Molecular Markers:
A molecular marker is a DNA sequence in the genome which can be located and identified. As a result of genetic alterations (mutations, insertions, deletions), the base composition at a particular location of the genome may be different in different plants/organism. These differences, collectively called as polymorphisms can be mapped and identified.

The molecular markers are highly reliable and advantageous in plant breeding programmes:

i. Molecular markers provide a true representations of the genetic makeup at the DNA level and are neutral (non-coding region)

ii. They are consistent and not affected by environmental factors.

iii. Molecular markers can be detected much before development of plants occur.

iv. A large number of markers can be generated as per the needs.

Basic principle of molecular marker detection:
Let us assume that there are two plants of the same species—one with disease sensitivity and the other with disease resistance. If there is DNA marker that can identify these two alleles, then the genome can be extracted, digested by restriction enzymes, and separated by gel electrophoresis. The DNA fragments can be detected by their separation. For instance, the disease resistant plant may have a shorter DNA fragment while the disease — sensitive plant may have a longer DNA fragment (Fig. 53.1).

Molecular markers are of two types:
1. Based on nucleic acid (DNA) hybridization (non-PCR based approaches).

2. Based on PCR amplification (PCR-based approaches).

Markers Based on DNA Hybridization:
The DNA piece can be cloned, and allowed to hybridize with the genomic DNA which can be detected. Marker-based DNA hybridization is widely used. The major limitation of this approach is that it requires large quantities of DNA and the use of radioactivity (labeled probes).

Restriction fragment length polymorphism (RFLP):

RFLP was the very first technology employed for the detection of polymorphism, based on the DNA sequence differences. RFLP is mainly based on the altered restriction enzyme sites, as a result of mutations and re-combinations of genomic DNA. An outline of the RFLP analysis is given in Fig. 53.2, and schematically depicted in Fig. 53.3.The procedure basically involves the isolation of genomic DNA, its digestion by restriction enzymes, separation by electrophoresis, and finally hybridization by incubating with cloned and labeled probes (Fig. 53.2).

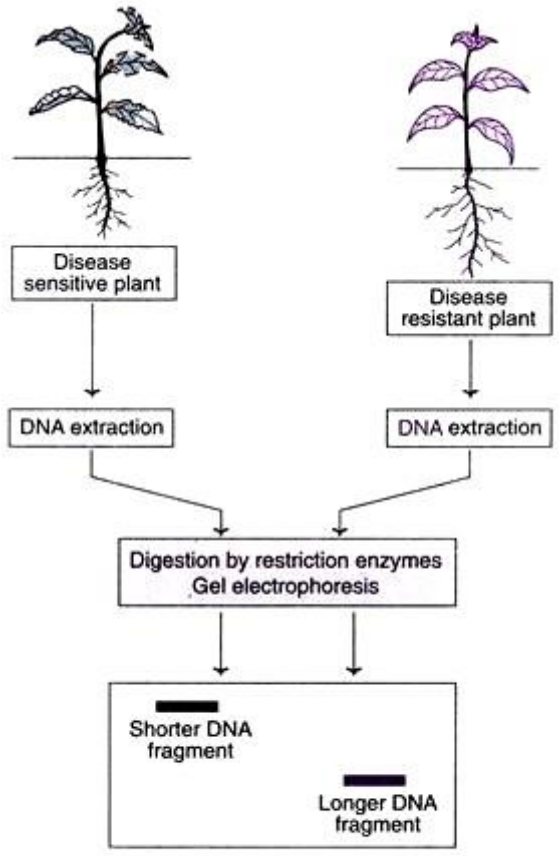

Fig. 53.1 : Basic principle of molecular marker detection (screening of genotypes for the identification of DNA markers).

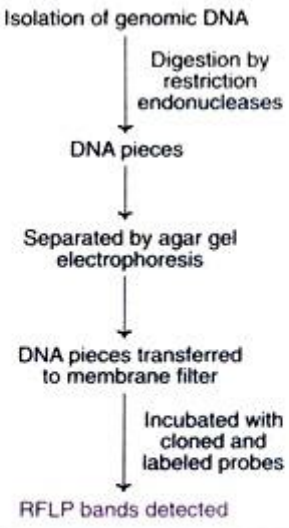

Fig. 53.2 : An outline of restriction fragment length polymorphism (RFLP) analysis as a molecular marker in plant breeding.

Based on the presence of restriction sites, DNA fragments of different lengths can be generated by using different restriction enzymes. In the Fig. 53.3, two DNA molecules from two plants (A and B) are shown. In plant A, a mutations has occurred leading to the loss of restriction site that can be digested by EcoRI.

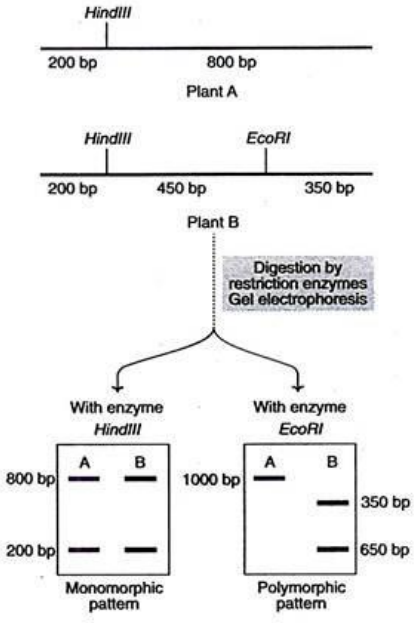

Fig. 53.3 : A schematic representation of restriction fragment length polymorphism (RFLP) analysis as molecular marker.

The result is that when the DNA molecules are digested by the enzyme Hindlll, there is no difference in the DNA fragments separated. However, with the

enzyme EcoRI, plant A DNA molecules is not digested while plant B DNA molecule is digested. This results in a polymorphic pattern of separation.

