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Research Paper

Plant DNA Amplification Fingerprinting: A Way to Look At a Plant's Genome

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Abstract *- DNA fingerprints have gained a lot of attention due to their tremendous practical relevance because they have multiple loci, considerable variability, and straightforward, consistent inheritance. DNA fingerprints have high variability and somatic stability, which may be used to identify people, which is extremely useful in forensics to identify criminals and in determining kinship between people. Other uses for DNA fingerprints include reliable variety-level identification and significant contributions to plant breeding. Animal evolutionary trends and the identification of animal kinds are two more fields where DNA fingerprints are frequently employed in study. All "higher" animals on Earth rely on plants for their survival. The structure, evolution, and function of complete plant genomes may now be studied using molecular genetics techniques rather of concentrating on particular genes. DNA-based markers were used to construct the precise genetic maps of model plants and all major crop species. The chemistry or sequencing data of a DNA sample can be used to characterize it for individual identity. Various terms for this type of DNA analysis include "identification testing," "fingerprinting," "profiling," "typing," and "genotyping." Applications of DNA fingerprinting include forensic identification, establishing familial relationships, genomic linkage mapping, prenatal diagnostics, locating disease loci, detecting genetic variation, molecular archaeology, and epidemiology.*

Keywords- *DNA Fingerprints, Mapping, Crop species, Plant breeding , Genotyping etc.*

I. Introduction-

It is very useful in plant breeding to be able to identify individuals thanks to the high diversity and somatic stability of DNA fingerprints. Because they offer strong technical and informational support for crop breeding, variety quality control, variety right protection, and molecular marker-assisted breeding, DNA fingerprint databases are crucial and significant instruments for plant molecular research. It takes a lot of time and resources to store, analyze, and retrieve the huge volumes of heterogeneous data needed to create a DNA fingerprint database. A database management system is urgently required to track samples and analyze data in order to process the massive volumes of data generated by laboratories and perform quality control. The plant international DNA-fingerprinting system (PIDS), which we built utilizing an open source web server and free software, offers automatic collecting, storage, and effective management features based on merging and comparison algorithms to handle large amounts of microsatellite DNA fingerprint data. Genetic analyses can also be done with PIDS. To upload fingerprint data to the server, this system may match a corresponding capillary electrophoresis image on each primer locus.

The process of finding, quantifying, or comparing genomic features—such as DNA sequence, structural variation, gene expression, and regulatory and functional element annotation—at the genomic scale is known as genomic analysis. On top of the molecular maps, physical maps and the first plant whole genome sequences were constructed. Comparative studies using genetic, cytogenetic, and physical maps as well as DNA sequence data have improved our understanding of the evolution of plant nuclear and organellar genomes. The mapping of factors impacting Mendelian and quantitative traits allowed for the identification and functional characterization of novel genes involved in plant growth, adaptability to biotic and abiotic stress, or the development of other agronomic properties. Data obtained from the simultaneous analysis of all transcripts, proteins, and metabolites present in plant cells or tissues may contribute to a better understanding of the genome function. The postfunctional study of inherent differences in gene function and their effects on phenotypic for use in plant breeding, adaptation, and ecology is anticipated to result in new diagnostic and therapeutic molecular tools.

There are two biological comparison techniques that don't rely on DNA-based technologies: blood group typing and isozyme analysis. However, direct DNA analysis has a lot of advantages. Since DNA is the vehicle of heredity, it immediately reflects the phylogeny or relatedness of the sample material. Since DNA chemistry (methylation, packaging, etc.) differs between cell types and individual cells inherit base pair mutations at every cell division that are themselves stably inherited, even single cells from a person can eventually be identified. The use of more recent techniques involving DNA amplification allows the examination of very small DNA samples acquired from just a few cells.

