

Lecture: - 19

BIOTECHNOLOGICAL TOOLS-DNA MARKERS AND MARKER ASSISTED SELECTION

DNA markers:-A DNA marker is a small region of DNA showing sequence polymorphism in different individuals within a species (or) among different species. A wide range of molecular techniques is now available to detect the polymorphism at DNA level. These have been grouped into the following.

- 1) Non PCR based approaches e.g.: RFLP
- 2) PCR based approaches Eg.: RAPD, AFLP, SSRs etc. (plants)

Restricted Fragment Length Polymorphism (RFLP):-This technique was first developed in 1980 and it was based on Southern hybridization technique.

- RFLP is used to describe the variation in the length of fragments obtained from the digestion of DNA from two or more organisms with the same endonuclease.
- The variation at DNA level is assayed by shearing the entire DNA with restriction enzymes. The length of each segment depends on the distance between two adjacent restriction sites.
- Simple base, pairs changes (or) large scale changes as a result of inversions, translocations, deletions (or) transposition may occur. This will result in loss (or) gain of recognition site and in turn lead to restriction fragments of different lengths.
- An RFLP is detected as a differential movement of a band on the gel lanes from different species and strains. Each such bond is regarded as single RFLP locus.
- The pattern of RFLP generated will depend mainly on :-The differentiation in DNA of selected strains (or) species, the restriction enzymes used and the DNA probe employed for southern hybridization.

RFLP analysis comprises the following basic steps:-

- Isolation of DNA
- Cutting of DNA into smaller fragments using restriction endonuclease enzymes

- Separation of DNA fragments by gel electrophoresis
- Transferring of DNA fragments on to a nylon and nitrocellulose membrane filter.
- Visualization of special DNA fragments by using labelled probes
- Analysis of results.

Application (or) uses of RFLP:

- Identification and isolation of any gene known to be linked with an RFLP locus.
- RFLP are co-dominant markers enabling heterozygotes to be distinguished from homozygotes
- The method is simple as no sequence specific information is required
- Used to measure genetic diversity between different populations or related species and also important in studies on evolution
- RFLP'S can be used to supplement regular plant breeding protocols through indirect selection
- Used as a marker in chromosomal mapping
- Used as a diagnostic tool for diseases.

Limitations:

- Requires relatively large amount of highly pure DNA
- Needs a good supply of probes that can reliably detect variation
- Laborious and expensive to identify a suitable marker restriction enzymes combination for genomic or cDNA libraries
- Time consuming as they are not amenable to automation.
- Required expertise in auto radiography because of using radio actively labelled probes.

Random Amplified Polymorphic DNA (RAPD): It was developed by Williams(1991).It is a PCR based molecule marker technique. Here, single short oligonucleotide primers are arbitrarily selected to amplify a set of DNA segments distributed randomly throughout the genome.

- It is a dominant marker.

- RAPD amplification is performed in condition resembling those of PCR using genomic DNA from the species of interest and a single short oligonucleotide (usually 10 base sequences) primer.

Applications:

- Construction of genetic maps
- Mapping of traits
- Analysis of genetic structure of populations
- Finger printing of individuals
- Targeting markers to specific region of the genome
- Identification of somatic hybrids
- A useful system for evolution and characterization of genetic resources.

Advantages of RAPD:

- Simple quick to perform
- Require relatively very small amounts of DNA
- Involves no radio activity.

Limitations: Unable to distinguish between homozygous and heterozygous variety.

Marker Assisted Selection:

- A method of selecting desirable individuals in a breeding scheme based on DNA molecular marker patterns instead of, or in addition to their trait values.
- A tool that can help plant breeders select more efficiently for desirable crop traits.
- MAS is not always advantageous, so careful analysis of the costs and benefits relative to conventional breeding methods is necessary.
- Molecular marker-assisted selection, often simply referred to as marker-assisted selection (MAS) involves selection of plants carrying genomic regions that are involved in the expression of traits of interest through molecular markers.
- MAS has become possible for traits both governed by major genes as well as quantitative trait loci (QTLs).
- In general, the success of a marker-based breeding system depends on three main factors:

- (i) A genetic map with an adequate number of uniformly-spaced polymorphic markers to accurately locate desired QTLs or major gene(s);
 - (ii) Close linkage between the QTL or a major gene of interest and adjacent markers;
 - (iii) Adequate recombination between the markers and rest of the genome; and
 - (iv) Ability to analyse a larger number of plants in a time-and cost-effective manner.
- Molecular marker analysis allows to identify genome segments, so -called quantitative trait loci (QTL), contributing to the genetic variance of a trait and thus to select superior genotypes at these loci without uncertainties due to genotype by environment interaction and experimental error.
 - DNA markers are highly reliable selection tools as they are stable, not influenced by environmental conditions and relatively easy to score in an experienced laboratory.
 - Molecular Breeding is used to describe plant breeding programmes that are supported by the use of DNA – based markers.
 - MAS is the breeding strategy in which selection for a gene is based on molecular markers closely linked to the gene of interest rather than the gene itself, and the markers are used to monitor the incorporation of the desirable allele from the donor source.

Advantages of MAS:

- It can be performed on seedling material.
- MAS is not affected by environmental condition.
- When recessive alleles determine traits of interest.
- Environmental variation in the field reduces a trait's heritability.
- It may be cheaper and faster than conventional phenotypic assay, depending on the trait.

Drawback of MAS:

- MAS may be more expensive than conventional techniques, especially for startup expenses and labor cost.
- Recombination between the marker and the gene of interest may occur, leading to false positives.