymorphic, functional motifs affecting plant phenotype can be identified. EST and genome sequence information derived from the genome sequencing projects serve as a raw material for developing SSR and SNPs closely linked to genes linked to economically important traits. The developed functional markers are validated before they are applied in crop improvement.

Functional markers v/s other marker types

Functional markers are generally considered superior to GTMs and RDMs in several applications owing to their complete linkage to functional motifs of genome. Because FMs allow reliable application of markers in populations without prior mapping, the use of markers in mapped populations without risk of information loss owing to recombination and better representation of genetic variation in natural or breeding populations. More generally, FMs are useful for: (i) more efficient fixation of alleles in populations; (ii) screening for alleles in natural as well as breeding populations; (iii) combination of FM alleles affecting identical or different traits in plant breeding.

Scope and limitations of functional markers

For the development of the functional markers several target genes have been identified in Rice Arabidopsis and other crops and allele sequencing projects are also underway in Arabidopsis as well as in other crop species. However, even in model species like Arabidopsis approximately 20% of all genes have been functionally characterized, whereas this number is less than 5% in other crops. Therefore, there is need to characterize more number of genes so the key genes responsible for the traits can be used to develop the markers. In addition, the criteria by which such genes have been functionally characterized might not be sufficient to establish gene function in an agronomic sense. Thus, further functional characterization might be necessary before proceeding to FM development. Since the functionally described target genes are available, the limiting step in FM development will be the choice and development of suitable plant materials and its thorough phenotypic characterization to distinguish minor phenotypic effects. At the same time development of FMs is expensive and cannot be undertaken for all the traits and in all crop species.

Advantages of MAS:

- **Independent of environment**
- **Makes selection more efficient**
- **Selection for recessive gene possible even in heterozygous condition**
- **Indirect selection by linkage**

Applications of molecular markers:

- Identification of breeding lines/varieties by fingerprinting
- Identification of resistance genes/gene tagging and mapping thus, make gene pyramiding easier
- Markers can be use for map based cloning
- Used for isolation of gene to transfer in different individual
- Can knockdown expression of undesirable genes
- Molecular markers are better solution for QTL traits

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