

Molecular marker techniques

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Abstract

Genetic markers, differences in the DNA sequence of chromosomes derived from different progenitors, can be visualised in several different ways. Morphological mutations, sometimes called visible markers can be visualised by just looking at the individual. Isozymes, or protein variants require separation by electrophoresis, and are visualised by calorimetric “activity assay” for the relevant enzyme in crude extracts from living tissues. DNA markers are visualised either by use of radioactivity (autoradiography), fluorescence, or by direct chemical staining of the DNA.

Over the last two decades a number of DNA markers have been developed and put into use for different purposes in both animal and plant genetic research. A lot has been written about the different methods and how they work. It is practically impossible to go in detail in to any one of the methods in a short presentation like this. Instead, I will try to briefly outline the basic principles of the techniques, their application, advantages and disadvantages. Looking at the last 15 to 20 years of progress in the development and use of molecular marker techniques for genetic characterisation of a broad range of crop species, the following four techniques emerge as the most popular. To understand the overall impact these techniques have on gene mapping, genome fingerprinting, estimation of genetic diversity and relatedness among germplasm, one needs only look at the amount of information generated every year on different plant species and appreciate how these techniques have revolutionized the study of genome organization and function in a short span of time. These techniques are:

1. Restriction Fragment Length Polymorphism (RFLP),
2. Randomly Amplified DNA Polymorphism (RAPD),
3. Amplified Fragment Length Polymorphism (AFLP), and
4. Simple Sequence Repeats (SSRs), or Microsatellites

In: B.I.G. Haussmann, H.H. Geiger, D.E. Hess, C.T. Hash, and P. Bramel-Cox (eds.). 2000. Application of molecular markers in plant breeding. Training manual for a seminar held at IITA, Ibadan, Nigeria, from 16-17 August 1999. International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru 502 324, Andhra Pradesh, India.

Restriction Fragment Length Polymorphism (RFLP) technique

The RFLP technique consists of DNA isolation from a suitable set of plants, digestion of the DNA with a restriction enzyme, separation of the restricted fragments by agarose gel electrophoresis, transfer of the separated restriction fragments to a filter membrane by a method known as Southern Blotting, detection of individual restriction fragments by nucleic acid hybridisation with labelled cloned probes, and scoring of RFLPs by direct observation of autoradiograms.

RFLP markers arise as a result of several different classes of mutations. The simplest event is substitution of a single nucleotide differentiating two genotypes. When a base substitution eliminates a restriction site, it changes the length of DNA fragment that can be detected by this method, and thus represents a discrete marker which is directly representative of an individual's genotype. Alternatively, rearrangements in the DNA intervening between two restriction sites, or two priming sites, can generate DNA markers. Such rearrangements might include deletion, insertion and/or transposition, or error in DNA replication. The RFLP technique uses molecular biological methods to detect these variations among individuals and populations. This technique is robust, and readily transferable between labs, but it also has limitations that restrict its wider acceptance as the only marker. The first limitation is the quantity of DNA required (50m-200 micrograms of DNA per individual) to generate a DNA fingerprint of the entire genome. Large-scale DNA extraction is tedious and laborious. In contrast PCR-based techniques require only small amounts of crude genomic DNA from each sample, results can be obtained in a short time, the necessary laboratory equipment is not expensive, and they are not technically difficult. The three methods that follow are PCR (polymerase chain reaction)-based techniques for detecting DNA markers much faster and simpler than RFLP technique.

PCR- based techniques

Randomly Amplified Polymorphic DNA (RAPD)

In this technique a single species of primer (10-base, at least 60%GC content) binds to the genomic DNA at two different sites on opposite strands of the DNA template. If these priming sites are within an amplifiable distance (2000 to 5000 bp) of each other, a discrete DNA product is produced through thermocyclic (PCR) amplification. The amplification yields many DNA fragments ranging in size from less than 100 bp to greater than 2 kb. These fragments are anonymous in the sense that their genomic origins are not known. Differences in the fragment patterns amplified from each genomic DNA sample are generally attributed to mutation at

primer binding sites, preventing the annealing of a primer. Because multiple fragments are produced by a single amplification, data are recorded as presence or absence of each fragment in the pattern. These fragments are scored as dominant Mendelian elements.

Amplified Fragment Length Polymorphism (AFLP)

This technique shares some characteristics with both RFLP and RAPD analysis. AFLP combines the specificity of restriction analysis with PCR amplification. The sequence variation detected is the same as that detected by RFLP analysis, but the number of polymorphisms detected per analysis is higher. AFLP uses restriction enzyme-digested genomic DNA as the template for a PCR reaction with primers that contain the restriction enzyme recognition site as well as additional 'arbitrary' nucleotides that extend beyond the restriction site. By varying the number of these additional 'arbitrary' nucleotides that extend beyond the restriction sites into the unknown sequence, it is possible to control the proportion of the ligated fragments that could be amplified. The amplified products are then resolved by polyacrylamide gel electrophoresis. In general, 75 to 150 fragments are amplified with each primer combination, and as each fragment represents a unique site, the proportion of the genome assayed with each primer combination is much higher than with any other DNA analysis approach.

Simple Sequence Repeats (SSRs) or Microsatellites

Microsatellites are tandem repeats of short sequence motifs that occur ubiquitously in eukaryotic genomes. One of the main feature of this class of repetitive DNA is high level of variation among taxa, mainly expressed as a variable copy number of tandem repeats. Length variation of individual microsatellite loci is analyzed by PCR with a pair of locus specific flanking primers. The DNA sequences flanking microsatellites are generally conserved within individuals of the same species, allowing the selection of PCR primers that will amplify the intervening SSR in all genotypes. Variation in the number of tandem repeats, n , results in different PCR product lengths. These repeats are highly polymorphic even among closely related cultivars, due to mutations causing variations in the number of repeating units. Unlike the other PCR-based marker techniques SSRs are inherited in a co-dominant fashion. This allows one to discriminate between homo- and heterozygous state, and increases the efficiency of genetic mapping and population genetic studies.