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Plant DNA amplification fingerprinting: a strategy for plant genomic analysis

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Abstract *- The survival of all "higher" animals on Earth depends on plants. Instead of focusing on individual genes, molecular genetics methods have made it possible to analyse the structure, evolution, and function of entire plant genomes. The precise genetic maps of model plants and all main crop species were created using DNA-based markers. A DNA sample's chemistry or sequencing information can be used to describe it for individual identity. This kind of DNA analysis has been referred to as "identification testing," "fingerprinting," "profiling," "typing," and "genotyping." Forensic identification, family relationship determination, genome linkage mapping, prenatal diagnosis, localization of disease loci, detection of genetic variation, molecular archaeology, and epidemiology are all applications of DNA fingerprinting.*

I. Introduction

Genomic analysis is the process of identifying, quantifying, or contrasting genome features at the genomic scale, such as DNA sequence, structural variation, gene expression, and regulatory and functional element annotation. Physical maps and the initial plant whole genome sequences were built on the molecular maps. The evolution of plant nuclear and organellar genomes was better understood thanks to comparative research based on genetic, cytogenetic, and physical maps as well as DNA sequence data. The cloning and functional characterisation of novel genes that function in plant growth, adaptation to biotic and abiotic stress, or in the production of other agronomic features were made possible by the mapping of variables affecting Mendelian and quantitative traits. The examination of all transcripts, proteins, and metabolites found in plant cells or tissues in parallel has produced data that could help us better understand how the genome works as a whole. New diagnostic and therapeutic molecular tools are expected to be produced by postfunctional study of natural variations in gene function and their impact on phenotypic for use in plant breeding, adaption, and ecology.

Blood group typing and isozyme analysis are two biological comparison methods that don't rely on DNA-based technologies. However, there are many benefits to DNA analysis done directly. DNA directly represents the relatedness or phylogeny of the sample material because DNA is the medium of heredity. Even single cells from a person can ultimately be identified since DNA chemistry (methylation, packaging, etc.) varies between cell types and individual cells inherit base pair mutations at every cell division that are themselves stably inherited. Very small DNA samples obtained from just a few cells can be examined using more current methods involving DNA amplification.

It has been used for genome linkage mapping, identity testing, determining family relationships and genetic variation, population and pedigree analysis, forensic identification, localising disease loci, and epidemiology. This process of characterising a DNA sample for individual identity based on its chemistry or sequence information is frequently referred to as DNA "fingerprinting." DNA-based procedures immediately reflect the relatedness or phylogeny of the sample material, in contrast to numerous methods for biological comparison, such as blood group typing and isozyme analysis. Additionally, because DNA chemistry differs across different types of cells and base pair mutations are persistently acquired during each cell division cycle, they may be able to tell one cell from another. DNA is the optimal analytical environment due to its versatility, ubiquity, and stability. DNA amplification, for instance, can be used to evaluate DNA from small, somewhat deteriorated forensic samples, DNA from a small number of cells, or ancient DNA from mummified or fossilised material. DNA's nucleotide sequence variation can be used to create distinctive fingerprints. The immense complexity of the DNA beginning material must frequently be reduced into basic yet recognisable patterns in order to evaluate or compare complete genomes.

The ability to distinguish between plant genotypes and/or to calculate the degree of variety and relatedness in a collection of genotypes is a prerequisite for many fields of botany. Such tasks have traditionally been carried out primarily using data on physical traits, but these have some drawbacks, including insufficient diversity among the genotypes under study, subjectivity in the data collection and handling, and plasticity caused by environmental variation. Molecular markers based on isoenzymes, or enzymes that catalyse the same

chemical process but differ in amino acid sequence and consequently in the speed at which they move across an electrophoretic gel, provided a more objective and neutral tool. Early in the 1960s, isoenzymes were introduced to plant research, and their significance significantly grew in the 1970s and 1980s. For studies of things like population structure, gene flow, isolation-by-distance (IBD), mating systems, and hybridization, co-dominant allozyme data—which are allelic enzymes coded by genes at the same locus—quickly gained a lot of popularity. However, protein extraction was frequently problematic, particularly for plants whose leaves had high polyphenol concentrations. Since proteins often need to be separated and purified within a short period of time from sampling, the examination of plants growing in remote places presented another challenge. The sometimes insufficient level of allozyme polymorphism among related genotypes was the third significant issue.

The DNA molecule is much more durable and manageable than proteins, and its capacity to produce polymorphic data is practically limitless. When the DNA-based restriction fragment length polymorphism (RFLP) technology was developed in the 1970s, botanists were able to examine samples taken from plants that were growing practically anywhere. Before being brought to a lab, samples, generally leaves, were often dried on silica gel so that they could be kept frozen until DNA isolation. The RFLP method required the isolation of genomic DNA from the collected material, cutting the DNA samples with restriction enzymes, transferring the fragments to a filter with Southern blotting, hybridising the filter-bound fragments with locus-specific probes, and finally using, for instance, autoradiography to detect the fragments. However, the main obstacle was the requirement to create species-specific hybridization probes for these investigations. As a result, the RFLP technology was mostly used on commercially significant crop species, with many active scientists and substantial grants. In these crops, RFLP markers were a highly valued tool for the creation of genetic maps, as well as occasionally for cultivar identification and genetic relatedness research. But even with the RFLP approach, a lack of available appropriate loci frequently led to insufficient polymorphism.