Markers Based on PCR Amplification:
Polymerase chain reaction (PCR) is a novel technique for the amplification of selected regions of DNA . The advantage with PCR is that even a minute quantity of DNA can be amplified. Thus, PCR-based molecular markers require only a small quantity of DNA to start with.

Random amplified polymorphic DNA (RAPD) markers:
RAPD is a molecular marker based on PCR amplification. An outline of RAPD is depicted in Fig. 53.4. The DNA isolated from the genome is denatured the template molecules are annealed with primers, and amplified by PCR.

Single short oligonucleotide primers (usually a 10-base primer) can be arbitrarily selected and used for the amplification DNA segments of the genome (which may be in distributed throughout the genome). The amplified products are separated on electrophoresis and identified.

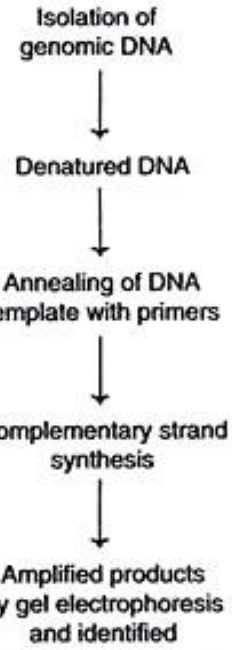

Fig. 53.4 : An outline of random amplified polymorphic DNA (RAPD) analysis as a molecular marker in plant breeding.

Based on the nucleotide alterations in the genome, the polymorphisms of amplified DNA sequences differ which can be identified as bands on gel electrophoresis. Genomic DNA from two different plants often results in

different amplification patterns i.e. RAPDs. This is based on the fact that a particular fragment of DNA may be generated from one individual, and not from others. This represents polymorphism and can be used as a molecular marker of a particular species.

Amplified fragment length polymorphism (AFLP):
AFLP is a novel technique involving a combination of RFLP and RAPD. AFLP is based on the principle of generation of DNA fragments using restriction enzymes and oligonucleotide adaptors (or linkers), and their amplification by PCR. Thus, this technique combines the usefulness of restriction digestion and PCR.

The DNA of the genome is extracted. It is subjected to restriction digestion by two enzymes (a rare cutter e.g. Msel; a frequent cutter e.g. EcoRI). The cut ends on both sides are then ligated to known sequences of oligonucleotides (Fig. 53.5).

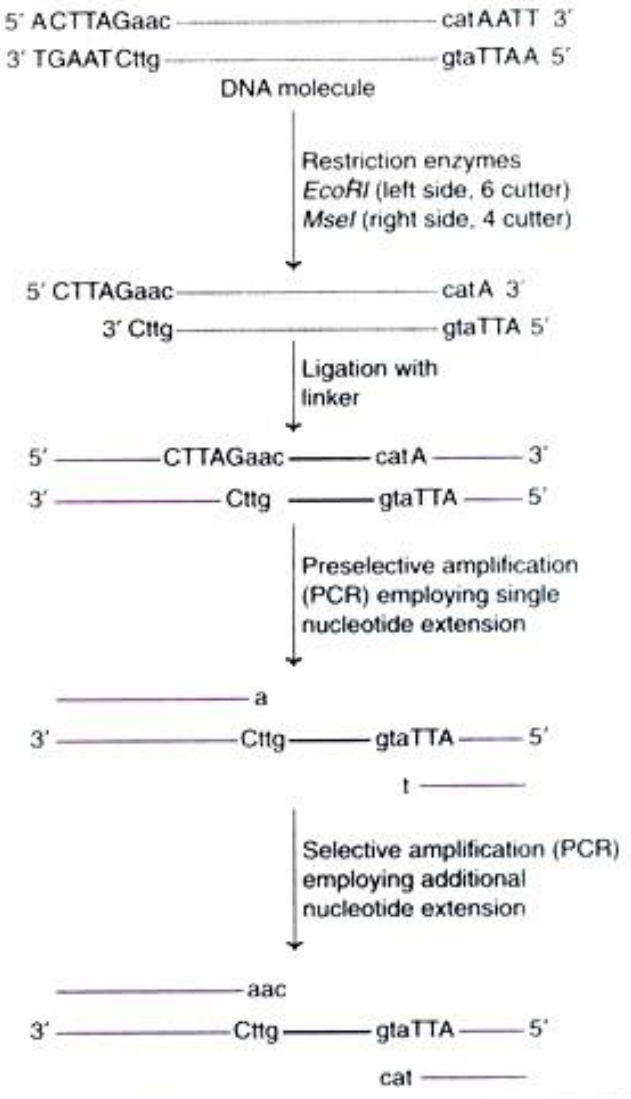

***Fig. 53.5** : A diagrammatic representation of the amplified fragment length polymorphism (AFLP)* ***(Note** : The lower case letters represent the sequences found within the amplified region; the coloured lines indicate linkers).*

PCR is now performed for the pre-selection of a fragment of DNA which has a single specific nucleotide. By this approach of pre-selective amplification, the pool of fragments can be reduced from the original mixture. In the second round of amplification by PCR, three nucleotide sequences are amplified.

This further reduces the pool of DNA fragments to a manageable level (< 100). Autoradiography can be performed for the detection of DNA fragments. Use of radiolabeled primers and fluorescently labeled fragments quickens AFLP.

AFLP analysis is tedious and requires the involvement of skilled technical personnel. Hence some people are not in favour of this technique. In recent years, commercial kits are made available for AFLP analysis. AFLP is very sensitive and reproducible. It does not require prior knowledge of sequence information. By AFLP, a large number of polymorphic bands can be produced and detected.