It has been utilized in forensic identification, population and pedigree analysis, genome linkage mapping, identity testing, identifying familial links and genetic variation, localizing disease loci, and epidemiology. DNA "fingerprinting" is the term used to describe the process of identifying a DNA sample for individual identity based on its chemical or sequence information. In contrast to many approaches for biological comparison, such as blood group typing and isozyme analysis, DNA-based procedures instantly reflect the relatedness or phylogeny of the sample material. They may also be able to distinguish one cell from another due to differences in DNA chemistry between various types of cells and the ongoing acquisition of base pair mutations throughout each cycle of cell division. Due to its adaptability, ubiquity, and stability, DNA is the ideal analytical environment. DNA amplification, for example, can be used to analyze ancient DNA from mummified or fossilized material, DNA from a small number of cells, or DNA from small, somewhat damaged forensic samples. Unique fingerprints can be made using the nucleotide sequence variation in DNA. To analyze or compare whole genomes, it is usually necessary to simplify the enormous complexity of the DNA starting material into simple but recognizable patterns.

For many areas of botany, it is necessary to be able to discriminate between plant genotypes and/or determine the degree of variation and relatedness in a group of genotypes. These tasks have historically been predominantly accomplished using data on physical features, but this has some limitations, including a lack of diversity in the genotypes being studied, subjectivity in the collection and administration of the data, and plasticity brought on by environmental variation. A more objective and impartial tool was offered by molecular markers based on isoenzymes, or enzymes that catalyze the same chemical reaction but differ in the amino acid sequence and subsequently in the rate at which they travel over an electrophoretic gel. Isoenzymes were first used in plant study at the beginning of the 1960s, and in the 1970s and 1980s, their importance considerably increased. Co-dominant allozyme data, which are allelic enzymes coded by genes at the same locus, quickly became very popular for investigations of things like population structure, gene flow, isolation-by-distance (IBD), mating systems, and hybridization. However, protein extraction was frequently difficult, especially for plants with high polyphenol contents in their leaves. The analysis of plants that grew in remote locations offered another issue because proteins frequently need to be isolated and purified within a short amount of time following sampling. The third important problem was the sometimes insufficient level of allozyme polymorphism among related genotypes.

Proteins are significantly more fragile and difficult to control than DNA molecules, and DNA has a practically infinite capacity to produce polymorphic data. Botanists were able to evaluate samples taken from plants that were growing almost anywhere when DNA-based restriction fragment length polymorphism (RFLP) technology was established in the 1970s. Samples, usually leaves, were frequently dried on silica gel before being sent to a lab so they could be maintained frozen until DNA isolation. According to the RFLP method, genomic DNA had to be extracted from the collected material, cut with restriction enzymes, transferred to a filter with Southern blotting, hybridized with locus-specific probes, and then detected, for example, by autoradiography. The requirement to develop species-specific hybridization probes for these investigations was the fundamental challenge, though. As a result, the RFLP technology was mostly used to crop species that were important from a commercial standpoint. For the construction of genetic maps in these crops, as well as sometimes for cultivar identification and genetic relatedness study, RFLP markers were a highly useful tool. But even with the RFLP method, insufficient polymorphism was frequently caused by the absence of suitable loci.

In the 1980s, the first genetic material to undergo RFLP analysis was the chloroplast DNA (cpDNA) molecule. To do this, DNA samples were digested with a single restriction enzyme or a combination of enzymes, followed by the hybridization of radiolabelled cpDNA-specific probes from one of the universal libraries derived from, for example, Petunia. The information was used to construct the restriction site maps of the cpDNA molecule. Since the highly conserved cpDNA molecule exhibits little intra-specific variation, the majority of cpDNA-based RFLP investigations have been conducted on an inter-specific level. While plant mitochondria have occasionally been used in molecular research. The primary reason is that while plant mitochondrial DNA (mtDNA) sequences are normally highly conserved, their size and structure can vary significantly among individual plants. The rates of mtDNA gene substitution may also vary significantly even among closely related plant species, according to current studies.

