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THE ABC'S OF PLANT RFLPs

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ABSTRACT

Traditionally, plant geneticists and plant breeders relied on easily recognizable phenotypic differences, such as flower color, in their studies. More recently, isozyme marker systems, which distinguish among different forms of enzymes, have increased the number of readily scoreable phenotypes. With the advent of restriction fragment length polymorphism (RFLP) markers, it is now possible to detect differences in DNA sequences that do not necessarily result in phenotypic differences. This is because RFLP markers are different among homologous DNA fragment lengths that result from base pair changes in the restriction enzyme recognition sites between genotypes. RFLP markers can be observed after the restriction fragments have been separated by agarose gel electrophoresis, Southern blotted, and hybridized with a radioactively labeled DNA probe which is homologous to these fragments (locus). Therefore, RFLPs serve as genetic markers.

INTRODUCTION

Most organisms found in nature have unique genotypes; the differences are reflected in the DNA sequences of each genotype. When comparing two genotypes, their similarities and differences can be assessed using morphological, isozymic, or restriction fragment length polymorphism (RFLP) markers (Bernatzky and Tanksley, 1986; Evola et al., 1986; Helentjaris et al., 1986; Tanksley et al., 1986). Traditionally, plant geneticists and plant breeders depended upon morphological markers to identify different genotypes and to develop genetic maps. A cultivated crop, such as tomato, has about 300 morphological markers mapped. The development of isozymic markers expanded the number of loci that could be mapped.

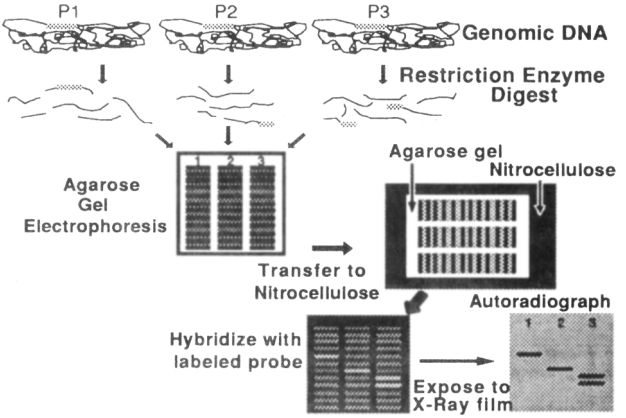
RFLP markers are a powerful tool that can be used to characterize plant genotypes (DNA fingerprinting)(Bunce et al., 1986; Helentjaris et al., 1985; Landry et al., 1987; Welker et al., 1986), and to locate genes that code for important agronomic traits (RFLP linkage mapping) (Beckman and Soller, 1983; Burr et al., 1983; Edwards et al., 1987; Helentjaris, 1987; Lander and Botstein, 1989; Landry et al., 1987), RFLP markers require that

DNA differences between genotypes result in changes in the relative locations of restriction enzyme recognition sites. These differences may or may not be within a gene. Morphological and isozyme markers are of more limited use because a mutation must occur within a gene to be observed.

Southern Blot

The RFLP technique is used to characterize plant genomes according to unique sets of restriction fragments visualized by Southern blotting. Southern blot analysis (Figure 1) begins by extracting the genomic DNA from each plant genotype. This DNA is digested with a restriction enzyme, and then subjected to gel electrophoresis to separate the DNA restriction fragments by size. The size fractionated DNA is then immobilized on nitrocellulose by blotting. The blotted DNA is hybridized with a radioactively labeled DNA probe, which allows for the hybridized DNA to be visualized by autoradiography. An informative Southern blot will produce different banding patterns between different genotypes.

Figure 1. Southern blot procedure.



Restriction Endonuclease

RFLP markers between two or more genotypes are different lengths of homologous fragments of the genomic DNA (which was cut into fragments by a restriction enzyme) that are observed as different banding patterns after Southern blotting. Only when the homologous fragments being compared are of different lengths can an RFLP be observed. The restriction enzyme that cuts the genomic DNA into fragments recognizes specific short sequences (usually

4-6 base pairs) which occur randomly throughout the genomes of plants. Due to the variation in DNA sequences that exist between plants in nature, every genotype should have enzyme recognition sites in different places. For example, if an area of DNA has restriction endonuclease recognition sites for Eco Ri (GAATTC) in one genotype, a second genotype may have a base pair change at that site (GATTTC). Because of the difference in this sequence, it is no longer an Eco Ri "site" and the enzyme will not cut the DNA, thereby resulting in different size fragments for that homologous DNA. Such base pair substitutions from genotype to genotype can occur throughout the DNA of the plant. But, it is only one way to explain why restriction enzyme recognition sites are different for each distinct genotype. For this reason, the way an individual genome can be cut into fragments by restriction enzyme is a unique property to that genotype.

