MOLECULAR MARKERS

Polymorphism involves the existence of different forms (alleles) of the same gene in plants or a population of plants. These differences are tracked as molecular markers to identify desired genes and the resulting trait. Most organisms are diploid, meaning they have two copies of each gene — one from each parent. One gene usually dominates the other thus determining the inherited trait.

Why Marker?

A breeder aims to improve the resistance of a cultivated form. Therefore, he/she performs a cross between the susceptible culitvated forms with a wild form that possess the required resistance. However, at least 6 backcrossing steps are necessary and the resistance is difficult to detect.

DNA-based molecular markers

Genetic polymorphism is classically defined as the simultaneous occurrence of a trait in the same population of two or more discontinuous variants or genotypes.

Although DNA sequencing is a straightforward approach for identifying variations at a locus, it is expensive and laborious. A wide variety of techniques have, therefore, been developed in the past few years for visualizing DNA sequence polymorphism.

Properties desirable for ideal DNA markers

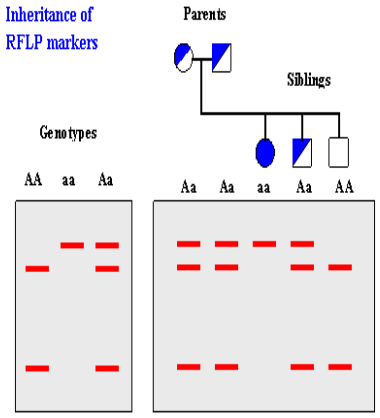
- Highly polymorphic nature
- Codominant inheritance (determination of homozygous and heterozygous states of diploid organisms)
- Frequent occurrence in genome
- Selective neutral behaviour (the DNA sequences of any organism are neutral to environmental conditions or management practices)
- Easy access (availability)
- Easy and fast assay
- High reproducibility
- Easy exchange of data between laboratories.

Types and description of DNA markers

Restriction fragment length polymorphism (RFLP):

RFLPs involves fragmenting a sample of DNA by a restriction enzyme, which can recognize and cut DNA wherever a specific short sequence occurs. A RFLP occurs when the length of a detected fragment varies between

individuals and can be used in genetic analysis.



Advantages:

- Variant are co dominant
- Measure variation at the level of DNA sequence, not protein sequence.

Disadvantage:

- Requires relatively large amount of DNA

Amplified fragment length polymorphism (AFLP)

AFLP, is a technique based on the detection of genomic restriction fragments by PCR amplification and can be used for DNAs of any origin or complexity. The fingerprints are produced, without any prior knowledge of sequence, using a limited set of generic primers. The number of fragments detected in a single reaction can be 'tuned' by selection of specific primer sets. AFLP technique is reliable since stringent reaction conditions are used for primer annealing. This technique thus shows an ingenious combination of RFLP and PCR techniques and is extremely useful in detection of polymorphism between closely related genotypes.

AFLP procedure mainly involves 3 steps

(a) Restriction of DNA using a rare cutting and a commonly cutting restriction enzyme simultaneously (such as MseI and EcoRI) followed by ligation of oligonucleotide adapters, of defined sequences including the respective restriction enzyme sites.

(b) Selective amplifications of sets of restriction fragments, using specifically designed primers. To achieve this, the 5' region of the primer is made such that it would contain both the restriction enzyme sites on either sides of the fragment complementary to the respective adapters, while the 3' ends extend for a few arbitrarily chosen nucleotides into the restriction fragments.

(c) Gel analysis of the amplified fragments.

AFLP analysis depicts unique fingerprints regardless of the origin and complexity of the genome. Most AFLP fragments correspond to unique positions on the genome and hence can be exploited as landmarks in genetic and physical mapping. AFLPs are extremely useful as tools for DNA fingerprinting and also for cloning and mapping of variety-specific genomic DNA sequences. Thus AFLP provides a newly developed, important tool for a variety of applications.

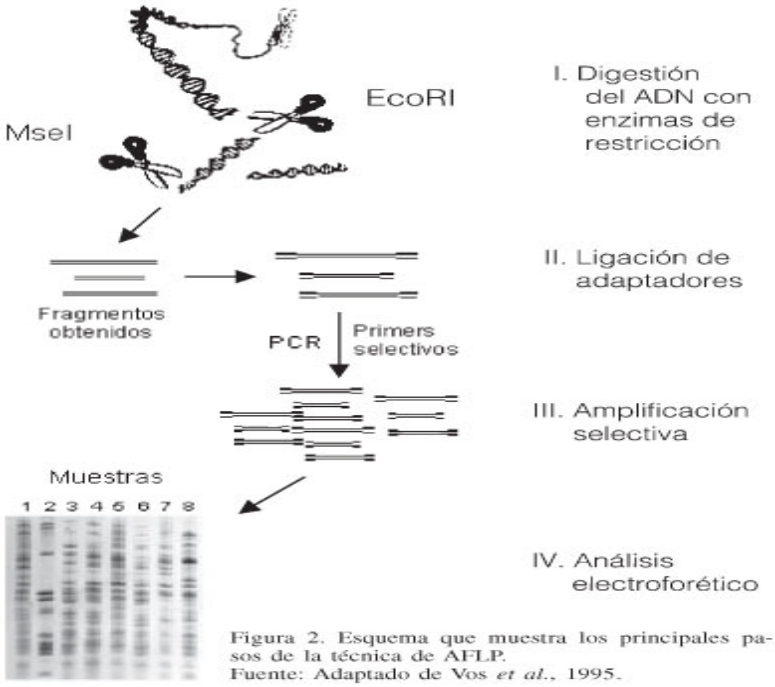


Figura 2. Esquema que muestra los principales pasos de la técnica de AFLP.
Fuente: Adaptado de Vos *et al.*, 1995.

Advantages:

- Fast
- Relatively inexpensive
- Highly variable

Disadvantage:

- Markers are dominant
- Presence of a band could mean the individual is either homozygous or heterozygous for the Sequence - can't tell

RAPD (Random amplification of polymorphic DNA)

Random Amplification of Polymorphic DNA. It is a type of PCR reaction, but the segments of DNA that are amplified are random.

Advantages:

- Fast
- Relatively inexpensive
- Highly variable

Disadvantage:

- Markers are dominant
- Presence of a band could mean the individual is either homozygous or heterozygous for the Sequence - can’t tell which?
- Data analysis more complicated

Micro satellite polymorphism, SSR or Simple sequences repeat

Microsatellites, Simple Sequence Repeats (SSRs), or Short Tandem Repeats (STRs), are repeating sequences of 1-6 base pairs of DNA.

Advantages:

- Highly variable
- Fast evolving
- Co dominant

Disadvantage:

Relatively expensive and time consuming to develop