Nobody foresaw that the so-called DNA fingerprinting method, which Jeffreys and colleagues initially introduced in their landmark works on RFLP experiments with probes derived from tandemly repeated DNA

sequences in human DNA, would also revolutionize plant research. However, as these novel minisatellite probes demonstrated a great potential for unveiling individual-specific DNA fingerprints both in other mammals and in birds, botanists quickly decided to study the prospective benefits of this technique in plants. Dallas was able to distinguish between various species of rice, Oryza sativa, by combining restriction-digested rice DNA with the human 33.6 minisatellite probe in a paper published in 1988. Rice is self-pollinating and has a high homozygosity, hence it was found that the offspring from a single plant were all fingerprinted identically. Additionally, Dallas was able to establish the Mendelian inheritance of DNA fragments from grandparents to second generation (F2) offspring. Shortly after Saiki and colleagues created the dazzling PCR technique, three PCR-based approaches to produce DNA fingerprints were published nearly simultaneously. Using single oligonucleotide primers with random sequences, all of these methods produced PCR fragments from genomic DNA, resulting in multi-locus banding patterns that could be observed by staining or radiography after electrophoretic separation. Random amplified polymorphic DNA (RAPD), a technology developed by Williams and colleagues, swiftly ascended to the top of this list of methods. The primary reasons for this fast success are the little amounts of sample DNA needed and the quick and simple procedures when compared to hybridization-based techniques.

The organelle that is used most commonly in studies on plant genetics is the chloroplast. In plastid genomes, recombination is infrequent or nonexistent, allowing "haplotypes" to be formed from all of a person's DNA polymorphisms. The number of detected band profiles (haplotypes) is usually substantially fewer than those of nuclear markers due to the relatively low frequency of intra-specific plastid DNA polymorphisms. On the plus side, because to the remarkable conservation of organellar DNA sequences, non-specific, or "universal," PCR primers have been developed that can amplify cpDNA introns and intergenic spacers in a range of plant species. Additionally, there are available universal primers for amplifying SSR sites in the chloroplast genome. There are many techniques for spotting polymorphisms in amplified fragments, including high-resolution electrophoresis for spotting length variations, sequencing for spotting sequence variants, and PCR-RFLP, which entails digesting the PCR findings with restriction enzymes.

Plastid DNA is especially useful for studies requiring a low mutation rate, such as the analysis of phylogenetic and phylogeographic patterns. Plastid and nuclear markers are routinely combined in the same investigation for additional data. Given that the pathway of plastid inheritance is typically maternal in angiosperms and paternal in gymnosperms, these markers also offer the possibility of following uni-parental lineages over considerable intervals of time and distance.

Applications of present-day DNA fingerprinting in plants

Genotype identification

DNA fingerprinting has advanced from its humble beginnings in 1988 to become an essential technique for genotype identification in both wild and farmed plant species. The reproductive properties of plants, such as the mode of reproduction and, for those that reproduce by seed, also in their breeding system (selfing or cross-pollinated), as well as the way that pollen and seeds are transported, differ widely among species. All of these factors have a substantial impact on the amount and distribution of genetic variability between and within various entities, such as populations and cultivars. These changes affect how certain plants or genotypes are identified using DNA markers.

While there are times when estimates of genetic closeness based on DNA closely match prior estimates based on morphology, there are also times when there are notable discrepancies. When the morphological features are mostly quantitative in nature, correlation with DNA marker estimations is often quite good compared to qualitative traits, which are more likely to reflect only a small number of mutation events. Additionally, it has been suggested that molecular data are better at differentiating between cultivated genotypes and their wild cousins according to origin and pedigree, but traditional pomological characterisation data are more directly linked to physiological properties.

Wild plant genotype identification

The precise identification of particular genotypes serves as a crucial foundation for a variety of studies based on wild plants. As was already mentioned, the number and distribution of genetic diversity are influenced by several life history factors. Inbreeding species, for instance, are ideally suited for forensic applications since they usually produce patches of plants that are almost or exactly the same size and possess the same genetic make-up. In contrast, outcrossing species have the property that every plant has a distinct genotype. Even though it could be very helpful, obtaining forensic evidence about a particular, unusual plant specimen is frequently very difficult. Whether through extensive vegetative propagation or apomixis, clonal plants typically produce a large number of offspring with the same genotype. Since they may cover large geographic areas, such genotypes are not exact enough to pinpoint the location of botanical data.