Agarose Gel Electrophoresis and Blotting

When the genomic DNA of different genotypes is digested with a restriction enzyme, the DNA is cut into many ($>10^6$) DNA fragments. These fragments are electrophoresed through an agarose gel. The fragments are separated by size and visualized as a smear (bands are contiguous, not discrete). There are fragments in the smears of each genotype that came from the same region of DNA (homologous) in each genotype. These size-fractionated DNA fragments are immobilized by blotting onto nitrocellulose. To locate those fragments within all the millions of other fragments present, the blot is hybridized with a probe.

What is a Probe?

A probe is a piece of cloned DNA which has been labeled. A clone may be selected from a genomic library. A genomic library is constructed from DNA isolated from a plant species or genotype. This DNA is digested with a restriction enzyme that cleaves at specific sequences, positioned randomly throughout the plant genome. A cloning vector is digested with the same restriction enzyme or with a restriction enzyme that will produce compatible or "sticky" ends. The plant DNA fragments to be cloned and the vector DNA are ligated (bonded) together with DNA ligase to form recombinant DNA molecules. These molecules are used subsequently to transform competent E. coli cells. After the transformation procedure, the bacterial cells are plated on a selective medium containing an antibiotic which allows only bacteria transformed with the vector DNA to grow. Bacteria that have been transformed with the recombinant DNA molecules can then be selected. Each of the transformed bacterial colonies which contains a recombinant plasmid will have a clone of a portion of the plant genome. All the clones together comprise the genomic library. Clones from this library serve as a source of probes. To be used as a probe the clone must be labeled, usually radioactively with ^{32}P.

Probe/blot Hybridization and Autoradiography

The labeled probe is denatured at 95°C for 10 minutes and hybridized to the blot overnight. The unbound probe is washed from the blot and the blot is exposed to X-ray film (autoradiography). Although only one type of probe is hybridized to the blot of digested genomic DNA at a time, millions of copies of this one probe have been radioactively labeled, so that the hybrid formed between the probe and the genomic DNA can be seen clearly on the autoradiograph. The purpose of the probe is to mark the fragment of genomic DNA containing the complementary (homologous) sequence to the probe found in each genotype and compare the fragment sizes of each genotype. Therefore, the areas on the blot where the hybridization occurred will be visible after development of the X-ray film.

If all of the fragments in all the genotypes that hybridize to the probe (marked) are the same size, then there is no polymorphism (RFLP) between the genotypes for that area of DNA. A probe with this result is not useful. Instead, if there are any differences in the fragment sizes that hybridize to the probe, then polymorphisms (RFLPs) do exist between the genotypes in that area of the DNA. A useful probe is one that marks fragments of different lengths, indicating that there are polymorphisms present within that area of the DNA of the genotypes being studied. Although RFLPs can provide insight into the DNA sequence of the genotypes tested, this technique is not and does not require DNA sequencing.

SUMMARY

RFLPs can be used to: 1) identify genotypes (DNA fingerprinting); 2) protect plant varieties; 3) evaluate genetic variation; 4) establish linkage maps; 5) study genome organization; 6) study gene action; 7) evaluate genetic diversity; 8) evaluate evolutionary relationships; 9) select desirable genotypes, molecular-marker-facilitated selection (MMFS); and 10) clone genes (chromosome walking and jumping) (Bunce et al., 1986; Gardiner et al., 1990; Helentjaris, 1987; Rommens et al., 1989; Tanksley et al., 1989).

One advantage of RFLP markers relative to morphological or isozymic markers is the potential for the identification of an almost unlimited number of RFLP loci in the genome (Tanksley et al., 1989). Consequently, the possibility exists to saturate a plant genome with RFLP markers. A second advantage is that RFLP markers have a heritability equal to one, with genotype equaling phenotype. Therefore, RFLP markers can be used for indirect selection of traits (MMFS) which are tedious to score/screen or are of low heritability (Tanksley et al., 1986).

The use of MMFS in crop improvement is based on the tight linkage of a unique RFLP band (allele) and a favorable allele, of

a locus coding for a trait of interest (eg. disease or insect resistance; Landry et al., 1987; Nienhuis et al., 1987). MMFS could prove to be a valuable tool for producing new cultivars by increasing the efficiency of parent and progeny selection in breeding programs, and introgression of useful traits from wild or other related species into cultivated species (Tanksley et al., 1989).

RFLP analysis could accelerate breeding progress by providing an increased understanding of the gene action that conditions important quantitative traits (Edwards et al., 1987; Lander and Botstein, 1989; Osborn et al., 1987; Paterson et al., 1988). RFLP analysis could be used to measure shifts in allelic and genotypic frequencies under various circumstances. To be able to assign real values to parameters of quantitative genetic theory would provide a powerful means for characterizing gene action and for evaluating different methods of selection and mating schemes for the production of varieties. Therefore, the integration of RFLP techniques into plant breeding promises to:

1) expedite the movement of desirable genes among varieties,

2) allow the transfer of novel genes from related wild species

3) make possible the analysis of complex polygenic characters as ensembles of single Mendelian factors, and

4) establish genetic relationships between sexually incompatible crop plants (Tanksley et al., 1989).

