

Classes of Genetic Markers and it's applications in crop breeding improvement

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Molecular markers could be appropriate choice to study and preserve the range in any germplasm. Molecular markers have diverse applications in crop breeding improvement, mainly in the areas of genetic diversification and varietal identification, sequence tagged analysis, biotic stress diagnostics, pedigree determination, hybrid detection, sex differentiation and MAS. The sequence of nucleotides in polymer of an individual is exclusive and so determines its identity. The ultimate distinction between people lies in the ester sequence of their polymer. These can be wont to diagnose the presence of the sequence while not having to attend for sequence effect to be seen.

Introduction

Linkage maps of the many plant species were restricted in size until the advent of molecular mapping. Primarily its difficult to construct linkage maps. This inability occurred as a result of the harmful effects of the expression of all mutant phenotypes within the single stock. as a result of normal deoxyribonucleic acid or protein molecules are used to score the genetic material, molecular markers are phenotypically neutral. This may be a major advantage as compared to the traditional markers. The three most common forms of markers used these days are RFLP, RAPD and isozymes. Of the three marker types, RFLPs are used extensively. Random Fregment Length Polymorphism markers have many benefits as compared with the RAPD and isozyme markers: 1) they're co-dominant and unaffected by the environment; 2) deoxyribonucleic acid is used for the analysis; and 3) several markers is mapped in a population that's not stressed by the results of phenotypical mutations. The primary disadvantage to RAPD markers is that they're dominant and don't allow the scoring of heterozygous individuals. The weakness of isozyme markers is that every source of the proteins that are being scored may not be expressed within the same tissue. Thus many samplings of the genetic population ought to be created.

RFLP: A molecular marker depended on the differential hybridisation of cloned Deoxyribonucleic acid to deoxyribonucleic acid fragments during a sample of restriction enzyme digested dnas; the marker is restricted to one clone/restriction enzyme combination.

RAPD: A molecular marker supported the differential PCR amplification of a sample of DNAs from short oligonucleotide sequences.

AFLP: A molecular marker generated by a combination of restriciton digestion and PCR amplification.

Isozyme: A molecular marker system supported the staining of proteinious outcomes with identical perform, but it have fully completely different electrophoretic mobilities.

RFLP Loci: RFLP analysis is an application of the Southern hybridization procedure. The principles are explained here which we will then discuss several papers to induce an extra thorough understanding of the procedure.

Clones, Enzymes and Informative Hybridizations.

RFLP markers are described by selected enzyme-probe combination. The primary step within the analysis is to derive a group of clones that may be used to determine RFLPs. Genomic clones that represent sequences randomly square measure a poor selection as interbreeding probes as a result of plant genomes contains a large proportion of recurrent sequences. However, variety of clones clones will contain repitiated sequences and hybridise with those clones contain repeated sequences to generate many hybridisation bands that are difficult to research genetically.

The two primary sources of these clones for RFLP mapping of plants are DNA clones and PstI-derived genomic clones. These two clone sources are usually represent expressed genes whice are in low copy range. Complementary deoxyribonucleic acid clones are DNA copies of expressed genes. PstI clones are based on the expressed genes which aren't methylated. We discussed earlier, in plants the gc and GXC methylation is that the most prominent type of methylation. The enzyme PstI enzyme is C-methylation sensitive. Therefore, the catalyst can only cut non-methylated sites. If a gene is expressed, then its sequence methylated and can be prone to PstI digestion and because they probably contain expressed sequences, these fragments would have a greater chance of being low copy number. Once a series of clones are derived, DNA from potential parental genotypes is digested with a series of enzymes and hybridized with the clones. A number of these hybridizations will generate fragments of only one size and are not polymorphic. Different hybridisations will provides a particular hybridization pattern for every parent. These polymorphisms occur as a result of the sequence of the probe is homologous to restriction fragments of various sizes. Those genotypes that are extremely polymorphic are individuals as parents from that a mapping population are derived.

RAPD Loci

RAPD markers have recently caught the fancy of the many individuals within the field of applied plant breeding. This molecular marker is based on the PCR amplification of random locations within the genome of the plant. With this technique, a single oligonucleotide is used to prime the amplification of genomic deoxyribonucleic acid. As a result of these primers are ten nucleotides long, they need the possibility of annealing at a number of locations within the genome. For amplification product to occur, the binding should be to inverted repeats sequences usually 150-4000 base pairs apart. The several number of amplification outcomes is directly related to the number of the several sequences and its orientation that are complementary to the primer with'n the genome.

Steps of RAPD PCR Amplification of Plant deoxyribonucleic acid

1. Isolate total deoxyribonucleic acid from the individuals of interest.
2. Establish the conditions for the amplification of the deoxyribonucleic acid from wer specific species. Many considerations are: MgCl₂, primer and dNTP concentrations and therefore the quality and concentration of the target deoxyribonucleic acid. The goals of RAPD experiments are to compare populations. This requires well to well and run to run consistency. Thus, we would like to make sure that every well of the cycler can produce identical result given the same, target DNA, primer and dNTP concentration. Without this management we'll not make sure that the amplification polymorphisms ar the results of population variability or cycler variability.

The conditions used in our lab for the amplification of bean DNA are:

- 25 ng DNA
- 200 mM dNTP
- 200 uM primer
- 1.5 mM MgCl₂

- 1X Promega Taq polymerase buffer
- 1 unit Taq polymerase
- Reaction volume: 10 ul overlaid with 35µ l mineral oil.

4. The PCR amplification steps are:

- 94°C, 1 min for denaturation
- 35°C, 30 sec for annealing
- 72°C, 2 min for extension
- This profile is performed forty five times then the product are completed by one extension for seven min at 72°C. (All of those conditions are a modification of published procedures.)