However, the variation in plant life cycle features can be viewed as a positive in other research areas; by using the appropriate tools and methods, a variety of biological issues can be resolved. Thus, genotype age in plant clones has been assessed using DNA marker studies, which typically showed out to be much larger and thus frequently older than predicted from past data. For instance, Steinger and colleagues looked at Carex curvula, a sedge plant that is common in the European Alps. A total of 15 multi-locus genotypes were discovered using RAPD analysis of 116 tillers from a small patch (2.0 0.4 m). It was discovered that more than half of the tillers in the population under study were a member of a single, substantial clone estimated to be around 2,000 years old. Invasive plants that occasionally generate exceptionally massive clones include the Japanese knotweed, Fallopia japonica, and the alligator weed, Alternanthera philoxeroides, both of which displayed a single RAPD phenotype despite being examined over extremely wide areas. Sometimes, DNA marker research has revealed more heterogeneity than was anticipated. As a result, it was shown through analysis of the RAPD profiles that each of the five Chinese populations of the invasive water hyacinth Eichhornia crassipes included at least three different clones.

Understanding clonal proliferation can be very helpful for identifying the factors that affect population structure. In the maritime eelgrass Zostera marina, a microsatellite-based study found a positive association between clonal size and heterozygosity. Outbreeding clones' size and number of blossoming shoots revealed that inbreeding depression had decreased vigor and fecundity. A unusually high level of genetic uniformity in the geophyte Gagea spathacea has lately been discovered. All but two of the 138 specimens that were examined showed identical AFLP profiles. 52 populations from over the whole distributional range in northern, central, and eastern Europe were represented by these specimens. This taxon, which is very polyploid, possibly resulted from a hybridization process. Bulbil production and dissemination allowed it to spread across a large region as opposed to seed production and seedling establishment.

The study of the reproductive system in animals with both sexual and asexual seed production methods (i.e., by apomixis) has also benefited from DNA fingerprinting. Because they contain triploid individuals that seem to reproduce by apomixis, many Taraxacum populations are clonal. The results of an AFLP research demonstrated that these clones can occasionally cover sizable areas. A comparison of SSR and AFLP data revealed that both marker types were capable of differentiating across Taraxacum's nine apomictic microspecies (defined on the basis of morphological features), but AFLP was more adept at detecting minor, mutation-derived alterations within each microspecies. AFLP revealed much less variation in apomictic lineages of Ranunculus carpaticola than at two dinucleotide repeat SSR loci. The lack of allele segregation in the investigated SSR locations provided support for a mutational origin rather than a recombinational origin. As a result, each lineage had the same number of alleles at each locus, and these alleles also formed classes of related allele sizes within each lineage.

Final Thoughts and Summary The polymerase chain reaction (PCR) has been effectively used in conventional DNA fingerprinting to enzymatically amplify particular DNA sequences. In PCR, two oligonucleotide primers, each 20 nucleotides long, selectively hybridize to opposing DNA strands on either side of a region that has to be amplified. A series of cycles including DNA denaturation, primer annealing, and extension of the annealed primers by DNA polymerase will amplify the target DNA region many times over, producing an amplified fragment whose termini are specified by the 5' ends of each primer. By using PCR, radiolabeled nucleotides have been incorporated into DNA fingerprinting probes. These probes have a very high specific activity, which makes them highly useful when there is a small ratio between probe and target size. Additionally, hypervariable regions inside designated loci can be amplified selectively using the PCR method. Changes in electrophoretic mobility can be used to spot single-base substitutions that impact DNA shape as well as changes in DNA sequence. These PCR amplified and tagged regions contain single-strand conformation polymorphisms (SSCPs), which are capable of detecting allelic polymorphism repetitions at distinct chromosomal loci.