REFERENCES

Beckman, J.S., and Soller, M. 1983. Restriction fragment length polymorphisms in genetic improvement: methodologies, mapping and costs. Theoretical and Applied Genetics 67:35-43.

Bernatzky, R., and Tanksley, S.D. 1986. Toward a Saturated linkage map of tomato based on isozyme and random cDNA sequences. Genetics 112:887-898.

Bunce, N.A.C., Forde, B.G., Kreis, M., and Shewry, P.R. 1986. NA restriction fragment length polymorphism at hordein loci: application to identifying and fingerprinting barley cultivars. Seed Sci. & Technol. 14:419-429.

Burr, B., Evola, S.V., Burr, F.A., and Beckman, J.S. 1983. The application of restriction fragment length polymorphism to plant breeding. In: Genetic Engineering: Principles and Methods. Vol. 5, pp. 45-59. J.K. Setlow and A. Hollaender (eds.). Plenum Press, New York.

Edwards, M.D., Stuber, C.W., and Wendel, J.F. 1987. Molecular-marker facilitated investigations of quantitative-trait loci in maize. I: Numbers, genomic distribution and types of gene action. Genetics 116:113-125.

Evola, S.V., Burr, F.A., and Burr, B. 1986. The suitability of restriction fragment polymorphisms as genetic markers in maize. Theoretical and Applied Genetics 71:765-771.

Gardiner, J., Hosisington, D., Coe, E., Ciao, S., and Melia-Hancock. 1990. Preferred set of RFLP probes. Maize Genetics Cooperation Newsletter 64:47-48.

Helentjaris, T. 1987. A genetic linkage map for maize based on RFLP's. Trends in Genetics 3:217-221 .

Helentjaris, T., King, G., Slocum, M., Siedenstrang C., and Wegman, S. 1985. Restriction fragment polymorphisms as probes for plant diversity and their development as tools for applied plant breeding. Plant Molecular Biology 5:109-118.

Helentjaris, T., Slocum, M., Wright, S., Schaefer, A., and Nienhuis, J. 1986. Construction of genetic linkage maps in maize and tomato using restriction fragment length polymorphisms. Theoretical and Applied Genetics 72:761-769.

Lander, E.S., and Botstein, D. 1989. Mapping mendelian factors underlying quantitative traits using RFLP linkage maps. Genetics 121:185-199.

Landry, B.S., Kesseli, R.V., Farrara, B., and Michelmore, R.W. 1987. A genetic map of lettuce (Lactuca sativa L.) with restriction fragment length polymorphism, isozyme, disease resistance and morphological markers. Genetics 116:331-337.

Landry, B.S., Kesseli, R., Leung, H., and Michelmore, R.W. 1987. Comparison of restriction endonucleases and sources of probes for their efficiency in detecting restriction fragment length polymorphisms in lettuce (Lactuca sativa L.). Theoretical and Applied Genetics 74:646-653.

Nienhuis, J., Helentjaris, T., Slocum, M., Ruggero, B., and Schaefer, A. 1987. Restriction fragment length polymorphism analysis of loci associated with insect resistance in tomato. Crop Sci. 27:797-803.

Osborn, T.C., Alexander, D.C., and Fobes, J.F. 1987. Identification of restriction fragment length polymorphisms linked to genes controlling soluble solids content in tomato fruit. Theoretical and Applied Genetics 73:350-356.

Paterson, A.P., Lander, E.S., Hewitt, J.D., Peterson, S., Lincoln, S.E., and Tanksley, S.D. 1988. Resolution of quantitative traits into mendelian factors using a complete map of restriction fragment length polymorphisms. Nature 335:721-726.

Rommens, J.M., Iannuzzi, M.C., Kerem, B., Drumm, M.L., Melmer, G., Dean, M. Rozmahel, R., Cole, J.L., Kennedy, D., Hidaka, N., Zsiga, M. Buchwald, M., Riordan, J.R., Tsui, L., and Collins, F.S. 1989. Identification of the cystic fibrosis gene: chromosome walking and jumping. Science 245:1059-1065.

Tanksley, S.D., Miller, J., Paterson, A., and Bernatzky, R. 1986. Molecular mapping of plant chromosomes. Proceedings of the 18th Stadler Genetics Symposium 157-173.

Tanksley, S.D., Young, N.D., Paterson, A.H., and Bonierbale, M.W. 1989. RFLP mapping in plant breeding: new tools for an old science. Bio/Technology 7:257-264.

Welker, D.L., Hirth, K.P., Romans, P., Noegel, A., Firtel, R.A., and Williams, K.L. 1986. The use of restriction fragments length polymorphisms and DNA duplications to study the organization of the actin multigene family in Dictyostelium discoideum. Genetics 112:27-42.