The chloroplast DNA (cpDNA) molecule was also the first genetic material to be subjected to RFLP analysis in the 1980s. To do this, DNA samples were digested using either a single restriction enzyme or a combination of enzymes, and then hybridised with radiolabelled cpDNA-specific probes from one of the universal libraries created from, for instance, Petunia. The cpDNA molecule's restriction site maps were created using the information that was discovered. Since there is little intra-specific variation in the highly conserved cpDNA molecule, most cpDNA-based RFLP experiments have been carried out on an inter-specific level. Plant mitochondria, on the other hand, have rarely been used in molecular investigations. The main cause is that although while the structure and size of mtDNA molecules can differ greatly among individual plants, the sequence of plant mitochondrial DNA (mtDNA) is typically well conserved. Furthermore, recent research showed that even among closely related plant species, the rates of mtDNA gene substitution might differ greatly.

Nobody anticipated that the so-called DNA fingerprinting technique, which Jeffreys and colleagues first described in their seminal articles on RFLP studies with probes made from tandemly repeated DNA sequences in human DNA, would likewise change the study of plants. Botanists soon opted to research the possible uses of this technology in plants, though, as these new minisatellite probes shown a high potential for disclosing individual-specific DNA fingerprints also in other mammals and in birds. By fusing restriction-digested rice DNA with the human 33.6 minisatellite probe, Dallas was able to differentiate between several varieties of rice, Oryza sativa, in a work published in 1988. Due to rice's self-pollinating nature and high homozygosity, it was discovered that the progeny from a single rice plant studied all had the same fingerprints. Dallas was also able to determine the Mendelian inheritance of DNA pieces from grandparents to the kids of the second generation (F2). Three PCR-based methods to create DNA fingerprints were published more or less simultaneously shortly after Saiki and colleagues developed the brilliant PCR procedure. All of these techniques generated PCR fragments from genomic DNA using single oligonucleotide primers with random sequences, resulting in multi-locus banding patterns that could be seen by staining or radiography following electrophoretic separation. The strategy known as random amplified polymorphic DNA (RAPD), created by Williams and colleagues, quickly rose to the top of this list of techniques. The modest amounts of sample DNA required and the quick and easy procedures compared to hybridization-based approaches are the main causes of this instant success.

The chloroplast is the organelle that is most frequently employed in genetic research on plants. Recombination occurs infrequently or not at all in plastid genomes, allowing the formation of "haplotypes" from all of an individual's DNA polymorphisms. Because intra-specific plastid DNA polymorphisms are rather uncommon, the number of observed band profiles (haplotypes) is frequently much lower than that of nuclear markers. On the plus side, because organellar DNA sequences are highly conserved, it has been possible to create non-specific, or "universal," PCR primers that can amplify cpDNA introns and intergenic spacers in a variety of plant species. There are also universal primers for the amplification of SSR sites in the chloroplast genome available. Numerous methods exist for detecting polymorphisms within amplified fragments, such as high-resolution electrophoresis for detecting length variations, sequencing for detecting sequence variations, or PCR-RFLP, which involves digesting the PCR results with restriction enzymes.

Plastid DNA is particularly helpful for research projects where a low mutation rate is desired, including the examination of phylogenetic and phylogeographic patterns. For supplementary data, plastid and nuclear markers are frequently integrated in the same study. These markers also offer the potential for tracking uni-parental lineages over significant periods of time and space, as the route of plastid inheritance is typically maternal in angiosperms and paternal in gymnosperms.

Applications of present-day DNA fingerprinting in plants

Genotype identification

Since its modest beginnings in 1988, DNA fingerprinting has evolved into a crucial tool for genotype identification in both wild and domesticated plant species. Plants vary greatly in their life cycle characteristics, including reproductive characteristics like the method of reproduction and, for those that reproduce by seed, also in their breeding system (selfing or cross-pollinated), as well as in the manner in which pollen and seeds are dispersed. The amount and distribution of genetic variability across and within different entities, such as cultivars and populations, are significantly influenced by all of these variables. These variations have an impact on how DNA markers are used to identify certain plants or genotypes.

In some instances, estimations of genetic similarity based on DNA show a pretty close correlation with earlier estimates based on morphology, but in other instances, there are also significant disparities. Comparatively to qualitative traits, which are more likely to reflect only a limited number of mutation events, correlation with DNA marker estimations is typically rather good when the morphological characters are largely quantitative in nature. Additionally, it has been proposed that while traditional pomological characterization data are more directly linked to physiological characteristics, molecular data are better at discriminating cultivated genotypes as well as their wild cousins according to origin and pedigree.