5. The amplification product are then separated on a 200th agarose gel, stained and photographed.

6. Variability is then scored as the presence or absence of a specific amplification product. For RFLP mapping, we first ought to determine the clone/restriction enzyme combinations that are polymorphic between the parents of wer mapping population. The analgous experiment with RAPD markers is to spot the primer and reaction conditions that are polymorphic between ancestors. These primers and conditions are used to amplify and score the product of wer segregating population.

Isozyme Loci

Isozymes are protein markers. The technique relies on the principal that allelomorphic variation exists from many various proteins. As an example, alleles of malic dehydrogenase would each perform the correct enzymatic perform, however the electrophoretic mobility of the two could differ. Therefore, two alternate forms of gene wouldn't migrate to a similar location during a starch gel. The procedures to identify isozyme variation is easy. A crude protein extract is formed from some tissue sources, typically leaves. The extracts are next separated by electrophoresis during a starch gel. The gel is then placed during a solution that contains reagents needed for the enzymatic activity of the enzyme we're monitoring. Additionally, the solution contains a dye that the enzyme will turn into a color reagent that stains the protein. During these manner allelomorphic variants of the protein is visualised in a gel.

Several drawbacks should be noted with regards to isozyme. First, the number of isozyme loci that may be scored is restricted. To date, only 40-50 reagent systems are developed that allow the staining of a specific protein in a starch. Moreover, not all of those reagent systems work efficiently with all plant species. Therefore, for several species only 15-20 loci is mapped. A second disadvantage is tissue variability. Some isozymes are better expressed in certain tissues comparable to roots, whereas other are best sampled in leaf tissue. Therefore, many samplings of the segregating population are necessary to attain all the available isozyme. As a result of neither of those drawbacks have an effect on RFLP or RAPD loci, isozyme loci are seldom scored today.

AFLP Loci

Amplified Fragment Length Polymorphism is the the most up-to-date molecular loci. These are evolved using a simple procedure that combines both restriction digestion and PCR amplification. The ability of this procedure is that we simply will obtain a large number of mappable loci with a single amplification. This may help we saturate a part of the genome rather quickly. The disadvantage is that procedure could be a bit time consuming and needs the running of a deoxyribonucleic acid sequencing gel.

Amplified fregment length polymorphism Procedure:

1. DNA samples digest with restriction endonucleases EcoRI and MseI.

5'-GAATTCN-----NTTAA-3'

3'-CTTAAGN-----NAATT-5"

↓
 AATTCN-----NT
 GN-----NAAT

2. EcoRI and MseI adaptors anneal to restriction products.

????AATTCN-----NTTA????

????TTAAGN-----NAAT????

(???? = unknown sequences that are unique for the primers)

3. by polymeric chain reaction amplification of preselected restricted products with "EcoRI + A" and "MseI +C" oligonucleotide primers.

EcoRI Primer+A

????AATTCN-----NTTA????

????TTAAGN-----NAAT????

C+MseI Primer

4. Earlier selected products are selective amplified through the polymeric chain reaction by using the endonucleases "EcoRI + 3'" and "MseI +3" oligonucleotide primers.

EcoRI Primer+AAC

????AATTCA-----GTTA????

????TTAAGN-----CAAT????

AAC+MseI Primer

5. The fragments are Separated by the denaturing polyacrylamide gel electrophoresis.

Detecting deoxyribonucleic acid Polymorphisms

Because any deoxyribonucleic acid molecule larger than ten base pairs contains essentially a similar mass-to-charge ratio, any procedure that separates the molecules based on mass alone are helpful to uncover deoxyribonucleic acid polymorphisms. Currently, gel electrophoresis is that the most often used procedure to detect these polymorphisms. However as we move into the post genomic era, techniques that rapidly screen large numbers of samples are emerging. The most recently wide applied procedure is capillary array electrophoresis. In the near future, matrix-assisted laser desorption/ionization time-of-flight mass spectrographic analysis (MALDI-TOF MS) could replace many of the current procedures.

Gel electrophoresis

Gel electrophoresis is most widely adapted technique for detection polymorphism. Samples are loaded into a gel and allowed to migrate in an electrical field. Since deoxyribonucleic acid is negatively charged, the samples are loaded close to the negative pole, and that they migrate toward the positive pole. Separation of the molecules is strictly supported size: the smallest fragments move farther within the gel as a result of they'll navigate through the small pores within the gel better than large molecules.

Agarose		Polyacrylamide	
%	Resolution (kb)	%	Resolution (bp)
0.9	0.5 - 0.7	3.5	1000 – 2000
1.2	0.4 - 6.0	5.0	80 – 500
1.5	0.2 - 3.0	8.0	60 – 400
2.0	0.1 - 2.0	12.0	440 – 200

The two gel matrices used to separate the molecules are agarose and polyacrylamide. The separation capabilities of every molecule is performed by the concentration of the polymer within the gel. The table below shows the resolution which will be obtained with different polymer concentrations.

We choose the different kind of polymers to use based on the range of fragment that needs to be distinguished. For RFLP and RAPD procedures, agarose is chosen. As a result of microsatellites and AFLPs procedures generate smaller fragments for comparison, polyacrylamide is often used. Following gel electrophoresis to separate molecules based on mass, the polymorphism is disclosed using a detection agent. The dye ethidium bromide is often used to reveal RAPD polymorphisms in agarose gels. Silver nitrate is a procedure used to find polymorphisms in polyacrylamide gels. This has been used for both microsatellites and AFLPs. With these genetic markers, it's an option to include a radio-labelled nucleotide during the polymeric chain reaction (PCR) step. If this feature is chosen, the gel is used to expose autoradiographic film. The film is used for analyzing to detect the polymorphism.

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