II. CONCLUSIONS

The detection of DNA polymorphisms using conventional fingerprinting techniques requires extensive experimental manipulation, prior knowledge of the DNA sequence, or cloned and specified probes. To get past these limitations, many labs have recently used a PCR-based technique to amplify short, arbitrary portions of DNA from a target genome. In these studies, amplification using a thermos table DNA polymerase driven by one or more oligonucleotides of arbitrary sequence resulted in a characteristic spectrum of products under less strict circumstances. The basis for genetic linkage analysis of genomes is the use of AFLPs as genetic markers in DNA recombination between pairs of maternally and paternally derived chromosome homologs during meiosis. A lot of work has gone into getting high-resolution RFLP genetic maps for different plant species, and in some cases, resolving several loci affecting quantitative traits.

References

[1]. Wall J.D., Cox M.P., Yong T.P., Mendez F.L., Woerner A., Severson T., Hammer M.F. A novel DNA sequence database for analyzing human demographic history. Genome Res. 2015;18:1354–1361.

[2]. Struyf P., De M.S., Vandeviver C., Renard B., Vander B.T. The effectiveness of DNA databases in relation to their purpose and content: A systematic review. Forensic Sci. Int. 2019;301:371–381.

[3]. Carew M.E., Nichols S.J., Batovska J., St C.R., Murphy N.P., Blacket M.J., Shackleton M.E. A DNA barcode database of Australia's freshwater macroinvertebrate fauna. Mar. Freshw. Res. 2017;68:1788–1802.

[4]. Mantelatto F.L., Terossi M., Negri M., Buranelli R.C., Robles R., Magalhaes T., Tamburus A.F., Rossi N., Miyazaki M.J. DNA sequence database as a tool to identify decapod crustaceans on the Sao Paulo coastline. Mitochondrial DNA Part A. 2018;29:805–815.

[5]. Zhou H.Y., Zhang P.H., Luo J., Liu X.Y., Fan S.X., Liu C.J., Han Y.Y. The establishment of a DNA fngerprinting database for 73 varieties of Lactuca sativa capitate L. using SSR molecular markers. Hortic. Environ. Biotechnol. 2018;60:95–103.

[6]. Backiyarani S., Chandrasekar A., Uma S., Saraswathi M.S. MusatransSSRDB (a transcriptome derived SSR database)—An advanced tool for banana improvement. J. Biosci. 2019;43:110–116.

[7]. Yu J.Y., Dossa K., Wang L.H., Zhang Y.X., Wei X., Liao B.S., Zhang X.R. PMDBase: A database for studying microsatellite DNA and marker development in plants. Nucleic Acids Res. 2017;45:1046–1053.

[8]. Pan Y.B. Development and Integration of an SSR-Based Molecular Identity Database into Sugarcane Breeding Program. Agronomy. 2016;6:28.

[9]. Sochorová J., Garcia S., Gálvez F., Symonová R., Kovařík A. Evolutionary trends in animal ribosomal DNA loci: Introduction to a new online database. Chromosoma. 2018;127:141–150.

[10]. Freixas-Coutin J.A., An S.Y., Postman J., Bassil N.V., Yates B., Shukla M., Saxena P.K. Development of a reliable Corylus sp. reference database through the implementation of a DNA fingerprinting test. Planta. 2019;6:1863–1874.

[11]. Li L., Fang Z.W., Zhou J.F., Chen H., Hu Z.F., Gao L.F., Chen L.H., Ren S., Ma H.Y., Lu L., et al. An accurate and efficient method for large-scale SSR genotyping and applications. Nucleic Acids Res. 2017;10:e88.

[12]. Molla M.R., Ahmed I., Rohman M.M., Hossain M.A., Chowdhury M.A.Z. Genetic diversity analysis and DNA fingerprinting of Mungbean (Vigna radiata L.) genotypes using SSR markers. J. Plant Sci. 2016;6:153–164.

[13]. Wilton R., Wheelan S.J., Szalay A.S., Salzberg S.L. The Terabase Search Engine: A large-scale relational database of short-read sequences. Bioinformatics. 2019;35:665–670.