Genotype identification in wild plants

Numerous research based on wild plants have an important foundation in the accurate identification of specific genotypes. As was already mentioned, different life history variables have an impact on the amount and distribution of genetic variation. For instance, inbreeding species are best suited for forensic applications because they frequently result in patches of plants that are almost or exactly genetically identical in size and have the same genetic makeup. As opposed to this, outcrossing species have a condition where each plant has a unique genotype. It is typically quite challenging to get forensic evidence regarding a specific, unique plant specimen, despite the fact that it could be incredibly instructive. Clonal plants usually generate a high number of offspring with the same genotype, whether as a result of intensive vegetative propagation or apomixis. Such genotypes are insufficiently precise to link botanical data to a specific location since they might span wide geographic ranges.

The heterogeneity in plant life cycle traits, however, can be seen as a benefit in other research domains; by selecting the right materials and techniques, a wide range of biological problems can be addressed. Thus, genotype age in plant clones, which frequently turned out to be significantly larger and thus frequently older than anticipated from prior data, has been estimated using DNA marker studies. For instance, Carex curvula, a sedge species widespread in the European Alps, was investigated by Steinger and colleagues. 116 tillers from a small patch (2.0 0.4 m) were analysed using RAPD to find a total of 15 multi-locus genotypes. More over half of the tillers in the studied population turned out to be part of a single, sizable clone thought to be about 2,000 years old. The Japanese knotweed, Fallopia japonica, and the alligator weed, Alternanthera philoxeroides, both of which revealed a single RAPD phenotype despite being tested over extremely broad areas, are examples of invasive species that occasionally form particularly enormous clones. Other times, DNA marker studies have shown greater heterogeneity than anticipated. Thus, it was demonstrated that each of the five examined Chinese populations of the invasive water hyacinth Eichhornia crassipes consisted of at least three distinct clones based on their RAPD profiles.

Finding out about clonal proliferation can be quite beneficial for figuring out the elements that influence population structure. A microsatellite-based study in the marine eelgrass Zostera marina revealed a favourable correlation between clonal size and heterozygosity. The size and number of blooming shoots in outbreeding clones showed that inbreeding depression had reduced vigour and fecundity. In the geophyte Gagea spathacea, a surprisingly high level of genetic homogeneity has just been reported. 138 specimens were analysed, and all but two of them had identical AFLP profiles. These specimens represented 52 populations across the full distributional range in northern, central, and eastern Europe. This extremely polyploid taxon most likely descended from a hybridogenic event. Rather than seed production and seedling establishment, bulbil production and spread allowed it to proliferate over a broad area.

Additionally, DNA fingerprinting has aided in the understanding of the reproductive system in animals that have both sexual and asexual seed production mechanisms (that is, by apomixis). Many Taraxacum populations are clonal because they contain triploid individuals that appear to reproduce through apomixis.

These clones can occasionally cover considerable territories, as was shown in an AFLP investigation. Both marker types were able to distinguish between Taraxacum's nine apomictic microspecies (identified on the basis of morphological traits), according to a comparison of SSR and AFLP data, but AFLP was more sensitive in spotting subtle, mutation-derived changes within each microspecies. In contrast, apomictic lineages of Ranunculus carpaticola showed significantly greater diversity at two dinucleotide repeat SSR loci than at AFLP. The absence of allele segregation in the examined SSR sites offered evidence for an origin via mutation as opposed to recombination. As a result, a locus in each lineage always included the same number of alleles, and these alleles within each lineage also formed classes of related allele sizes.

Conclusions and Summary- Conventional DNA fingerprinting has successfully employed the polymerase chain reaction (PCR) to enzymatically amplify specific DNA sequences. Two 20 nucleotide long oligonucleotide primers are used in PCR, and they selectively hybridise to opposing DNA strands that are on either side of an area that needs to be amplified. The target DNA area will be amplified many times over by a series of cycles involving DNA denaturation, primer annealing, and extension of the annealed primers by DNA polymerase, resulting in an amplified fragment whose termini are determined by the 5' ends of each primer. Radiolabeled nucleotides have been included into DNA fingerprinting probes by the use of PCR. These probes are very helpful when there is a small ratio between probe and target size since they have a very high specific activity. Additionally, the PCR procedure can be used to specifically amplify hypervariable areas inside predetermined loci. Changes in electrophoretic mobility can be used to identify changes in DNA sequence and single-base substitutions that affect DNA conformation. These single-strand conformation polymorphisms (SSCPs), which can detect allelic polymorphism repetitions at various chromosomal loci, are found in PCR amplified and tagged areas.

Even with the aid of PCR, it takes a lot of experimental manipulation, prior knowledge of the DNA sequence, or cloned and defined probes to detect DNA polymorphisms using standard fingerprinting techniques. Recently, numerous labs have employed a PCR-based method to amplify brief, arbitrary sections of DNA from a target genome in order to get around these restrictions. In these research, under less stringent conditions, amplification employing a thermostable DNA polymerase driven by one or more oligonucleotides of arbitrary sequence produced a distinctive spectrum of products. AFLPs are used as genetic markers in DNA recombination between pairs of maternally and paternally derived chromosome homologs during meiosis provides the foundation for genetic linkage analysis of genomes. In a variety of plant species, significant efforts have been made to obtain high-resolution RFLP genetic maps and, in some cases, to resolve multiple loci controlling quantitative features.

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